Molecular pathways underlying lung-brain axis signaling in asthma: Relevance for psychopathology and neuroinflammation

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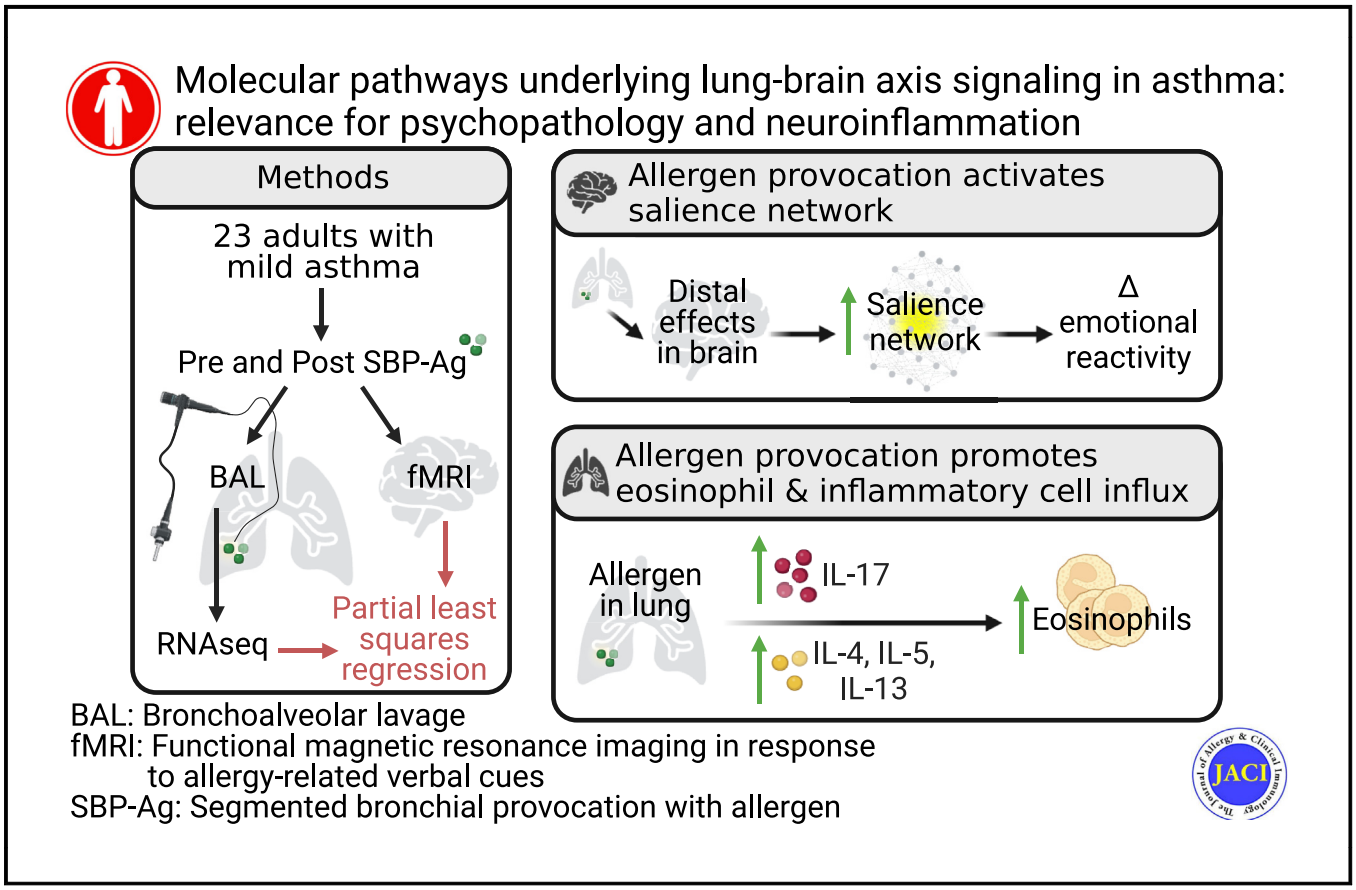
GRAPHICAL ABSTRACT



Capsule summary: Systemic effects of airway inflammation on function in the brain's salience network are related to signaling in T_H17-related pathways, which may give rise to symptoms of depression and long-term impacts on brain health.

