ORIGINAL ARTICLE

Association of Differential Mast Cell Activation with Granulocytic Inflammation in Severe Asthma

Angelica Tiotiu[1,2*], Yusef Badi[1,3*], Nazanin Zounemat Kermani[3], Marek Sanak[4], Johan Kolmert[5,6,7], Craig E. Wheelock[6], Philip M. Hansbro[8], Sven-Erik Dahlén[5,7], Peter J. Sterk[9], Ratko Djukanovic[10], Yike Guo[3], Sharon Mumby[1], Ian M. Adcock[1,3‡], and Kian Fan Chung[1,3‡]; on behalf of the U-BIOPRED Consortium Project Team

[1]National Heart and Lung Institute and [3]Data Science Institute, Department of Computing, Imperial College London, London, United Kingdom; [2]Department of Pulmonology, University Hospital of Nancy, Nancy, France; [4]Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland; [5]The Institute of Environmental Medicine and [6]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; [7]Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden; [8]Centre for Inflammation, Centenary Institute, University of Technology Sydney, School of Life Sciences, Faculty of Science, Sydney, New South Wales, Australia; [9]Amsterdam University Medical Centres, University of Amsterdam, Amsterdam, the Netherlands; and [10]Faculty of Medicine, University of Southampton, Southampton, United Kingdom

ORCID IDs: 0000-0002-8113-0653 (C.E.W.); 0000-0003-2101-8843 (I.M.A.); 0000-0001-7101-1426 (K.F.C.).

Abstract

Rationale: Mast cells (MCs) play a role in inflammation and both innate and adaptive immunity, but their involvement in severe asthma (SA) remains undefined.

Objectives: We investigated the phenotypic characteristics of the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes) asthma cohort by applying published MC activation signatures to the sputum cell transcriptome.

Methods: Eighty-four participants with SA, 20 with mild/moderate asthma (MMA), and 16 healthy participants without asthma were studied. We calculated enrichment scores (ESs) for nine MC activation signatures by asthma severity, sputum granulocyte status, and three previously defined sputum molecular phenotypes or transcriptome-associated clusters (TACs) 1, 2, and 3 using gene set variation analysis.

Measurements and Main Results: MC signatures except unstimulated, repeated FcεR1-stimulated and IFN-γ–stimulated signatures were enriched in SA. A FcεR1-IgE–stimulated and a single-cell signature from asthmatic bronchial biopsies were highly enriched in eosinophilic asthma and in the TAC1 molecular phenotype. Subjects with a high ES for these signatures had elevated sputum amounts of similar genes and pathways. IL-33– and LPS-stimulated MC signatures had greater ES in neutrophilic and mixed granulocytic asthma and in the TAC2 molecular phenotype. These subjects exhibited neutrophil, NF-κB (nuclear factor-κB), and IL-1β/TNF-α (tumor necrosis factor-α) pathway activation. The IFN-γ–stimulated signature had the greatest ES in TAC2 and TAC3 that was associated with responses to viral infection. Similar results were obtained in an independent ADEPT (Airway Disease Endotyping for Personalized Therapeutics) asthma cohort.

Conclusions: Gene signatures of MC activation allow the detection of SA phenotypes and indicate that MCs can be induced to take on distinct transcriptional phenotypes associated with specific clinical phenotypes. IL-33–stimulated MC signature was associated with severe neutrophilic asthma, whereas IgE-activated MC was associated with an eosinophilic phenotype.

Keywords: mast cell; eosinophils; neutrophils; IL-33; IgE-FcεRI

(Received in original form February 11, 2021; accepted in final form November 22, 2021)

*Co–first authors.

‡Co–senior authors.

A complete list of U-BIOPRED Consortium Project Team members may be found before the beginning of the References.

Supported by the European Union's Seventh Framework Programme (FP7/2007-2013) Innovative Medicines Initiative Joint Undertaking (grant 115010). U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes) was also supported by European Federation of Pharmaceutical Industries and Associations companies' in-kind contribution (www.imi.europa.eu). K.F.C. and I.M.A. are funded by UK Research and Innovation. K.F.C. is an Emeritus Senior Investigator of the UK National Institute for Health Research. P.M.H. is funded by the National Health and Medical Research Council of Australia. C.E.W. is funded by the Swedish Heart Lung Foundation (grants HLF 20180290 and HLF 20200693).

Am J Respir Crit Care Med Vol 205, Iss 4, pp 397–411, Feb 15, 2022

Originally Published in Press as DOI: 10.1164/rccm.202102-0355OC on November 23, 2021
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Mast cells play a role in inflammation and both innate and adaptive immunity, but their involvement in severe asthma remains undefined.

What This Study Adds to the Field: Gene signatures of mast cell activation allow the detection of severe asthma phenotypes and indicate that mast cells can be induced to take on distinct transcriptional phenotypes associated with specific clinical phenotypes. The IL-33–stimulated mast cell signature was associated with severe neutrophilic asthma, whereas the IgE-activated mast cell signature was associated with an eosinophilic phenotype.

Patients with severe asthma (SA) are those who require treatment with high-dose inhaled corticosteroids plus a second controller to prevent it from becoming "uncontrolled" or those who remain uncontrolled despite this treatment (1, 2). The mast cell (MC) represents an important heterogeneous population identified by cell morphology, histochemical properties, protease content, piecemeal or anaphylactic types of degranulation, and surface receptor expression (3). The close vicinity of MCs to blood vessels and terminal nerve endings exposes them to antigens or incoming pathogens or locally released mediators. This results in MCs becoming involved in acquired allergic reactions and also acting as critical sentinel cells in innate immunity (4). Consequently, MCs may drive different pathophysiologic aspects of asthma according to their response to their immediate environment, leading to the induction of specific activation pathways (3, 5).

Information on the role of MCs in asthma is limited because of the difficulty in isolating sufficient numbers from tissues for *ex vivo* analysis. In bronchial biopsies, MCs in the airways of patients with SA detected by conventional anti-tryptase immunohistochemical staining are reduced compared with those in healthy control subjects (6), which means that the activation status of MCs is likely to be more important. In SA, tryptase-positive submucosal MCs predominate in the airway submucosa and epithelium, and their increased activation has been deduced by increased amounts of prostaglandin D2 (PGD_2), a specific product of MCs (7). The availability of *–omics* platforms represents a powerful tool to analyze the potential role of MCs and has identified MC genes such as *TPSB2* (tryptase β2) and *CPA3* (carboxypeptidase A3) as being highly expressed in the sputum of SA patients with sputum eosinophilia (8). MC activation may result from exposure to allergen but also to bacterial or viral infections (9, 10).

MC mediators including tryptase, chymase, and CPA3 are elevated in BAL fluid in both severe atopic, T-helper cell type 2 (Th2)-high, and nonatopic Th2-low/neutrophilic asthma (11, 12). The expression of eight MC-related genes in sputum samples correlated with MC/basophil numbers in severe Th2-high asthma (13), but drivers of non-IgE MC activation remain unclear. We have previously reported molecular phenotypes or transcriptomic-associated clusters (TACs) that provide transcriptomic information regarding Th2-associated eosinophilic (TAC1), inflammasome-associated neutrophilic (TAC2), and paucigranulocytic (TAC3) asthma (8).

We hypothesized that MC activation signatures will not only provide insight into determining asthma severity but also identify different asthma driver mechanisms because of their differential sensitivity to local inflammatory stimuli. We examined the contribution of distinct Th2 and non-Th2 MC activation pathways in determining the inflammatory phenotype of asthma. We used previously identified gene signatures of MCs activated not only by IgE interaction with its high-affinity IgE receptor, FcεRI, but also by the alarmin IL-33, the TLR4 (Toll-like receptor 4)–activator LPS, and the innate and adaptive immune cytokine, IFN-γ, to examine MC activation status in the sputum cell transcriptome of the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes) cohort (14).

Methods

Subjects

We analyzed sputum transcriptomic data from 104 participants with SA who were nonsmokers (Sans), who were smokers or ex-smokers (Sasms), or who had mild/moderate asthma (MMA) and from 16 healthy participants (HVs) from the European U-BIOPRED cohort (14) (Table E1 in the online supplement). Airflow limitation was defined as post-bronchodilator FEV_1 <75% of predicted and an FEV_1/FVC ratio <0.70 (15). TAC classification was defined previously (8). The study was approved by the local ethics committees, and participants gave written informed consent.

Sputum Collection and Microarray Transcriptomics

Sputum induction was performed and differential cell counts in sputum plugs were measured (16). Sputum supernatants were assayed for proteins, cytokines, and eicosanoids. Urine eicosanoid concentrations were also measured (17). Eleven sputum and urinary eicosanoids and 12 serum markers were measured because of biological interest and relevance to severe airway disease. Details of eicosanoid measurements are given in the Supplementary Methods. Transcriptomic analysis was performed using Affymetrix U133 Plus 2.0 (Affymetrix) (8), and the expression matrix was derived using *affy* R package (18). The raw

Author Contributions: A.T., Y.B., N.Z.K., and S.M. analyzed the data; J.K., C.E.W., S.-E.D., and M.S. were involved in the eicosanoid assays in sputum supernatants and urine samples; Y.G. was responsible for the U-BIOPRED dataset; I.M.A. and K.F.C. conceived the idea with contributions from S.M. and P.M.H.; I.M.A., K.F.C., P.J.S., and R.D. obtained the funding for the U-BIOPRED project; A.T., Y.B., I.M.A., and K.F.C. wrote the manuscript; all authors contributed to its finalization.

Correspondence and requests for reprints should be addressed to Kian Fan Chung, M.D., D.Sc., National Heart and Lung Institute, Imperial College London, Dovehouse Street, London SW3 6LY, UK. E-mail: f.chung@imperial.ac.uk.

This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

transcriptomics data have been used in previous publications (8, 19). Data were hosted on tranSMART, an open-source knowledge management platform (20). We defined neutrophilic asthma as ⩾73.6% of neutrophils and eosinophilic asthma as ⩾1.49% of eosinophils in sputum (19).