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Molecular pathways underlying lung-brain axis signaling in asthma: Relevance for psychopathology and neuroinflammation

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Background: Accumulating evidence indicates that asthma has systemic effects and affects brain function. Although airway inflammation is proposed to initiate afferent communications with the brain, the signaling pathways have not been established.

Objective: We sought to identify the cellular and molecular pathways involved in afferent lung-brain communication during airway inflammation in asthma.

Methods: In 23 adults with mild asthma, segmental bronchial provocation with allergen (SBP-Ag) was used to provoke airway inflammation and retrieve bronchoalveolar lavage fluid for targeted protein analysis and RNA sequencing to determine gene expression profiles. Neural responses to emotional cues in nodes of the salience network were assessed with functional magnetic resonance imaging at baseline and 48 hours after SBP-Ag.

Results: Cell deconvolution and gene coexpression network analysis identified 11 cell-associated gene modules that changed in response to SBP-Ag. SBP-Ag increased bronchoalveolar lavage eosinophils and expression of an eosinophil-associated module enriched for genes related to T_H17-type inflammation (eg, *IL17A*), as well as cell proliferation in lung and brain (eg, *NOTCH1*, *VEGFA*, and *LIF*). Increased expression of genes in this module, as well as several T_H17-type inflammation–related proteins, was associated with an increase from baseline in salience network reactivity.

Conclusions: Our results identify a specific inflammatory pathway linking asthma-related airway inflammation and emotion-related neural function. Systemically, T_H17-type inflammation has been implicated in both depression and neuroinflammation, with impacts on long-term brain health. Thus, our data emphasize that inflammation in the lung in asthma may have profound effects outside of the lung that may be targetable with novel therapeutic approaches. (J Allergy Clin Immunol 2024;153:111-21.)

Key words: *Asthma, eosinophils, salience network, T_H17, IL-17, depression, gene expression network analysis, fMRI*

Abbreviations used

ACQ: Asthma Control Questionnaire
BAL: Bronchoalveolar lavage
BDI: Beck Depression Inventory
BOLD: Blood oxygen level dependent
CPM: Count per million
FDR: False discovery rate
FENO: Fraction of exhaled nitric oxide
fMRI: Functional magnetic resonance imaging
ROI: Regions of interest
SBP-Ag: Segmental bronchial provocation with allergen
SN: Salience network
sPLS: Sparse partial least squares
WLAC: Whole-lung allergen challenge

Airway inflammation is a cardinal feature of asthma and serves as the primary therapeutic target. However, accumulating evidence suggests that inflammation in asthma extends beyond the airway to result in systemic inflammation,[1] which may give rise to cognitive and affective impairments.[2,3] A long history of both clinical and experimental research has connected asthma with psychological comorbidities, and our previous work sought to uncover the underlying neural mechanisms. In several previous studies, we have demonstrated that allergen provocation of eosinophilic airway inflammation in asthma has an impact on function in the components of the salience network (SN)—the insula, anterior cingulate cortex, and amygdala, in particular. Activity in this brain network shows differential sensitivity to emotional cues during the development of eosinophilic airway inflammation[4,5] and is important in modulating cognitive, affective, and autonomic functions to shift attention toward important information in the body or in the environment,[6,7] in support of homeostatic maintenance.[6] This network has been implicated in the pathophysiology of both depression and dementia,[8,9] which have increased prevalence in asthma,[2,10-13] elevating the clinical importance of efforts to understand how the SN is functionally affected by airway inflammation and to define the signaling pathways involved.