Gene Set Variation Analysis

Gene set variation analysis (GSVA) allows identification of differences in the expression of predefined sets of genes between heterogeneous groups by calculating a sample-wise enrichment score (ES) ranging from −1 to 1 for each gene set for each subject (21). The nine gene set signatures were derived from *in vitro* studies of human MCs (Table E2). The first two gene sets represented baseline unactivated MCs and MCs activated by a single exposure to IgE receptor (FcεRI) cross-linking for 2 hours (signatures 1 and 2) (22). Support-vector machine learning enhanced the specificity of signatures from unstimulated human cord blood–derived MCs or after FcεRI activation (23) against 20 other immune cells (22), with MC genes enriched by overlap with MCs stimulated by adenosine A3 receptor and by T-cell contact (24). Signatures 3 and 4 were from cord blood progenitor–derived MCs sensitized with IgE for 24 hours and FcεRI activated with anti-human IgE for 6 hours once or repeatedly over 2 weeks (25), and signature 5 was from human umbilical cord blood $CD34^+$ progenitor–derived MCs sensitized with IgE overnight and FcεR activated with anti-human IgE for 2 hours (26). Signature 6 was from single-cell RNA sequencing of MCs from asthma bronchial biopsies (MCscbb) (27). Signature 7 was obtained from blood $CD34^+$ progenitor–derived MCs cultured with stem cell factor and IL-6 and exposed to IL-33 (28). Finally, adult blood progenitor–derived MCs activated by LPS and IFN-γ provided signatures 8 and 9, respectively (29).

To ensure the specificity of these various signatures, we removed overlapping genes across the MC, granulocyte (30, 31), and TAC signatures to ensure <10% gene overlap. The only exceptions were the overlap between the IgE-activated and MCscbb signatures (16.7%, 5 of 30 genes), the IL-33–stimulated and LPS- (18.6%, 16 of 86 genes) and IFN-γ–stimulated (12.8%, 11 of 86 genes) signatures, and the LPS- and IFN-γ–stimulated signatures (12.6%, 18 of 143 genes).

ADEPT Cohort

Results from U-BIOPRED cohort were validated using an external dataset from the Airway Disease Endotyping for Personalized Therapeutics (ADEPT) cohort of patients with mild asthma ($n = 52$), moderate asthma ($n = 55$), and SA ($n = 51$) (32).

Statistical Analysis

Analyses were performed primarily in R version 3.5.0 (33). Comparisons of clinical data were performed between "high"- and "low"-enriched groups of patients defined using ES cutoffs of greater than or equal to 0 or less than 0, respectively. All categorical variables were analyzed using Fisher exact test. Student's *t* test was used for continuous variables with normal distribution; otherwise, Wilcoxon rank sum test was used. To correct for testing of multiple clinical variables between groups, *P* values were adjusted for by using an optimized version of the false discovery rate through the qvalue package (34). Pairwise Wilcoxon rank sum tests were used to analyze the ES differences between groups with Hommel multiple testing correction using the rstatix package (https://cran.r-project.org/web/packages/rstatix/index.html). Plots were generated in ggplot2 (35), and statistics were displayed using the ggpubr package (36).

We identified differentially expressed genes (DEGs) in sputum between high- and low-enriched groups using Limma (Ritchie ME Nucleic acid Res). Statistical significance was determined by $\log_2$ fold change greater than or equal to 1 or less than or equal to −1 and a Benjamini-Hochberg false discovery rate of less than or equal to 0.05. Pathway analysis was performed using STRING database (www.string-db.org) and Enrichr (https://maayanlab.cloud/Enrichr/) and visualized in Cytoscape (https://cytoscape.org).

Results

Expression of MC Signatures according to Severity

Only the 2-hour FcεRI-activated MC (signature 2) and the MCscbb (signature 6) signatures were enriched in subjects with MMA compared with those in HVs (Figure 1). In contrast, all MC signatures were significantly enriched in subjects with SA compared with those with MMA and HVs except for the repeated unstimulated, the FcεR1-stimulated, and the IFN-γ–stimulated signatures (Figure 1). There was no significant difference between the ES of any MC signature between Sans and Sasms (Figure E1 in the online supplement). All MC-activated signatures were enriched in Sans compared with those with MMA and/or HVs with the exception of the IFN-γ–stimulated, the repeated FcεR1-stimulated, and the unstimulated signatures. Furthermore, signature 2 (2-h FcεRI activated), signature 3 (6-h single FcεR1 activated), signature 5 (2-h FcεR1 activated), and signature 8 (LPS stimulated) were enriched in Sasms compared with subjects with MMA and/or HVs (Figure E1).

All MC signatures were significantly enriched in subjects with asthma with airflow limitation compared with those without airflow limitation, with the exception of the unstimulated (signature 1), the repeated FcεR1-stimulated (signature 4), and IFN-γ–stimulated (signature 9) MC signatures (Figure E2). The unstimulated MC signature (signature 1) and the MCscbb signature (signature 6) ESs did not correlate with sputum MC numbers ($r = 0.028$ and $r = -0.014$, respectively) or percentages ($r = 0.0102$ and $r = -0.018$, respectively) (Figure E3). Furthermore, these signatures are clearly detecting distinct entities, as they do not align with respect to asthma severity, sputum granulocyte status, or molecular phenotype enrichment.

Other recently published single-cell MC signatures (37, 38) did not correlate with sputum MC numbers, suggesting that the signatures represent distinct MC activation states (Figure E3). However, the Dwyer MC2 and MC4 single-cell signatures, but not the IL-4–stimulated MC signature, (37) gave a better correlation with airway smooth muscle–associated, but not epithelial- or submucosal-associated, MC numbers, but these did not reach significance (Figure E3). The nine MC signatures all had weak correlations with tissue MCs. The murine single-cell MC signature from Hamey and coworkers (38) provided the only significant correlation with tissue MC numbers in airway smooth muscles ($r = 0.5988$; $P = 0.005$). This signature has a 45% overlap with the human single-cell MC signatures, suggesting that the nonoverlap genes may drive this correlation.

MC Signatures according to Sputum Granulocytic Inflammation

MC signatures were enriched in subjects with eosinophilic, neutrophilic, and mixed granulocytic asthma

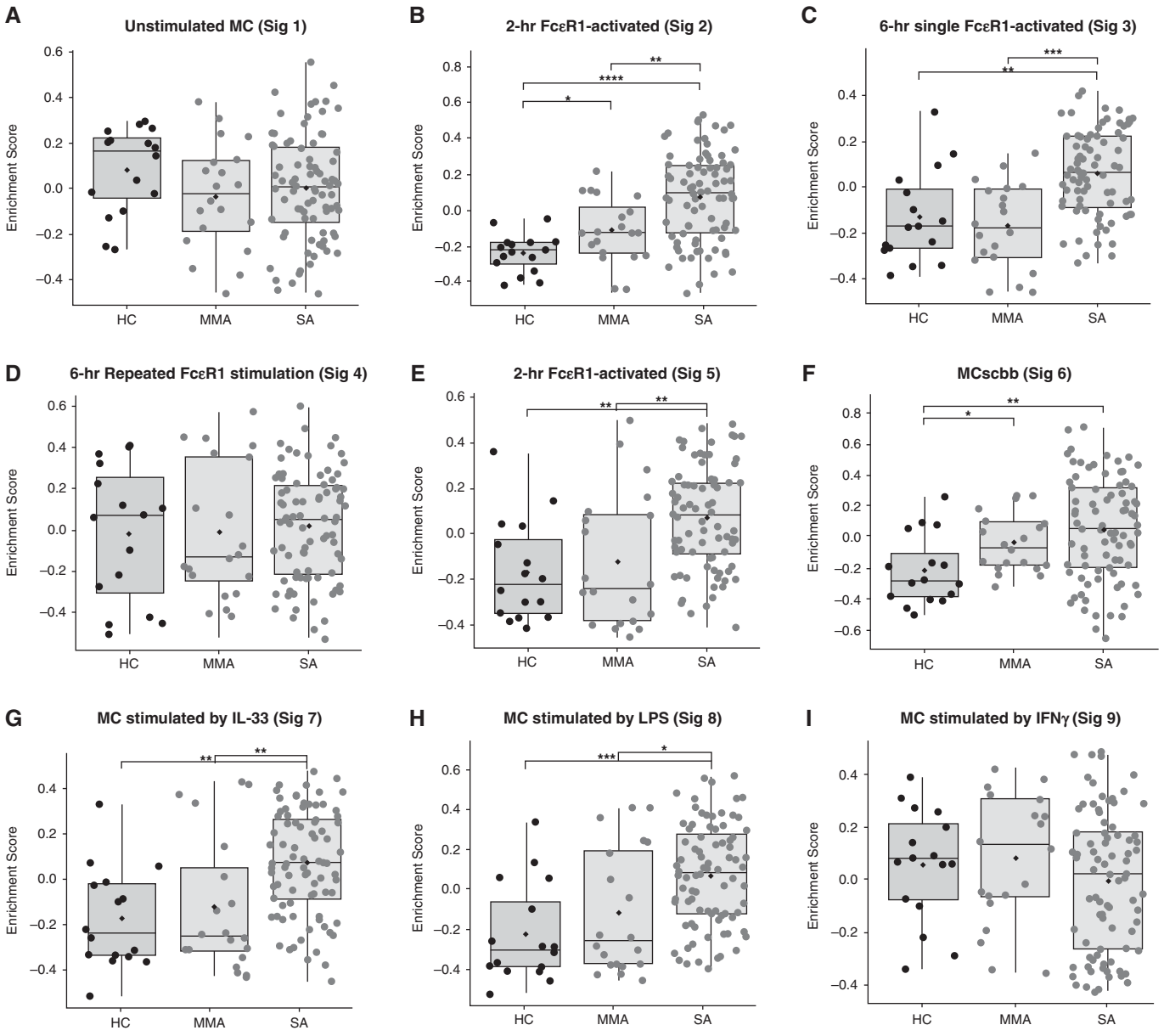

Figure 1. Box plot of enrichment scores for mast cell (MC) signatures according to clinical category. Box plots of gene set variation analysis show enrichment scores for gene signatures according to sputum transcriptomic data of individual subjects using the following signatures: (*A*) unstimulated MCs, (*B*) 2-hour FcεRI-activated MCs, (*C*) single FcεR1–IgE receptor–stimulated (24-h IgE sensitization/6-h FcεRI activation) MCs, (*D*) repeated FcεR1-stimulated MCs, (*E*) FcεRI-activated MCs (overnight IgE sensitization/2-h FcεRI activation), (*F*) single MC bronchial biopsy signature, (*G*) MCs stimulated by IL-33, (*H*) MCs stimulated by LPS, and (*I*) MCs stimulated by IFN-γ. The box-and-whisker plots show the median and interquartile range, with diamonds representing the mean and whiskers representing the maximum and minimum values. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$, and $^{****}P < 0.00005$ between groups. HC = healthy nonsmoking control subject; MCscbb = single-cell RNA sequencing of MCs from asthma bronchial biopsies; MMA = mild/moderate asthma; SA = severe asthma; Sig = signature.

(Figures 2A–2E). In particular, the acute 2-hour FcεRI-activated (signature 2) (Figure 2B) and the single 6-hour FcεR1-activated (signature 3) (Figure 2C) MC signatures were enriched in the groups with eosinophil and mixed granulocytic asthma compared with those in subjects with paucigranulocytic asthma, whereas the MCscbb signature was enriched in the eosinophil and mixed groups compared with that in HVs (Figure 2F). As expected, the LPS-stimulated MC signature was highly significantly enriched in the subjects with neutrophilic and mixed granulocytic asthma compared with that of both HVs and subjects with paucigranulocytic asthma (Figure 2H), and a similar enrichment in groups with neutrophilic and mixed asthma was observed with the repeated FcεR1, single

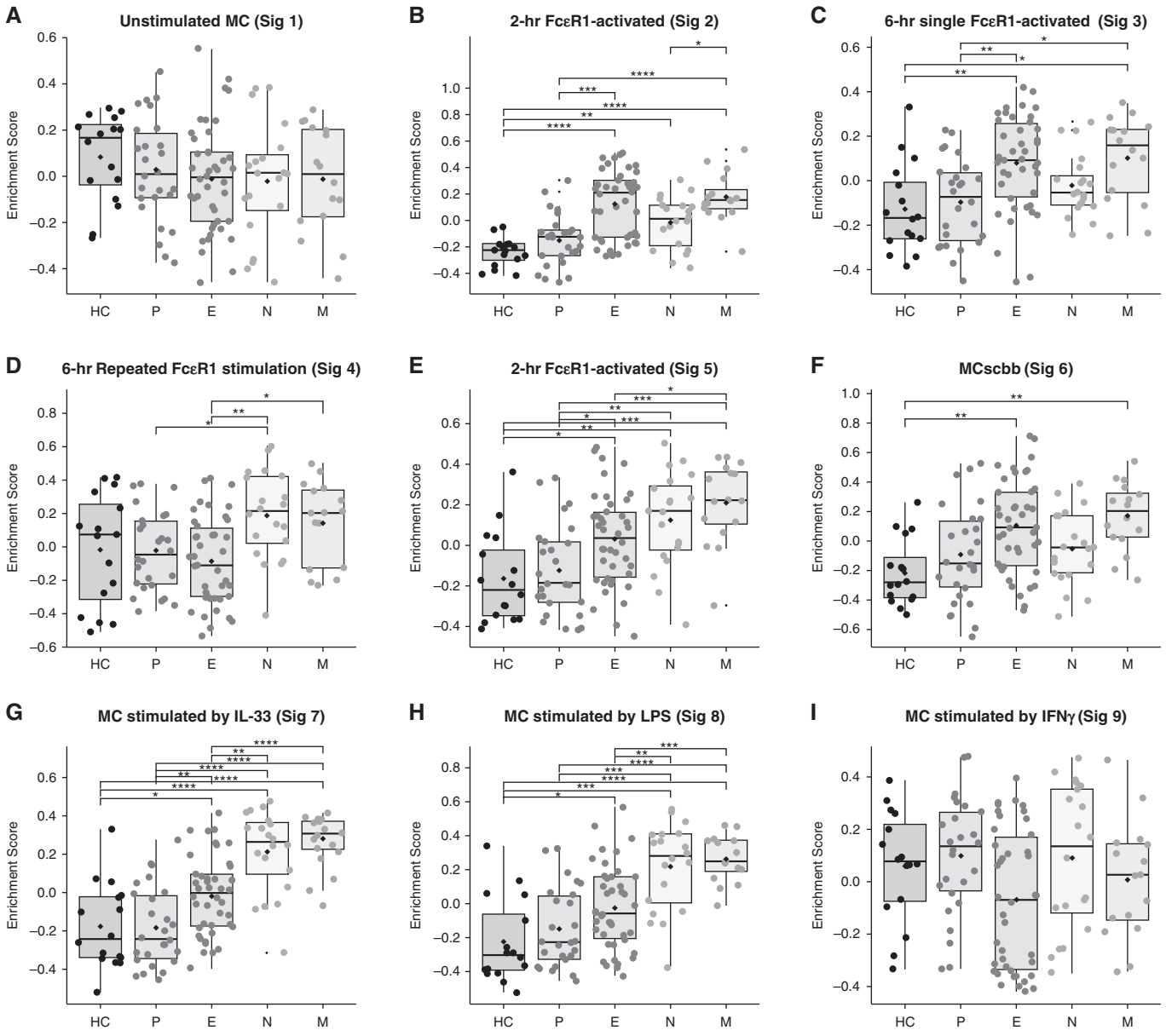

Figure 2. Box plot of mast cell (MC) signature enrichment scores according to sputum granulocytes in the U-BIOPRED cohort. Gene set variation analysis plots of U-BIOPRED participants' individual enrichment scores for (*A*) unstimulated MCs, (*B*) 2-hour FcεRI-activated MCs, (*C*) single FcεR1-activated MCs (24-h IgE sensitization/6-h FcεRI activation), (*D*) repeated FcεR1-stimulated MCs, (*E*) FcεRI-activated MCs (overnight IgE sensitization/2-h FcεRI activation), (*F*) single MC bronchial biopsy signature, (*G*) MCs stimulated by IL-33, (*H*) MCs stimulated by LPS, and (*I*) MCs stimulated by IFN-γ are shown according to sputum granulocyte status. The box-and-whisker plots show the median and interquartile range, with diamonds representing the mean and whiskers representing the maximum and minimum values. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$, and $^{****}P < 0.00005$. E = eosinophilic; HC = healthy control subject; M = mixed; MCscbb = single-cell RNA sequencing of MCs from asthma bronchial biopsies; N = neutrophilic; P = paucigranulocytic; Sig = signature.

FcεR1-activated (2-hour) (signature 5), and IL-33–stimulated MC signatures (Figures 2D, 2E, and 2G). Both the IL-33– and LPS-stimulated signatures were also enriched in subjects with eosinophilic asthma compared with those in HVs. Unstimulated and IFN-γ–stimulated signatures were not enriched in any group.

MC Signature Enrichment in the ADEPT Cohort

We showed similar trends regarding the enrichment of specific MC signatures in this cohort. The 2-hour FcεRI-activated signature (signature 2) was enriched in patients with eosinophilic asthma compared with that in HVs and patients with paucigranulocytic asthma (Figure E4B). The MCscbb signature was highest in the patients with eosinophilic asthma and showed a significant reduction in patients with neutrophilic asthma (Figure E4F). However, no enrichment was seen with the single FcεR1-activated (6-h) stimulated signature (signature 3) or with the unstimulated or IFN-γ–stimulated signatures (Figures E4A, E4C, and E4I). In contrast, the

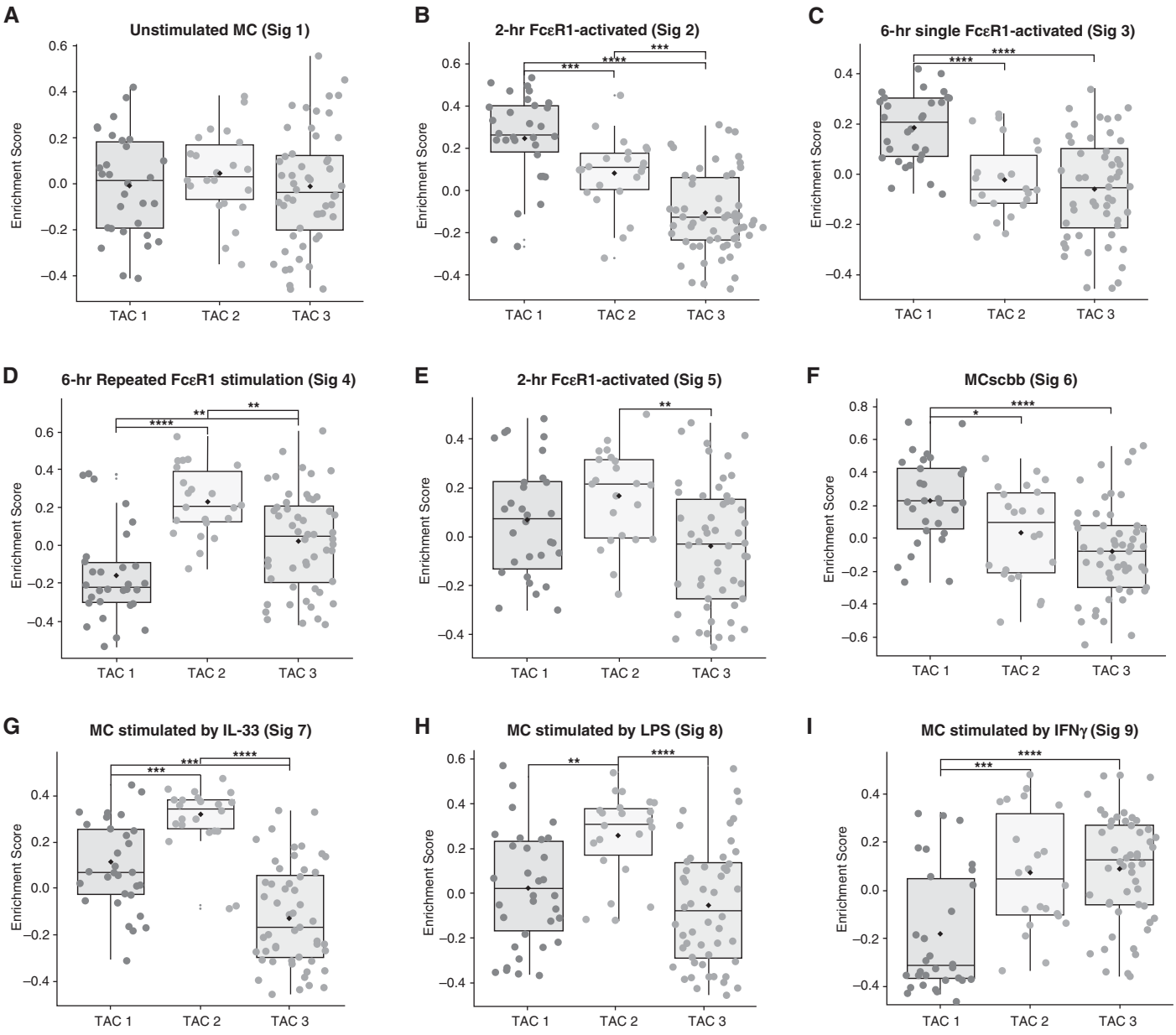

Figure 3. Mast cell (MC) signature enrichment scores according to TAC. Gene set variation analysis plots of U-BIOPRED participants' individual enrichment scores are shown for (*A*) unstimulated MCs, (*B*) 2-hour FcεRI-activated MCs, (*C*) single FcεR1-activated MCs (24-h IgE sensitization/6-h FcεRI activation), (*D*) repeated FcεR1-stimulated MCs, (*E*) FcεRI-activated MCs (overnight IgE sensitization/2-h FcεRI activation), (*F*) single MC bronchial biopsy signature, (*G*) MCs stimulated by IL-33, (*H*) MCs stimulated by LPS, and (*I*) MCs stimulated by IFN-γ. The box-and-whisker plots show the median and interquartile range, with diamonds representing the mean and whiskers representing the maximum and minimum values. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$, and $^{****}P < 0.00005$. MCscbb = single-cell RNA sequencing of MCs from asthma bronchial biopsies; Sig = signature; TAC = transcriptomic-associated cluster.