Although our previous work was successful in identifying the key neural components in lung-brain communication in asthma,[4,5,14,15] the molecular pathways engaged during allergic airway inflammation that drive neural modulation remain largely

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unknown. On the basis of our previous work, pathways associated with type 2 inflammation were central to our hypotheses. In addition, we hypothesized that T_H17-associated pathways would be important because IL-17 promotes depression-like behavior in mice[16] and is associated with depression in humans.[17]

To interrogate the relationship between specific molecular changes in the airway provoked by allergen and subsequent changes in SN function, we used bronchoscopy to directly challenge a single segment of the lower airways of patients with asthma. This approach generates an intense airway inflammatory response that is restricted to the challenged segment, resulting in minimal impact on lung function. We used a novel combination of functional magnetic resonance imaging (fMRI) to measure concurrent changes in SN function, coupled with network analysis of bronchoalveolar lavage (BAL) fluid gene and protein expression changes resulting from allergen provocation. By identifying the specific molecular pathways that link communication between the lung and the brain, it is our goal to ultimately identify novel therapeutic targets and potentially prevent the psychological and neurologic consequences associated with the chronic airway inflammation of asthma.

METHODS

Twenty-three participants with a physician diagnosis of asthma were recruited from Madison, Wis. Participants ranged in age from 19 to 41 years (mean, 26.74 years). All participants had mild asthma and required only bronchodilator medication for asthma management. Participants had allergic sensitization, a positive skin test result for common aeroallergens to be used in provocation procedures, prealbuterol FEV_1 greater than or equal to 70%, and FEV_1 albuterol reversibility greater than or equal to 12% or methacholine provocative concentration causing a 20% fall in FEV_1 less than or equal to 8 mg/mL. Patient characteristics are presented in Table I. Participants were free of respiratory illnesses for 1 month. Individuals with a history of claustrophobia, ferromagnetic implants, traumatic brain injury, neurologic or seizure disorder, or psychosis were excluded. Those requiring psychotropic medication for depression or anxiety were on a stable dose for 6 weeks before enrollment. The University of Wisconsin-Madison Health Sciences Institutional Review Board (Madison, Wis) approved the study.

Our experimental protocol involved 3 visits to the laboratory. During the first visit, participants provided written informed consent, were screened for inclusion and exclusion criteria, and underwent skin testing and a screening whole-lung allergen challenge (WLAC). During the second visit, fMRI data were acquired at baseline, followed by segmental bronchial provocation with allergen (SBP-Ag). During the third visit, 48 hours after the second visit, the identical fMRI protocol was administered, followed by bronchoscopy without allergen. Measures of lung function and airway inflammation were obtained before and after each bronchoscopy. Data were collected across all seasons between August 2016 and July 2019.

Participants underwent WLAC, as previously described,[18] to determine the allergen provocation dose resulting in a 20% reduction in FEV_1 ($AgPD_{20}$). SBP-Ag was performed using $AgPD_{20}$. A bronchoscopy, typically of the right middle lobe, was performed to retrieve BAL fluid at baseline, followed by SBP-Ag. Bronchoscopy was repeated 48 hours after SBP-Ag to retrieve BAL samples from the same lobe, at a time associated with a maximal inflammatory response.

Lung function was quantified using spirometry, according to the American Thoracic Society standards[19] and indexed as FEV_1% predicted. Asthma control was assessed using the 6-item Asthma Control Questionnaire (ACQ-6).[20] Depressive symptoms were measured using the Beck Depression Inventory (BDI).[21] Fraction of exhaled nitric oxide (FENO; NIOX System; Aerocrine, Solna, Sweden) was measured in exhaled breath before and after bronchoscopy, according to the American Thoracic Society guidelines[22] and BAL cell differential was determined in pre– and post–SBP-Ag samples. Protein quantification of 65 cytokines, chemokines, and growth factors in BAL fluids was performed using 2 Multiplex Immunoassay kits (HCP2MAG-62 K and HYCTMAG-60K; Millipore, Temecula, Calif) according to the manufacturer's instructions. A Luminex 100 instrument (Luminex Corp, Austin, Tex) was used to generate quantitative data, and abundance was log_{10}-transformed (see Table E1 in this article's Online Repository at www.jacionline.org).