signatures for repeated FcεR1 stimulation (signature 4), single FcεR1-activated (2-h signature 5), and IL-33– (signature 7) and LPS-stimulated (signature 8) signatures all showed enrichment in the subjects with neutrophilic and mixed granulocytic asthma but did not reach significance except for the repeated FcεR1-stimulated signature (Figure E4).

Expression of MC Signatures according to TACs

As described above, TACs are sputum transcriptomic phenotypes that are associated with Th2-associated eosinophilic (TAC1), inflammasome-associated neutrophilic (TAC2), and paucigranulocytic (TAC3) asthma. The unstimulated MC signature (signature 1) was not enriched in any TAC group (Figure 3A). We discerned greater differences among the TACs, with a general enrichment of FcεRI-activated MCs and MCscbb in TAC1 (Figures 3B, 3C, and 3F). In contrast, TAC2 participants had a greater enrichment for MCs stimulated by repeated FcεR1 challenge or overnight IgE sensitization/2-hour FcεRI activation (signatures 4 and 5) (Figures 3D and 3E),

Table 1. Clinical and Biological Parameters between the Group with High and Low ESs for FcεRI MC–activated Signature 2

Variable	High-ES 2-h FcεRI-activated MC ($n = 57$)	Low-ES 2-h FcεRI-activated MC ($n = 47$)	*P* Value	Adj. *P* Value*
Age, yr	52.8 ± 12	49.6 ± 14.8	NS	NS
Sex, F	33	27	NS	NS
BMI, kg/m^2	27.2 ± 4.4	28.6 ± 6.05	NS	NS
Atopy (positive)	27	16	NS	NS
Nonsmoker	33	31	NS	NS
Comorbidities				
Allergic rhinitis	24	18	NS	NS
Eczema	17	16	NS	NS
Nasal polyps	23	11	NS	NS
Severe exacerbations per year	1.61 ± 1.95	1.47 ± 2.09	NS	NS
ACQ-5	1.97 ± 1.17	1.99 ± 1.42	NS	NS
OCS daily	29	11	7.89×10^{-3}	3.58×10^{-2}
Lung function				
FEV_1 (% predicted) prebronchodilator	61.9 ± 21.1	74 ± 23.4	7.04×10^{-3}	3.58×10^{-2}
FEV_1/FVC ratio	55.2 ± 12.8	64 ± 11.1	2.88×10^{-4}	2.47×10^{-3}
F_{ENO}, ppb	43.7 ± 40.9	32.9 ± 29.1	NS	NS
Sputum cells, %				
MC	0.0326 ± 0.0863	0.0202 ± 0.0712	NS	NS
Eosinophils	18.3 ± 21.8	2.99 ± 6.81	1.19×10^{-6}	1.84×10^{-5}
Neutrophils	61.9 ± 26.3	51.7 ± 24.3	3.57×10^{-2}	NS
Lymphocytes	1.23 ± 1.25	1.56 ± 1.18	4.45×10^{-2}	NS
Macrophages	18.5 ± 15	44.2 ± 23.5	4.10×10^{-8}	1.58×10^{-6}
Blood cell counts, 10^3/μl				
Eosinophils	0.361 ± 0.249	0.28 ± 0.31	8.78×10^{-3}	3.76×10^{-2}
Neutrophils	5.48 ± 2.31	4.6 ± 2.25	2.31×10^{-2}	NS
Total serum IgE, IU/ml	295 ± 494	243 ± 551	NS	NS
Biomarkers				
Periostin (serum), ng/ml	56.4 ± 18.7	44.6 ± 11.8	1.21×10^{-3}	9.34×10^{-3}
Eotaxin (plasma), pg/ml	128 ± 82.9	120 ± 50.1	NS	NS
hCRP (serum), mg/L	7.16 ± 15.8	4.23 ± 6.9	NS	NS
IFN-γ (plasma), pg/ml	10.5 ± 9.85	8.16 ± 11.9	3.75×10^{-2}	NS
TNF-α (plasma), pg/ml	1.88 ± 0.561	2.04 ± 0.7	NS	NS
IL-13 (serum), pg/ml	1.14 ± 1.54	0.566 ± 0.384	2.01×10^{-3}	1.41×10^{-2}
IL-6 (plasma), pg/ml	1.26 ± 1.08	1.05 ± 0.941	NS	NS
IL-18 (serum), pg/ml	223 ± 92.2	249 ± 126	NS	NS
Galectin3 (serum), pg/ml	6,120 ± 1,970	5,610 ± 1,570	NS	NS
MCP4 (serum), pg/ml	171 ± 90.7	142 ± 83.1	2.55×10^{-2}	NS
MMP3 (serum), ng/ml	20.2 ± 14.7	20.5 ± 19.9	NS	NS
Serpin E1 (serum), ng/ml	104 ± 29.9	88.1 ± 23.8	2.65×10^{-3}	1.57×10^{-2}
Sputum eicosanoids, pg/ml				
11-dehydro-TXB2	139 ± 155	121 ± 197	NS	NS
12-HETE	2,420 ± 1,580	2,210 ± 2,600	2.07×10^{-2}	NS
15-HETE	8,800 ± 10,100	3,450 ± 6,030	1.66×10^{-4}	1.60×10^{-3}
5-HETE	1,210 ± 1,290	2,720 ± 5,300	NS	NS
6-keto-PGF1α	59.9 ± 30.1	52.3 ± 19.4	NS	NS
LTB4	728 ± 737	1,610 ± 3,240	NS	NS
LTE4	591 ± 700	307 ± 658	5.78×10^{-5}	6.37×10^{-4}
PGD2	272 ± 272	171 ± 198	4.73×10^{-3}	2.61×10^{-2}
PGE2	376 ± 435	284 ± 343	NS	NS
Tetranor-PGDM	61.7 ± 65.5	64.6 ± 54.7	NS	NS
Tetranor-PGEM	79.4 ± 65.9	78.9 ± 61.2	NS	NS
Urine eicosanoids, ng/mmol				
11-dehydroTXB2	14.3 ± 9.22	10.2 ± 7.08	7.70×10^{-3}	3.58×10^{-2}
2,3-dinor-11β-PGF2α	77.3 ± 36.5	68.6 ± 56.2	NS	NS
2,3-dinor-8-iso-PGF2α	248 ± 119	241 ± 223	NS	NS
2,3-dinor-TXB2	60.4 ± 38.6	50.4 ± 34.7	NS	NS
8,12-iso-iPF2α-VI	387 ± 224	436 ± 301	NS	NS
8-iso-PGF2α	28.2 ± 11.2	30 ± 15.8	NS	NS

(*Continued*)

Table 1. (*Continued*)

Variable	High-ES 2-h FcεRI-activated MC ($n = 57$)	Low-ES 2-h FcεRI-activated MC ($n = 47$)	*P* Value	Adj. *P* Value*
LTE4	13.5 ± 21	10.4 ± 17.1	1.37×10^{-2}	NS
PGE2	20.3 ± 19	16.6 ± 17.9	NS	NS
PGF2α	117 ± 61.9	138 ± 88.5	NS	NS
Tetranor-PGDM	340 ± 164	316 ± 216	NS	NS
Tetranor-PGEM	1,140 ± 894	1,190 ± 954	NS	NS

Definition of abbreviations: 8,12-iso-iPF2α-VI = isoprostane; ACQ-5 = Asthma Control Questionnaire score (mean of 5); Adj. = adjusted; BMI = body mass index; ES = expression score; Fe_{NO} = fractional exhaled nitric oxide; hCRP = high-sensitivity C-reactive protein; HETE = hydroxyeicosatetraenoic acid; LTB4 = leukotriene B4; LTE4 = leukotriene E4; MC = mast cell; MCP4 = monocyte chemoattractant protein 4; MMP3 = matrix metalloproteinase-3; NS = not significant; OCS = oral corticosteroid; PGD2 = prostaglandin D2; PGDM = prostaglandin DM; PGE2 = prostaglandin E2; PGEM = prostaglandin E metabolite; PGF1α = prostaglandin F1α; PGF2α = prostaglandin F2α; TNF-α = tumor necrosis factor-α; TXB2 = thromboxane B2.
Data are shown as mean ± SD; otherwise, data with only one number represent number of subjects.
*Adjusted for false discovery rate.

IL-33 (signature 7), and LPS (signature 8) (Figures 3G and 3H) compared with TAC1 and TAC3 subjects. The IFN-γ–stimulated MC signature had the greatest ES in TAC2 and TAC3 compared with TAC1 (Figure 3I).

Characteristics of IgE-activated MC Signature High/Low

We first examined the clinical characteristics between participants with asthma with a high versus low expression of IgE-activated MCs (Table 1). The participants with asthma enriched for the FcεRI-activated MC signature (signature 2) had lower lung function (FEV_1% predicted and FEV_1/FVC, higher sputum and blood eosinophils, and higher amounts of serum periostin and IL-13) (Table 1). Sputum eicosanoid amounts of 15-hydroxyeicosatetraenoic acid (15-HETE), leukotriene E_4 (LTE4), and PGD2 were also elevated in the subjects highly enriched for this 2-hour FcεRI-activated MC signature (Table 1).

These clinical differences were associated with the 35 upregulated DEGS in sputum (Table E3) that were enriched for pathways such as cytokine–cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, selective expression of chemokine receptors during T-cell polarization, purinergic signaling, and inflammation mediated by chemokine and cytokine signaling pathway. Significant gene ontologies included regulation of immune system process, response to stimulus, cellular response to stimulus, and regulation of T-cell activation and signaling (Figure 4A and Table E4).

Other MC High/Low Signature Enrichment Associated with Eosinophilic Asthma

The patients with asthma highly enriched compared with those lowly enriched for the single FcεRI-activation (6-h) MC signature (signature 3) had similar clinical features to those highly enriched for 2-hour FcεRI-activated MCs (signature 2) (Table E7). In particular, these patients had a lower lung function, higher sputum and blood eosinophil counts, and higher amounts of sputum LTE_4. The 12 upregulated sputum DEGs that differentiated high- versus low-enriched patients for the single FcεRI-IgE receptor–stimulated (6-h) MC signature (signature 3) (Figure E5A) were mostly a subset of those within the 2-hour FcεRI-stimulated DEGs (signature 2) (Table E3). These DEGs are associated with type 2 (T2) inflammatory pathways including MC activation without degranulation and eosinophil survival by cytokine signaling together with gene ontologies linked to endopeptidase activity and receptor signaling via JAK/STAT (Janus kinase/signal transducers and activators of transcription) (Figure E5A).

Signature 6 (MCscbb) was also enriched in eosinophilic asthma and had greater amounts of sputum eosinophils, reduced expression of sputum lymphocytes, increased serum periostin, and elevated amounts of sputum 15-HETE and LTE_4 (Table E8).

The nine significantly upregulated sputum DEGs that differentiated high- and low-enriched patients for signature 6 (MCscbb) (Figure E5B) were a subset of those seen with 2-hour FcεRI-activated MCs (signature 2) and represented pathways including metabolism of α-linolenic acid, eicosanoid synthesis, eicosanoid metabolism via lipoxygenases, synthesis of leukotrienes and eoxins, and IL-5 regulation of apoptosis. Gene ontologies were highly enriched for regulation of activated T-cell proliferation, regulation of signal transduction, and negative regulation of adaptive immune response (Figure E5B).

Characteristics of IL-33–activated MC Signature High/Low

The IL-33–stimulated MC signature was associated with neutrophilic asthma rather than eosinophilic asthma. The subjects with a high IL-33 MC signature ES, compared with the subjects with a low IL-33 MC signature ES, had lower lung function, higher sputum neutrophils, lower sputum macrophages, and elevated amounts of serum CRP (C-reactive protein) and MCP4 (monocyte chemoattractant protein-4) (Table 2). These differences were associated with 110 upregulated DEGs in sputum (Table E7) which were enriched for pathways such as NF-κB (nuclear factor-κB) signaling pathway, cytokine–cytokine receptor interaction, TNF (tumor necrosis factor) signaling pathway, glucocorticoid receptor pathway, IL-1 signaling, and cytokine signaling in immune system (Table E8). Significant gene ontologies include neutrophil degranulation and activation, response to LPS, and responses to bacteria (Figure 4B and Table E8).

Other MC High/Low Signature Enrichment Associated with Neutrophilic Asthma

Four signatures in addition to that for IL-33–stimulated MCs were also enriched in neutrophilic asthma (Figure 2). The subjects

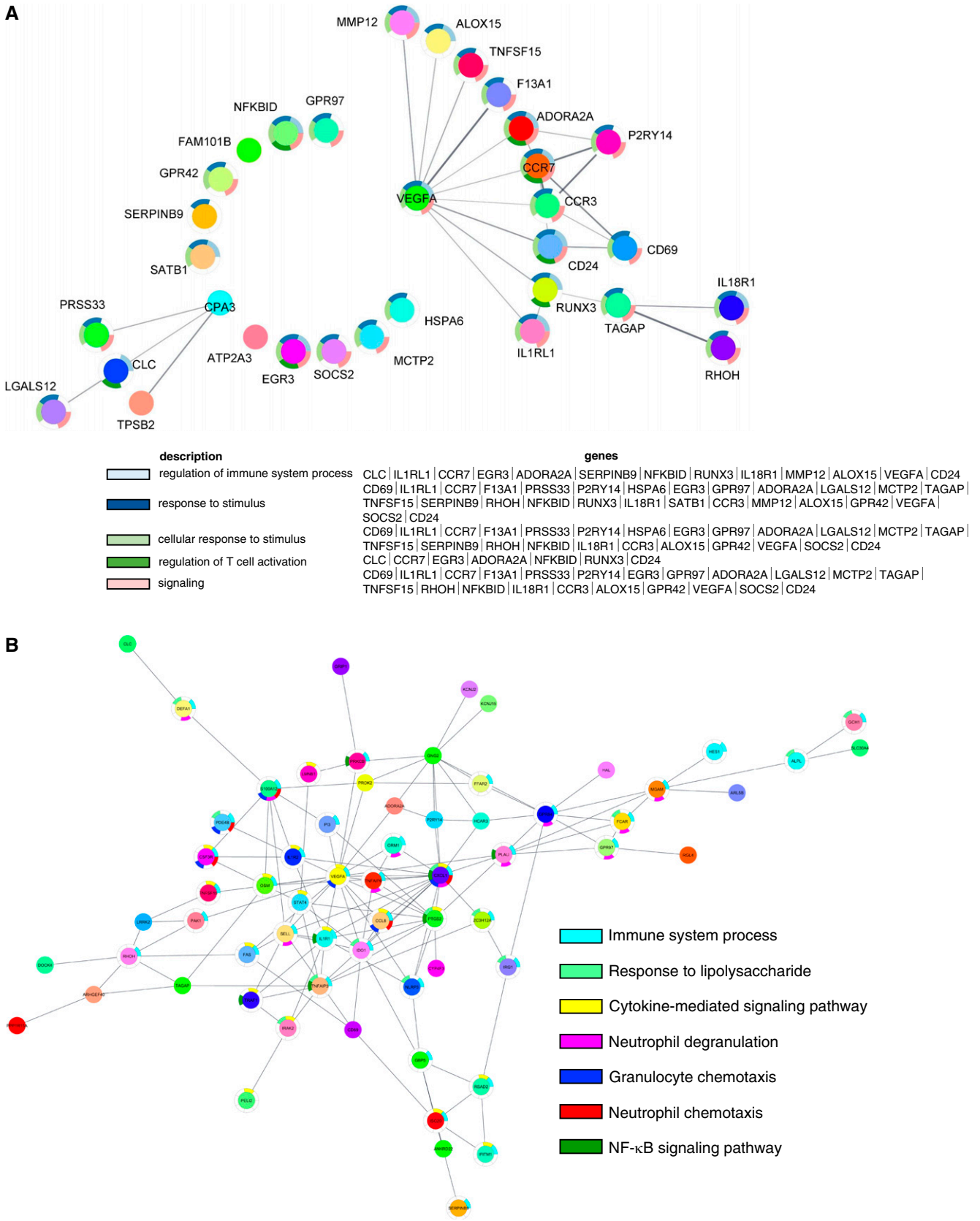

Figure 4. Protein–protein interaction network analysis of differentially expressed genes in sputum. (*A* and *B*) The enriched pathways identified by differentially expressed genes in the sputum from participants with asthma with high enrichment compared with low enrichment for mast cell

with high versus low ES for the repeated FcεRI-stimulated (signature 4) only showed elevated sputum neutrophils (Table E9). There were 76 upregulated sputum DEGs that distinguished these subjects with high versus low enrichment, associated with gene ontologies including IL-8 signaling, TNF-α regulation, and IFN production (Figure E6A). Similarly, the patients with asthma who had high ESs for FcεRI-stimulated (overnight IgE sensitization, 2-h FcεRI activation) MCs (signature 5) had sputum neutrophilia and reduced sputum macrophages (Table E10). There were 63 upregulated sputum DEGs associated with IL-1 activation together with pathways indicating immune and T-cell activation and cytokine release and regulation of the T2 immune response (Figure E6B).

The patients with asthma who were highly enriched compared with those lowly enriched for the LPS-stimulated MC signature (signature 8) had lower lung function and higher sputum neutrophils but lower amounts of sputum lymphocytes and macrophages (Table E11), with higher amounts of serum CRP and sputum 11-dehydro-thromboxane B2 (11-dehydro-TXB2).

There were 122 upregulated sputum DEGs that differentiated patients who were highly versus lowly enriched for LPS-stimulated MCs signature, and these reflected pathways associated with LPS responses, TLR signaling, Th1 cytokine production, and neutrophil function, as well as IL-1β, IL-6, IL-18, and TNF-α expression (Figure E6C).

The IFN-stimulated MC signature (signature 9) was also enriched in subjects with neutrophilic asthma in the most enriched compared with the less enriched subjects, as the LPS-stimulated MCs had elevated amounts of sputum neutrophils, reduced amounts of sputum macrophages, and enhanced amounts of sputum 11-dehydro-TXB2 (Table E12). There were 55 upregulated sputum DEGs that differentiated patients with high versus low enrichment for IFN-stimulated MC signature. These reflected pathways associated with expected IFN responses, including regulation of RNase activity, cellular responses to IFN-α, type 1 IFN signaling, viral response, response to IFN-γ, and response to LPS (Figure E6D).

Association of MC Signatures with Sputum and Blood Granulocytes

To address the question as to whether these MC signatures merely detect eosinophils or neutrophils, we compared the gene overlap between the nine MC signatures and published peripheral blood eosinophil (30) and neutrophil signatures (31) and correlated these MC and granulocyte signatures with sputum eosinophil and neutrophil cell counts. There was no significant gene overlap between any of the MC signatures with neutrophil and eosinophil signatures (all gene overlaps ranged from 0 to <5%) (Table E13).

Although there was no overlap between the MC signatures and transcriptomic signatures from purified neutrophils or eosinophils, there is a correlation between MC signatures and the proportion of eosinophils (signatures 2, 3, and 6) or neutrophils (signatures 4, 5, 7, and 8) in sputum but not in blood (Table E14). We also show that the MC activation status identified using MC gene signatures does not correlate with sputum MC counts (Table E14).