For RNA-sequencing analyses, BAL cells were stored in buffer RLT (Qiagen, Valencia, Calif) at −80°C before RNA extraction. Total RNA was extracted from BAL cell pellets (N = 22) using RNeasy Mini Kit (Qiagen). RNA quantity and quality were assessed using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Mass) and RNA electrophoresis (Agilent, Santa Clara, Calif). Sequencing libraries were constructed using SMART-Seq v4 Ultra Low Input RNA Kit (Takara, Kusatsu, Shiga, Japan) and clustered onto a flow cell using a cBOT amplification system with a HiSeq SR v4 Cluster Kit (Illumina, San Diego, Calif). Single-read sequencing was performed on a HiSeq2500 sequencer (Illumina), using a HiSeq SBS v4 Kit to generate 58-base reads. Reads were processed using workflows managed on the Galaxy platform including trimming to a minimum quality score of 30, aligning to the GRCh38 reference genome using STAR (v2.4.2a),[23] and quantifying using HTSeq-count (v0.4.1)[24] with ensembl release 91.[25]

Anatomical and functional MRI images were acquired using a GE MR750 3.0-Tesla high-speed imaging device with a 32-channel head coil (General Electric Medical Systems, Milwaukee, Wis). The key elements of the fMRI methods include sagittal echo planar image slices (44 × 3 mm) covering the whole brain (interslice gap, 0.5 mm; in-plane resolution, 3.5 × 3.5 mm; field of view, 224 mm; repetition time, 2000 ms; echo time, 20 ms; and flip angle, 75°). A high-resolution T1-weighted anatomical scan (3-dimensional T1-weighted inversion recovery fast gradient echo image; inversion time, 450 ms; in-plane resolution, 256 × 256 mm; field of view, 256 mm; and axial slices, 192 × 1.0 mm) was acquired for spatial coregistration of functional data. One participant was removed from analysis because of excessive movement.

During fMRI acquisition, participants performed a modified version of the Stroop task[26] to probe the neural response to emotional stimuli that vary in salience. Participants identified the color of asthma-relevant (As; eg, wheeze), negative (Ng; eg, loneliness), and valence-neutral (Ne; eg, curtains) words using an MRI-compatible response box. The As words were words associated with the experience of asthma, generated by individuals with asthma. The Ng and Ne words were selected from the Affective Norms for English Words data set.[27] Thirty-two words

TABLE I. Patient characteristics

Characteristics	Pre–SBP-Ag	Post–SBP-Ag	*P* value*
Age (y)	26 ± 6.7		
Sex: female (%)	52		
Race (%)			
White	83		
Black	8.7		
Mixed	8.7		
FEV_1 screening WLAC	2.5 ± 0.47		
FEV_1% predicted (%)	91 ± 11	91 ± 9.7	.99
FENO	46 ± 29	65 ± 40	4.1×10^{-5}
BAL cell percentage†			
Eosinophil	0.65 ± 0.69	31 ± 29	1.6×10^{-4}
Monocyte	83 ± 9.1	48 ± 29	1.4×10^{-5}
Neutrophil	2.1 ± 2.4	8.0 ± 12	.053
Lymphocyte	12 ± 6.9	13 ± 5.3	.47
Epithelial	2.6 ± 2.9	0.17 ± 0.39	3.1×10^{-3}

Data are presented as mean ± SD, unless specified otherwise.
*Paired *t* test.
†Benjamini-Hochberg–corrected *P* value across 5 cell types.

of each category were presented in 1 of 4 colors. A 10-second baseline period flanked each run. In each of 2 runs, 48 stimuli (16 per category) were presented for 2 seconds each, with a pseudorandomized inter-stimulus interval of 4 to 8 seconds.

Data analysis

To examine the impact of SBP-Ag on lung function and airway inflammation, we used paired *t* tests with Benjamini-Hochberg correction of *P* values to evaluate the change from baseline in FEV_1% predicted, FENO, and cell differentials. Pre– to post–SBP-Ag changes in ACQ and BDI scores were also tested using paired *t* tests.

Cleaning and analysis of RNA-sequencing data were performed in R (v4.0.2)[28] using tidyverse (v1.3.0).[29,30] RNA-sequencing libraries were filtered for quality at median coefficient of variance coverage of less than 0.65, mapped duplicate reads greater than 0.8, and total sequences greater than 1 million (see Table E2 in this article's Online Repository at www.jacionline.org). No batch effects were detected in principal-component analysis. Gene counts were normalized for RNA composition using trimmed mean of *M* value normalization, filtered to protein-coding genes with at least 1 count per million (CPM) in at least 10% of libraries, and converted to $\log_2$ CPM with quality weights using voom[31] (Table E1). This resulted in 14,346 genes for analysis in 22 paired pre- and postchallenge samples.