Discussion

MCs are one of the most transcriptionally variable cell types of the immune system, being responsive to environmental factors such as IgE, IL-33, LPS, and IFN-γ within their vicinity (39). We showed differential patterns of distribution and amounts of enrichment of nine different MC signatures in relation to asthma severity, granulocytic inflammation, and molecular phenotype. All the MC signatures except for unstimulated (signature 1), repeated FcεR1-stimulated (signature 4), and IFN-stimulated (signature 9) had higher ESs in groups with SA compared with those with MMA or healthy subjects. Smoking had no significant impact on the ES for SA. Only the 2-hour FcεRI-activated (signature 2) and the MCscbb (signature 6) signatures were enriched in patients with MMA compared with healthy subjects. Greater segregation was observed when analyzed according to sputum granulocyte status and TAC molecular phenotype. Single FcεR1-IgE receptor–stimulated (signatures 2 and 3) and MCscbb signatures were enriched in eosinophilic and mixed granulocytic asthma and the TAC1 molecular phenotype. In contrast, there was higher enrichment of IL-33–stimulated (signature 7), LPS-stimulated (signature 8), repeated FcεR1-stimulated, and FcεRI-activated (signature 5) signatures in neutrophilic and mixed granulocytic asthma, and together with the IFN-γ–stimulated signature, these are enriched in the TAC2 phenotype (8). These findings were validated in the ADEPT cohort of patients with moderate asthma to SA.

A refined 11-gene single-cell MC signature acts as a surrogate for MC numbers in asthmatic bronchial biopsies (40). We were unable to correlate either the unstimulated (signature 1) or the single-cell (MCscbb) signature ES with sputum MC numbers or percentages. This may reflect the fact that sputum MCs represent many different states of activation compared with those in sputum, which have trafficked through the airway and may sense distinct environments compared with that in the bronchial biopsies. The MCscbb signature is derived from patients who have allergies and asthma (27), which may explain why this signature is expressed preferentially in those with severe eosinophilic or TAC1 asthma. Whether single-cell signatures from other asthma subtypes will reflect different patient characteristics will need to be confirmed in future studies.

This concept is further supported by the indication that neutrophils or eosinophils purified from blood may have a transcriptome that is quite different from their counterparts in sputum as previously suggested (41, 42). These blood cell–derived granulocyte signatures have only a weak correlation with blood cell counts, again suggesting that the local environment is distinct from that "seen" by the isolated cells. This suggests that single-cell RNA-sequencing analysis should be used to determine MC and granulocyte signatures from the sputum and blood of healthy subjects to enable a better comparison with sputum and blood signatures in subjects with asthma. Although the current analysis associates some MC signatures with MC

Figure 4. (*Continued*). signatures of 2-hour FcεRI-activated (signature 2) mast cells (*A*) and IL-33–activated mast cells (*B*). Pathways were identified in the STRING database (www.string-db.org) and visualized in Cytoscape 3.8.2 (https://cytoscape.org/). The nodes are labeled with the gene names, and key gene ontologies and pathways are indicated by the colored arcs around the hubs. A list of genes within each ontology is also shown in *A*, identifying genes linked to eosinophils and mast cells representative of allergic asthma. IL-33–associated pathways include those associated with neutrophil and cytokine function.

Table 2. Clinical and Biological Parameters between the Group with High and Low ESs for MC Stimulated by IL-33 Signature (Signature 7)

Variable	High-ES IL-33–activated MC ($n = 57$)	Low-ES IL-33–activated MC ($n = 47$)	*P* Value	Adj. *P* Value*
Age, yr	52.7 ± 12.5	49.6 ± 14.4	NS	NS
Sex, F	38	22	NS	NS
BMI	28.2 ± 4.73	27.3 ± 5.83	NS	NS
Atopy (positive)	26	17	NS	NS
Nonsmoker	36	28	NS	NS
Comorbidities				
Allergic rhinitis	25	17	NS	NS
Eczema	16	17	NS	NS
Nasal polyps	23	11	NS	NS
Severe exacerbations per year	1.75 ± 2	1.29 ± 2.02	NS	NS
ACQ-5	2.14 ± 1.21	1.77 ± 1.37	NS	NS
OCS daily	27	13	NS	NS
Lung function				
FEV_1 (% predicted) pre-salb	60.5 ± 22.2	76.4 ± 20.7	2.93×10^{-4}	4.47×10^{-3}
FEV_1/FVC ratio	55.5 ± 13.3	63.9 ± 10.5	4.94×10^{-4}	6.70×10^{-3}
$F_{E_{NO}}$, ppb	39 ± 39.1	38.7 ± 32.7	NS	NS
Sputum cells, %				
MC	0.0278 ± 0.0814	0.026 ± 0.0784	NS	NS
Eosinophils	10.6 ± 15.7	12.4 ± 21.5	NS	NS
Neutrophils	70.2 ± 21.3	40.4 ± 21.1	6.26×10^{-9}	2.55×10^{-7}
Lymphocytes	1.16 ± 1.24	1.67 ± 1.15	1.85×10^{-3}	1.95×10^{-2}
Macrophages	18.1 ± 14.5	45.9 ± 22.8	1.29×10^{-9}	7.87×10^{-8}
Blood cell counts, 10^3/μl				
Eosinophils	0.362 ± 0.298	0.278 ± 0.248	NS	NS
Neutrophils	5.46 ± 2.41	4.61 ± 2.11	3.22×10^{-2}	NS
Total serum IgE, IU/ml	302 ± 648	232 ± 265	NS	NS
Biomarkers				
Periostin (serum), ng/ml	54.1 ± 18.8	47.9 ± 14.1	NS	NS
Eotaxin (plasma), pg/ml	129 ± 78.3	118 ± 57.3	NS	NS
hCRP (serum), mg/L	7.68 ± 15.6	3.48 ± 6.89	1.92×10^{-3}	1.95×10^{-2}
IFN-γ (plasma), pg/ml	10.4 ± 9.42	8.15 ± 12.5	1.58×10^{-2}	NS
TNF-α (plasma), pg/ml	1.97 ± 0.619	1.92 ± 0.648	NS	NS
IL-13 (serum), pg/ml	1.08 ± 1.6	0.665 ± 0.371	NS	NS
IL-6 (plasma), pg/ml	1.37 ± 1.19	0.891 ± 0.646	1.69×10^{-2}	NS
IL-18 (serum), pg/ml	245 ± 124	220 ± 83.9	NS	NS
Galectin3 (serum), pg/ml	6,290 ± 1,980	5,360 ± 1,420	1.39×10^{-2}	NS
MCP4 (serum), pg/ml	177 ± 101	133 ± 60.8	5.60×10^{-3}	4.88×10^{-2}
MMP3 (serum), ng/ml	19.7 ± 16.6	21.0 ± 17.9	NS	NS
Serpin E1 (serum), ng/ml	100.0 ± 30.0	93.7 ± 26.0	NS	NS
Sputum eicosanoids, pg/ml				
11-dehydro-TXB2	179 ± 219	71.9 ± 57.9	1.22×10^{-4}	2.13×10^{-3}
12-HETE	2,230 ± 2,090	2,440 ± 2,130	NS	NS
15-HETE	7,150 ± 9,270	5,350 ± 8,320	NS	NS
5-HETE	1,620 ± 1,760	2,250 ± 5,290	NS	NS
6-keto-PGF1α	58.9 ± 30.3	53.3 ± 19.0	NS	NS
LTB4	824 ± 912	1,510 ± 3,250	NS	NS
LTE4	553 ± 732	347 ± 630	1.75×10^{-2}	NS
PGD2	273 ± 278	168 ± 184	1.88×10^{-2}	NS
PGE2	415 ± 447	234 ± 299	6.61×10^{-3}	NS
Tetranor-PGDM	66.5 ± 69	58.8 ± 48.6	NS	NS
Tetranor-PGEM	76.6 ± 61.5	82.3 ± 66.3	NS	NS
Urine eicosanoids, ng/mmol				
11-dehydroTXB2	13.3 ± 8.35	11.3 ± 8.75	NS	NS
2,3-dinor-11β-PGF2α	72.2 ± 32.9	74.8 ± 60	NS	NS
2,3-dinor-8-iso-PGF2α	242 ± 126	250 ± 222	NS	NS
2,3-dinor-TXB2	59.8 ± 39.8	50.8 ± 32.9	NS	NS

(*Continued*)

Table 2. (*Continued*)

Variable	High-ES IL-33–activated MC ($n = 57$)	Low-ES IL-33–activated MC ($n = 47$)	*P* Value	Adj. *P* Value*
8,12-iso-iPF2α-VI	395 ± 229	428 ± 300	NS	NS
8-iso-PGF2α	28.8 ± 11.9	29.3 ± 15.4	NS	NS
LTE4	14.8 ± 24.5	8.64 ± 7.6	NS	NS
PGE2	18.9 ± 18.9	18.3 ± 18.0	NS	NS
PGF2α	129 ± 83.5	122 ± 64.3	NS	NS
Tetranor-PGDM	356 ± 172	294 ± 206	1.44×10^{-2}	NS
Tetranor-PGEM	1,170 ± 947	1,140 ± 886	NS	NS

Definition of abbreviations: 8,12-iso-iPF2α-VI = isoprostane; ACQ-5 = asthma control questionnaire score (mean of 5); Adj. = adjusted; BMI = body mass index; ES = expression score; F_{ENO} = fractional exhaled nitric oxide; hCRP = high-sensitivity C-reactive protein; HETE = hydroxyeicosatetraenoic acid; LTB4 = leukotriene B4; LTE4 = leukotriene E4; MC = mast cell; MCP4 = monocyte chemoattractant protein 4; MMP3 = matrix metalloproteinase-3; NS = not significant; OCS = oral corticosteroid; PGD2 = prostaglandin D2; PGDM = prostaglandin DM; PGE2 = prostaglandin E2; PGEM = prostaglandin E metabolite; PGF1α = prostaglandin F1α; PGF2α = prostaglandin F2α; salb = salbutamol; TNF-α = tumor necrosis factor-α; TXB2 = thromboxane B2.
Data are shown as mean ± SD; otherwise, data with only one number represent number of subjects.
*Adjusted for false discovery rate.

counts, use of single-cell RNA-sequencing signatures would also enable confirmation as to whether the signatures we have used represent distinct MC states that reflect the local environment or whether they are derived not from MCs themselves but from coisolated cell types.