For gene module analysis, all genes were linear-modeled for SBP-Ag and filtered to 6863 genes with a false discovery rate (FDR) of less than 0.3 (see Table E3 in this article's Online Repository at www.jacionline.org). This subset was then modeled separately against eosinophil or neutrophil percentages. Genes were assigned to cell types at an FDR of less than 0.3 and slope greater than 0, resulting in 2787 eosinophil- and 428 neutrophil-associated genes (Table E3). Cell-associated genes were then grouped into modules using Weighted Gene Coexpression Network Analysis[32] with minimum *R*-squared of 0.8, resulting in 9 eosinophil and 2 neutrophil modules (Table E1). Gene modules were associated with functions using STRING protein-protein interaction networks[33] and hypergeometric enrichment of Broad MSigDB gene sets.[34]

All fMRI data preprocessing and analyses were completed using FSL[35] v5.0, with the following steps: (1) removal of the first 5 volumes, (2) motion correction using MCFLIRT,[36] (3) brain extraction using BET,[37] and (4) registration of the individual functional and anatomical data using the boundary-based registration approach.[38] A 12–degree of freedom affine transformation, using FLIRT,[36] followed by FNIRT nonlinear transformation, was used to register functional data to standard space (Montreal Neurological Institute 152). Individual participant data for each run were analyzed using a general linear model with separate regressors for each condition (ie, As, Ng, and Ne), formed by convolving a stimulus boxcar function with an ideal hemodynamic response function. The model included motion parameters and their derivatives to adjust for motion-related artifact. The general linear model yielded a set of contrast maps (As-Ne, Ng-Ne, and As-Ng) for each individual. These contrast maps were spatially blurred using a 5-mm full-width–at–half-maximum Gaussian spatial filter.

A priori regions of interest (ROI) in the SN were selected on the basis of our previous published work.[4,5,15,39] The insular cortex was a central focus of these analyses, given its prominence as a hub in the SN and its consistent presence in pathways activated by peripheral inflammation.[4,5,40-45] Functional subregions of the insula were defined by Deen et al.[46] We identified an additional insula ROI on the basis of increased regional responsiveness to the Stroop task during airway inflammation.[5] The anterior cingulate cortex was selected on the basis of its involvement in brain-body communication during inflammation, both in our own work and in that of others,[4,40,44,45,47] and subdivisions were defined on the basis of the Harvard-Oxford Atlas. In addition, a perigenual anterior cingulate cortex ROI was defined (dilated 2×) by results described by Rosenkranz et al.[4] Finally, the amygdala, defined anatomically by the Harvard-Oxford Atlas, was selected on the basis of its sensitivity to peripheral inflammation and important role in emotion processing.[15,44,48]

Changes in response to SBP-Ag were calculated as post– minus pre–SBP-Ag values within-participant for gene modules, proteins, and fMRI blood oxygen level dependent (BOLD) percent signal change. Sparse partial least squares (sPLS) regression was used to select gene modules and proteins that most strongly associate with changes in fMRI (mixOmics[49]) in the subset of participants with all data types (N = 14). Models were tuned using axis loadings and mean absolute error. Two axes were chosen because this explained most (66%) of the variation in BOLD response. In total, 14 predictor features were mapped to component 1 and 2 predictor features to component 2. Correlations were calculated using the Pearson method (Table E3). Sequences are available in the National Center for Biotechnology Information Gene Expression Omnibus (GSE182733), and R code can be found at https://github.com/altman-lab/P337_MINA_BAL_public. The graphical abstract was created using Biorender.com.

RESULTS

Lung function, depression, asthma control, and airway inflammation

FEV_1 and BDI scores showed no significant change from pre– to post–SBP-Ag ($P > .1$). In contrast, ACQ scores increased

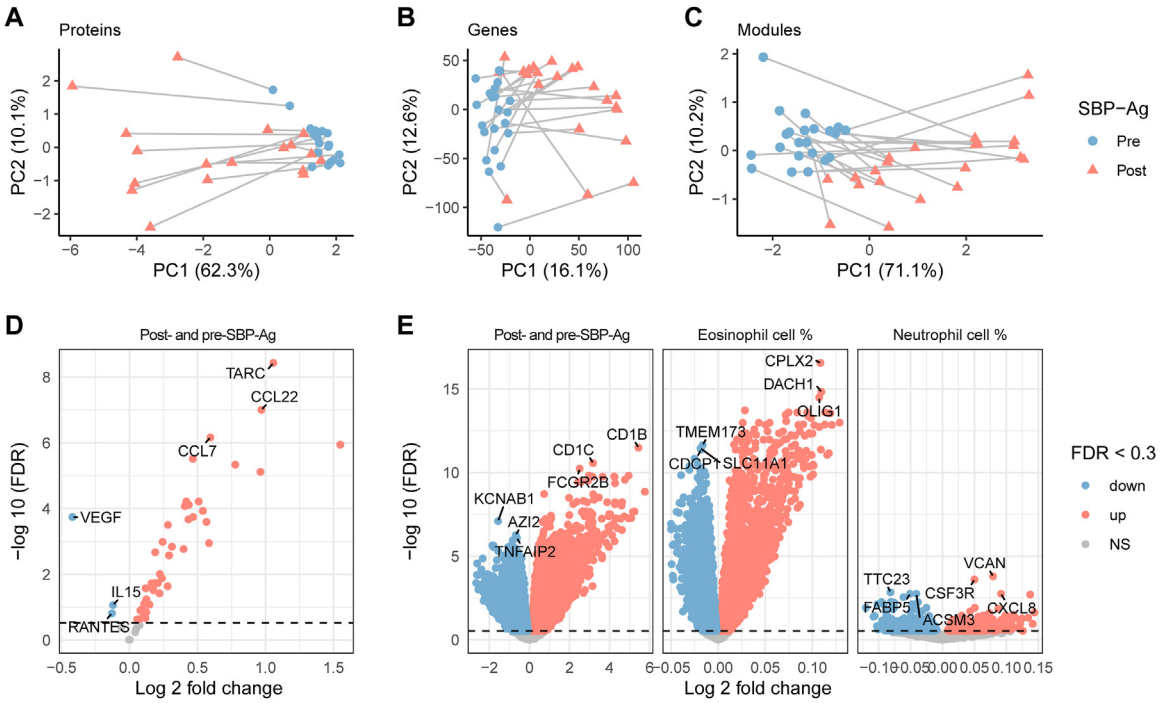

FIG 1. Proteins, genes, and gene modules affected by SBP-Ag. **A-C,** Principal-component analysis of 55 cytokine proteins (log_{10} pg/mL) (Fig 1, *A*), 14,346 genes (log_2 CPM) (Fig 1, *B*), and 11 eosinophil- and neutrophil-associated gene modules (mean log_2 CPM) (Fig 1, *C*). Sample color and shape indicate pre– and post–SBP-Ag with paired samples from the same donor connected by a line. **D** and **E,** Volcano plots of cytokine proteins pre– and post–SBP-Ag (Fig 1, *D*) and genes pre– and post–SBP-Ag as well as against eosinophil and neutrophil cell percentages (Fig 1, *E*). *Orange circles* indicate proteins and genes with significantly higher expression post–SBP-Ag or positively associated with cell percentages (FDR < 0.3). *Blue circles* indicate proteins and genes with significantly lower expression. The top 3 most significant up and down proteins and genes are labeled. *NS,* Not significant.

significantly (0.72 ± 0.61 to 0.91 ± 0.52; $P = .02$), indicating a reduction in asthma control, as did FENO (46 ± 29 ppb to 65 ± 40 ppb; $P = 4.1 \times 10^{-5}$). Cell differentials revealed that the cellular response to SBP-Ag was dominated by increases in the percentage of eosinophils (from 0.65% to 31.3%; FDR-adjusted $P = 1.56 \times 10^{-4}$) and relative decreases in monocytes (from 83.1% to 47.6%; FDR $= 1.40 \times 10^{-5}$) and epithelial cells (from 2.61% to 0.17%; FDR $= 3.12 \times 10^{-3}$). Neutrophils (FDR = 0.053) and lymphocytes (FDR = 0.466) were not affected by SBP-Ag (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Cytokine protein abundance showed global changes (Fig 1, *A*), with most proteins significantly affected (47 of 55; FDR < 0.3; Fig 1, *B*; see also Table E4 in this article's Online Repository at www.jacionline.org). Among these, most SBP-Ag–affected proteins were also positively associated with either eosinophils (44 of 47) and/or neutrophils (43 of 47; FDR < 0.3; Table E4).