The three MC signatures enriched in severe eosinophilic asthma (2-h FcεRI-activated [signature 2], 6-h FcεR1-activated [signature 3], and MCscbb) were associated with similar, but not identical, clinical characteristics associated with eosinophilic asthma, including low lung function, sputum and blood eosinophilia, and the presence of sputum and blood. For example, the 2-hour FcεRI-activated signature (signature 2) was linked with enhanced serum amounts of periostin and IL-13 and with increased sputum 15-HETE, PGD2, and LTE4. Urinary LTE4 and PGD2 metabolites, biomarkers of T2 asthma (17), were also elevated. These patients also required increased oral corticosteroid use in agreement with these signatures being enriched in TAC1 phenotype characterized by eosinophilic inflammation with airflow obstruction and high usage of oral corticosteroid se (8). The similarity in the clinical features associated with these three signatures was reflected in the sputum DEGs and activated pathways detected. The DEGs for the latter two signatures were subsets of those seen in the patients enriched for the 2-hour FcεRI-activated MC signature (signature 2) and contained genes linked to MCs (*CPA3*, *TPSB2*, and *PRSS33* [serine protease 33]) and others linked to eosinophilia (*CLC* [Charcot-Leyden crystal] and *IL1RL1* [IL-1 receptor like 1]), whereas eosinophils are likely to be the source of LTE4 and 15-HETE.

Subjects with asthma enriched for the MC signatures 2 and 5 (26) also exhibited a degree of enrichment for sputum neutrophilic inflammation and a trend toward higher serum amounts of CRP. The presence of both eosinophilic and neutrophilic inflammation in these subjects with asthma reflects cross-talk between MCs and eosinophils with concomitant release of neutrophil chemoattractants such as IL-6 and CXCL8 (C-X-C motif chemokine ligand 8), under the control of MAPK (mitogen-activated protein kinase) and NF-κB signaling pathways (43, 44). The specific stimulus and its duration appear to affect this cross-talk, and future studies should investigate this further.

Apart from FcεRI receptors through which IgE binds, MCs express other receptors that allow them to respond to other stimuli, such as IL-33, LPS, and IFN-γ, enabling the induction of an MC-driven innate immune response to external infections, including bacteria, viruses, and fungi (9, 45). The alarmin IL-33 is the ligand of the ST2 receptor complex expressed on MCs (46). It is released by epithelial cells exposed to fungi (47) and necrotic cells (48) and induces the production of proinflammatory mediators such as IL-1β, TNF, and PGD_2 (49), together with MC degranulation (50).

The highest enrichment of the IL-33–MC signature was associated with higher blood and sputum neutrophil counts, sputum 11-dehydro-TXB2 and PGE2, and serum CRP amounts. Elevated sputum 11-dehydro-TXB2 amounts are associated with neutrophilic patients with asthma (51), and sputum PGE2 amounts have been correlated with sputum neutrophilia (52). This is compatible with the network analysis of the DEGs between the high and low groups showing IL-1β, TNF, and NF-κB signaling and, importantly, with neutrophil degranulation and activation. Thus, we did not observe the exacerbation of allergen-driven Th2 inflammation that occurs in asthma with IL-33–mediated MC activation, as previously reported (53). IL-33 activation of a noneosinophilic response corresponds with the recent clinical trial results with the anti-ST2 antibody Astegolimab, which showed a reduction in exacerbation rates in T2-low patients with low blood eosinophil counts (54).

Analysis of LPS- and IFN-γ–stimulated MC signatures enabled us to investigate MC activation in response to potential bacterial and viral infections (9, 55). We found high enrichment of the LPS-activated MC signature in subjects with SA compared with that in healthy volunteers. High expressors were characterized by a greater neutrophilic inflammatory response with high serum CRP and sputum 11-dehydro-TXB2 and greater airflow obstruction and reduced lung function. DEGs in these highly enriched patients indicate enhanced expression of LPS response, TLR signaling, and neutrophil activation pathways. This indicates changes that would occur with activation of the TLR4 receptor in the presence of a gram-negative bacterial infection potentially in response to bacterial dysbiosis (56). There was no difference, however, in the expression of the MC-activated IFN-γ signature across asthma severity with enrichment in TAC2 and TAC3

compared with TAC1 patients. The group expressing a high IFN-γ signature had evidence of high sputum 11-dehydro-TBX2 amounts and IFN and antiviral responses validating the enrichment, although no measures of viral infection were recorded in these patients. The observations related to LPS agree with previous findings that LPS enhances the production of type 1–associated cytokines such as IL-6 and TNF with IgE sensitization of MCs (57, 58). LPS and IFN-γ may inhibit the baseline state of T2 activation induced by MCs in subjects with SA by mechanisms involving the inhibitory effect of type 1 cytokines on T2 inflammation.

Although GSVA is a powerful approach to examine cell activation states according to overall gene expression enrichment and provides useful insights into the role of MCs in SA, these findings need to be confirmed in additional cohorts, and ideally, protein signatures need to be examined. Our data also emphasize the importance of using optimized GSVA signatures with minimal overlap with those representing other cell types or activation states. This will necessitate single-cell analysis of MCs, together with wet laboratory experiments of MCs isolated from the airways/lungs of patients with SA compared with patients with MMA and healthy volunteers. In addition, although we have used a variety of MC stimulators, there are other activators of MCs not covered in this analysis that are likely playing a role in SA. The activation of MCs during exacerbations after viral or bacterial infections and the effect on cross-talk between TLRs, FcεR1, IFN-γ receptor, and ST2 receptors should be examined. This approach should also be undertaken in other obstructive lung diseases such as chronic obstructive pulmonary disease to elucidate the role of differentially activated MCs.

In summary, we show that the detection of MCs by transcriptomic analysis has a greater power to detect relevant subsets of patients with SA than counting sputum MC cell numbers *per se*. We assume that the transcriptomic signatures measured in the sputum reflect MC activity and therefore indicate that MCs can be induced to take on distinct transcriptional phenotypes that reflect the local microenvironment and are associated with distinct clinical phenotypes of SA. Thus, patients with high ESs for FcεRI-activated MC signatures have distinct characteristics associated with severe eosinophilic asthma, whereas IL-33–activated MCs are associated with a severe neutrophilic phenotype. Future studies using single-cell RNA sequencing should confirm whether these transcriptional phenotypes involve different sputum MC subsets or different MC states induced by discrete extracellular signals. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the whole U-BIOPRED team for their contribution.

U-BIOPRED Consortium Project Team members: Uruj Hoda and Christos Rossios, Airways Disease, National Heart and Lung Institute, Imperial College London, United Kingdom, and Biomedical Research Unit, Royal Brompton and Harefield NHS Trust, London, United Kingdom; Elisabeth Bel, Faculty of Medicine, University of Amsterdam, Amsterdam, the Netherlands; Navin Rao, Janssen Research and Development, High Wycombe, Buckinghamshire, United Kingdom; David Myles, Respiratory Therapy Area Unit, GlaxoSmithKline, Stockley Park, United Kingdom; Chris Compton, Discovery Medicine, GlaxoSmithKline, Stockley Park, United Kingdom; Marleen Van Geest, AstraZeneca R&D, Molndal, Sweden; Peter Howarth and Graham Roberts, Faculty of Medicine, Southampton University, Southampton, United Kingdom, and NIHR Southampton Respiratory Biomedical Research Unit, University Hospital Southampton, Southampton, United Kingdom; Diane Lefaudeux, European Institute for Systems Biology and Medicine, CNRS-ENS-UCBL, Université de Lyon, Lyon, France; Bertrand De Meulder, European Institute for Systems Biology and Medicine, CNRS-ENS-UCBL, Université de Lyon, Lyon, France; Aruna T. Bansal, Acclarogen Ltd., St John's Innovation Centre, Cambridge, United Kingdom; Richard Knowles, Knowles Consulting, Stevenage Bioscience Catalyst, Stevenage, United Kingdom; Damijn Erzen, Boehringer Ingelheim Pharma, Ingelheim am Rhein, Germany; Scott Wagers, BioSci Consulting, Maasmechelen, Belgium; Norbert Krug, Immunology, Allergology and Clinical Inhalation, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; Tim Higenbottam, Corporate Clinical Development, Chiesi Pharmaceutics Ltd., Cheadle, United Kingdom (Current address: Allergy Therapeutics, West Sussex, United Kingdom); John Matthews, Genentech Inc., South San Francisco, CA; Veit Erpenbeek, Translational Medicine — Respiratory Profiling, Novartis Institutes for BioMedical Research, Basel, Switzerland; Leon Carayannopoulos, Merck Inc., Kenilworth, New Jersey; Amanda Roberts, U-BIOPRED Patient Input Platform, ELF, Sheffield, United Kingdom; David Supple, U-BIOPRED Patient Input Platform, ELF, Sheffield, United Kingdom; Pim deBoer, U-BIOPRED Patient Input Platform, ELF, Sheffield, United Kingdom; Massimo Caruso, Department of Clinical and Experimental Medicine Hospital University, University of Catania, Catania, Italy; Pascal Chanez, Département des Maladies Respiratoires, Laboratoire d'immunologie, Aix Marseille Université, Marseille, France; Sven-Erik Dahlén, The Centre for Allergy Research, The Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; Ildikó Horváth, Department of Pulmonology, Semmelweis University, Budapest, Hungary; Jacek Musial, Department of Medicine, Jagiellonian University Medical College, Krakow, Poland; and Thomas Sandström, Department of Medicine, Respiratory and Allergy Unit, University Hospital, Umeå, Sweden.