RNA sequencing

From 14,346 pass-filter genes, 6,863 showed a change in expression after SBP-Ag (FDR < 0.3; Table E3; Fig 1, *C* and *D*). This subset of genes was then compared separately for relationships to eosinophil or neutrophil percentages. Genes were associated with cell types for which they had an FDR of less than 0.3 and a positive slope, resulting in 2,787 eosinophil- and 428 neutrophil-associated genes (Table E3). Next, cell-associated genes were grouped into modules using Weighted Gene Coexpression Network Analysis.[32] In total, 2,818 SBP-Ag– and cell-associated genes were collapsed into 9 eosinophil- and 2 neutrophil-associated modules. Mean gene expression in modules captured much of the gene-level changes pre– to post–SBP-Ag (Fig 1, *E*; Table E3).

fMRI analyses

The neural response to asthma-relevant words relative to valence-neutral words showed wide variability (Fig E1, *B*). Overall, there was no main effect of challenge on mean BOLD response in any of the ROI examined (FDR > 0.3; see Table E5 in this article's Online Repository at www.jacionline.org), which is not surprising in a sample of this size. However, in confirmation of our previous work,[4,5] we found the expected positive association between the change in the proportion of BAL eosinophils and BOLD response to asthma cues in the SN, most prominently in the anterior insula ($R = 0.46$; $P < .05$; Fig E1, *C*).

In sPLS analyses examining key associations between allergen-induced changes in gene and protein expression in the airway with changes in SN activity, component 1 represented significant positive correlations among allergen-induced increases in a gene expression module (EOS02) containing 416 eosinophil-associated genes and 13 proteins (most strongly FLT3LG and IL-17A) with increased activity in SN nodes (Fig 2). This result explained 36.7% of the