References

1. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343–373.
2. Chung KF, Adcock IM. Precision medicine for the discovery of treatable mechanisms in severe asthma. *Allergy* 2019;74:1649–1659.
3. Arthur G, Bradding P. New developments in mast cell biology: clinical implications. *Chest* 2016;150:680–693.
4. Olivera A, Beaven MA, Metcalfe DD. Mast cells signal their importance in health and disease. *J Allergy Clin Immunol* 2018;142:381–393.
5. Bradding P, Arthur G. Mast cells in asthma—state of the art. *Clin Exp Allergy* 2016;46:194–263.
6. Wilson SJ, Ward JA, Sousa AR, Corfield J, Bansal AT, De Meulder B, *et al.*; U-BIOPRED Study Group. Severe asthma exists despite suppressed tissue inflammation: findings of the U-BIOPRED study. *Eur Respir J* 2016;48:1307–1319.
7. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, *et al.* Mast cell phenotype, location, and activation in severe asthma. Data from the severe asthma research program. *Am J Respir Crit Care Med* 2011;183:299–309.
8. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, *et al.*; U-BIOPRED Study Group. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J* 2017;49:443–455.
9. Marshall JS, Portales-Cervantes L, Leong E. Mast cell responses to viruses and pathogen products. *Int J Mol Sci* 2019;20:4241.
10. Kim HS, Kawakami Y, Kasakura K, Kawakami T. Recent advances in mast cell activation and regulation. *F1000 Res* 2020;9: F1000.

11. Hinks TS, Zhou X, Staples KJ, Dimitrov BD, Manta A, Petrossian T, *et al.* Innate and adaptive T cells in asthmatic patients: relationship to severity and disease mechanisms. *J Allergy Clin Immunol* 2015;136: 323–333.
12. Maun HR, Jackman JK, Choy DF, Loyet KM, Staton TL, Jia G, *et al.* An allosteric anti-tryptase antibody for the treatment of mast cell-mediated severe asthma. *Cell* 2019;179:417–431.e19.
13. Winter NA, Qin L, Gibson PG, McDonald VM, Baines KJ, Faulkner J, *et al.* Sputum mast cell/basophil gene expression relates to inflammatory and clinical features of severe asthma. *J Allergy Clin Immunol* 2021;148:428–438.
14. Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, *et al.*; U-BIOPRED Study Group. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J* 2015;46:1308–1321.
15. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC; Official Statement of the European Respiratory Society. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. *Eur Respir J Suppl* 1993;16:5–40.
16. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation [editorial]. *Thorax* 1997;52: 498–501.
17. Kolmert J, Gómez C, Balgoma D, Sjödin M, Bood J, Konradsen JR, *et al.*; U-BIOPRED Study Group, on behalf of the U-BIOPRED Study Group. Urinary leukotriene E_4 and prostaglandin D_2 metabolites increase in adult and childhood severe asthma characterized by type 2 inflammation. A clinical observational study. *Am J Respir Crit Care Med* 2021;203:37–53.
18. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, *et al.* Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249–264.
19. Rossios C, Pavlidis S, Hoda U, Kuo CH, Wiegman C, Russell K, *et al.*; Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes (U-BIOPRED) Consortia Project Team. Sputum transcriptomics reveal upregulation of IL-1 receptor family members in patients with severe asthma. *J Allergy Clin Immunol* 2018;141: 560–570.
20. Athey BD, Braxenthaler M, Haas M, Guo Y. tranSMART: an open source and community-driven informatics and data sharing platform for clinical and translational research. *AMIA Jt Summits Transl Sci Proc* 2013; 2013:6–8.
21. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics* 2013;14:7.
22. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, *et al.* Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015;12:453–457.
23. Chtanova T, Newton R, Liu SM, Weininger L, Young TR, Silva DG, *et al.* Identification of T cell-restricted genes, and signatures for different T cell responses, using a comprehensive collection of microarray datasets. *J Immunol* 2005;175:7837–7847.
24. Baram D, Dekel O, Mekori YA, Sagi-Eisenberg R. Activation of mast cells by trimeric G protein Gi3; coupling to the A3 adenosine receptor directly and upon T cell contact. *J Immunol* 2010;184:3677–3688.
25. Suurmond J, Habets KLL, Tatum Z, Schonkeren JJ, Hoen PAC, Huizinga TWJ, *et al.* Repeated FcεRI triggering reveals modified mast cell function related to chronic allergic responses in tissue. *J Allergy Clin Immunol* 2016;138:869–880.
26. Jayapal M, Tay HK, Reghunathan R, Zhi L, Chow KK, Rauff M, *et al.* Genome-wide gene expression profiling of human mast cells stimulated by IgE or FcepsilonRI-aggregation reveals a complex network of genes involved in inflammatory responses. *BMC Genomics* 2006;7:210.
27. Vieira Braga FA, Kar G, Berg M, Carpaij OA, Polanski K, Simon LM, *et al.* A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat Med* 2019;25:1153–1163.
28. Nagarkar DR, Ramirez-Carrozzi V, Choy DF, Lee K, Soriano R, Jia G, *et al.* IL-13 mediates IL-33-dependent mast cell and type 2 innate lymphoid cell effects on bronchial epithelial cells. *J Allergy Clin Immunol* 2015;136:202–205.
29. Okumura S, Kashiwakura J, Tomita H, Matsumoto K, Nakajima T, Saito H, *et al.* Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcepsilonRI. *Blood* 2003;102:2547–2554.
30. Sridhar S, Liu H, Pham TH, Damera G, Newbold P. Modulation of blood inflammatory markers by benralizumab in patients with eosinophilic airway diseases. *Respir Res* 2019;20:14.
31. Abbas AR, Baldwin D, Ma Y, Ouyang W, Gurney A, Martin F, *et al.* Immune response in silico (IRIS): immune-specific genes identified from a compendium of microarray expression data. *Genes Immun* 2005;6:319–331.
32. Loza MJ, Djukanovic R, Chung KF, Horowitz D, Ma K, Branigan P, *et al.*; ADEPT (Airways Disease Endotyping for Personalized Therapeutics) and U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium) investigators. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respir Res* 2016;17:165.
33. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.
34. Storey JD, Bass AJ, Dabney A, Robinson D. qvalue: Q-value estimation for false discovery rate control. R package version 2.24.0; 2021.
35. Wickham JD. ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag; 2009.
36. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;43:e47.
37. Dwyer DF, Ordovas-Montanes J, Allon SJ, Buchheit KM, Vukovic M, Derakhshan T, *et al.* Human airway mast cells proliferate and acquire distinct inflammation-driven phenotypes during type 2 inflammation. *Sci Immunol* 2021;6:eabb7221.
38. Hamey FK, Lau WWY, Kucinski I, Wang X, Diamanti E, Wilson NK, *et al.* Single-cell molecular profiling provides a high-resolution map of basophil and mast cell development. *Allergy* 2021; 76:1731–1742.
39. Dwyer DF, Barrett NA, Austen KF; Immunological Genome Project Consortium. Expression profiling of constitutive mast cells reveals a unique identity within the immune system. *Nat Immunol* 2016; 17:878–887.
40. Jiang J, Faiz A, Berg M, Carpaij OA, Vermeulen CJ, Brouwer S, *et al.* Gene signatures from scRNA-seq accurately quantify mast cells in biopsies in asthma. *Clin Exp Allergy* 2020; 50:1428–1431.
41. Besteman SB, Callaghan A, Langedijk AC, Hennus MP, Meyaard L, Mokry M, *et al.* Transcriptome of airway neutrophils reveals an interferon response in life-threatening respiratory syncytial virus infection. *Clin Immunol* 2020;220:108593.
42. Conese M, Castellani S, Lepore S, Palumbo O, Manca A, Santostasi T, *et al.* Evaluation of genome-wide expression profiles of blood and sputum neutrophils in cystic fibrosis patients before and after antibiotic therapy. *PloS One* 2014;9:e104080.
43. Maurer M, Köberle M, Metz M, Biedermann T. Mast cells: Promoters of health and modulators of disease. *J Allergy Clin Immunol* 2019;144: S1–S3.
44. Stassen M, Hartmann AK, Delgado SJ, Dehmel S, Braun A. Mast cells within cellular networks. *J Allergy Clin Immunol* 2019;144:S46–S54.
45. Katsoulis-Dimitriou K, Kotrba J, Voss M, Dudeck J, Dudeck A. Mast cell functions linking innate sensing to adaptive immunity. *Cells* 2020;9: 2538.
46. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479–490.
47. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. *J Immunol* 2011; 186:4375–4387.
48. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* 2016;17:122–131.
49. Moulin D, Donzé O, Talabot-Ayer D, Mézin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* 2007;40:216–225.

50. Hoppe A, Katsoulis-Dimitriou K, Edler HJ, Dudeck J, Drube S, Dudeck A. Mast cells initiate the vascular response to contact allergens by sensing cell stress. *J Allergy Clin Immunol* 2020;145:1476–1479.e3.
51. Badi YE, Pavel AB, Pavlidis S, Riley JH, Bates S, Kermani NZ, *et al.*; U-BIOPRED Study Group. Mapping atopic dermatitis and anti-IL-22 response signatures to type 2-low severe neutrophilic asthma. *J Allergy Clin Immunol* [online ahead of print] 20 Apr 2021; DOI: 10.1016/j.jaci.2021.04.010.
52. Kontogianni K, Bakakos P, Kostikas K, Hillas G, Papaporfyriou A, Papiris S, *et al.* Levels of prostaglandin E(2) and Cysteinyl-leukotrienes in sputum supernatant of patients with asthma: the effect of smoking. *Clin Exp Allergy* 2013;43:616–624.
53. Sjöberg LC, Nilsson AZ, Lei Y, Gregory JA, Adner M, Nilsson GP. Interleukin 33 exacerbates antigen driven airway hyperresponsiveness, inflammation and remodeling in a mouse model of asthma. *Sci Rep* 2017;7:4219.
54. Kelsen SG, Agache IO, Soong W, Israel E, Chupp GL, Cheung DS, *et al.* Astegolimab (anti-ST2) efficacy and safety in adults with severe asthma: a randomized clinical trial. *J Allergy Clin Immunol* 2021;148:790–798.
55. Avila M, Gonzalez-Espinosa C. Signaling through Toll-like receptor 4 and mast cell-dependent innate immunity responses. *IUBMB Life* 2011;63:873–880.
56. Abdel-Aziz MI, Brinkman P, Vijverberg SJH, Neerincx AH, Riley JH, Bates S, *et al.*; U-BIOPRED Study Group. Sputum microbiome profiles identify severe asthma phenotypes of relative stability at 12 to 18 months. *J Allergy Clin Immunol* 2021;147:123–134.
57. Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. FcepsilonR1 and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood* 2006;107:610–618.
58. Takenaka H, Ushio H, Niyonsaba F, Jayawardana ST, Hajime S, Ikeda S, *et al.* Synergistic augmentation of inflammatory cytokine productions from murine mast cells by monomeric IgE and toll-like receptor ligands. *Biochem Biophys Res Commun* 2010;391:471–476.