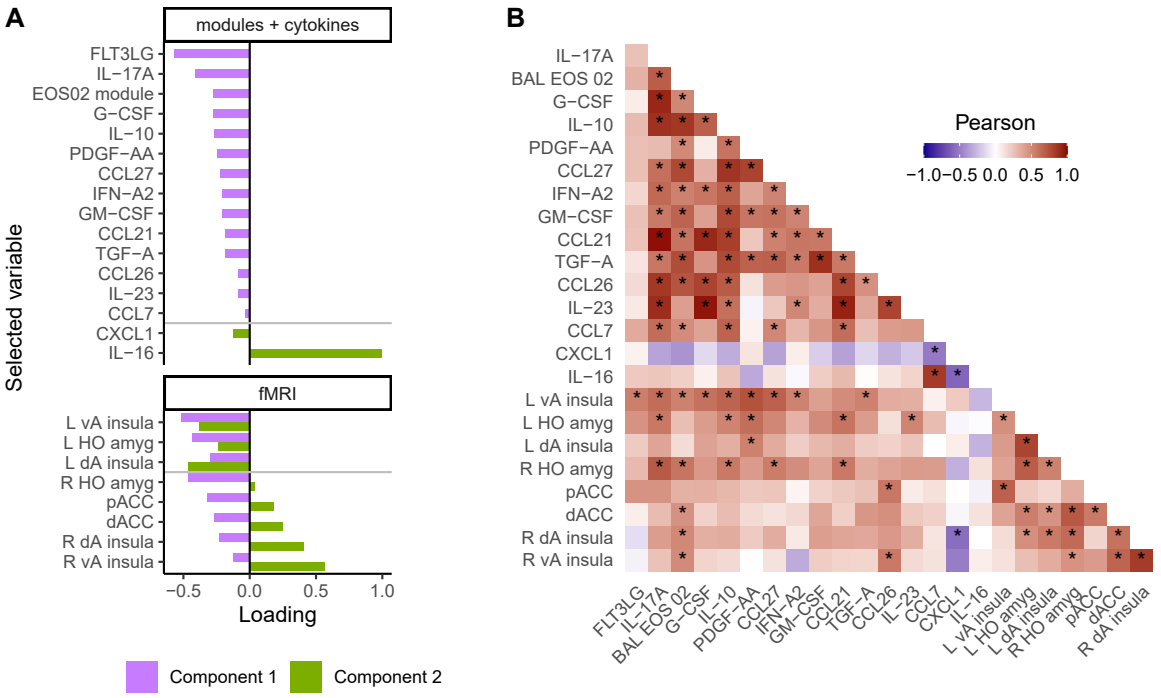

FIG 2. sPLS regression of SBP-Ag–induced changes in the lung and brain. Measures were assessed as post–minus pre–SBP-Ag. **A,** Axis loadings for gene modules and cytokine proteins against fMRI outcomes. The first 2 components explain more than 45% of variation in module and cytokine data as well as more than 65% of fMRI. *Horizontal gray lines* note where component 2 trends change. **B,** Pearson correlation of sPLS-selected variables. Variables are ordered as in Fig 2, *A. amyg*, Amygdala; *dA*, dorsal anterior; *HO*, Harvard-Oxford Atlas–defined; *L*, left; *p/dACC*, pregenual/dorsal anterior cingulate cortex; *R*, right; *vA*, ventral anterior. *Pearson $P < .05$.

variability in change in gene and protein levels and 45.4% of the variability in change in BOLD response in the SN with a correlation R value of 0.7. sPLS component 2 represented mixed negative (CXCL1) and positive (IL-16) correlations of change in protein levels with right-sided insula activity specifically (Fig 2). This result explained an additional 11.1% of the variability in change in gene and protein levels and 20.9% of the variability in change in BOLD response with a correlation R value of 0.9.

In identification of functional gene networks, we focused on component 1 because of its robust and consistent correlations across SN nodes. We found that the SBP-Ag–induced genes and proteins selected by sPLS have known functional interactions as evidenced by a significant interaction network of high confidence interactions in the STRING database[33] ($P = 1.2 \times 10^{-5}$; Fig 3). The most central proteins in this network included mitogen-activated protein kinase 3 (degree = 30; betweenness = 3989), VEGFA (degree = 30; betweenness = 3297), CDK3 (degree = 20; betweenness = 2286), and NOTCH1 (degree = 16; betweenness = 1814). Collectively, this network was functionally enriched for pathways related to cytokine signaling, leukocyte chemotaxis, and tissue growth (Table E3). It includes key components of T_H17 inflammation, including IL-17A, IL-23A, and FLT3LG, a cytokine critical to sustaining expansion of T_H17 cells in the lung,[50] as well as multiple molecules with overlapping roles in neuronal, vascular, and lung cell proliferation (eg, NOTCH1, VEGFA, and LIF).[51-53]

Expression of these genes and proteins increased with SBP-Ag, with the magnitude of change positively relating to both the directionality and the magnitude of changes in SN activity, as exemplified by the relationship between the EOS02 module and IL-17A protein levels and responsivity of the left ventral anterior insula to asthma-specific cues (Pearson $R \geq 0.54$; $P < .05$; Fig 4). These associations were stronger than those between the SN and eosinophil cell proportions alone (Pearson $R = 0.46$; Fig 4) and stronger than associations with type 2 inflammatory pathways represented by the EOS01 module, mast cell genes, or IL-5 and IL-13 proteins (see Fig E2 in this article's Online Repository at www.jacionline.org). In addition, although TNFA protein itself increased significantly with SBP-Ag, TNFA abundance was not significantly correlated with changes in the SN, nor was IL-6 and other related genes and proteins selected in sPLS analysis (Fig E2).

DISCUSSION

The eosinophil-associated pathways reflected in the EOS02 module and associated proteins emerged as the molecular signature of airway inflammation most closely associated with affect-related changes in SN function. These data are consistent with previous work and importantly advance our understanding of the specific biology linking lower airway inflammation with neural changes in patients with asthma but also add an important level of molecular specificity. The genes and proteins functionally

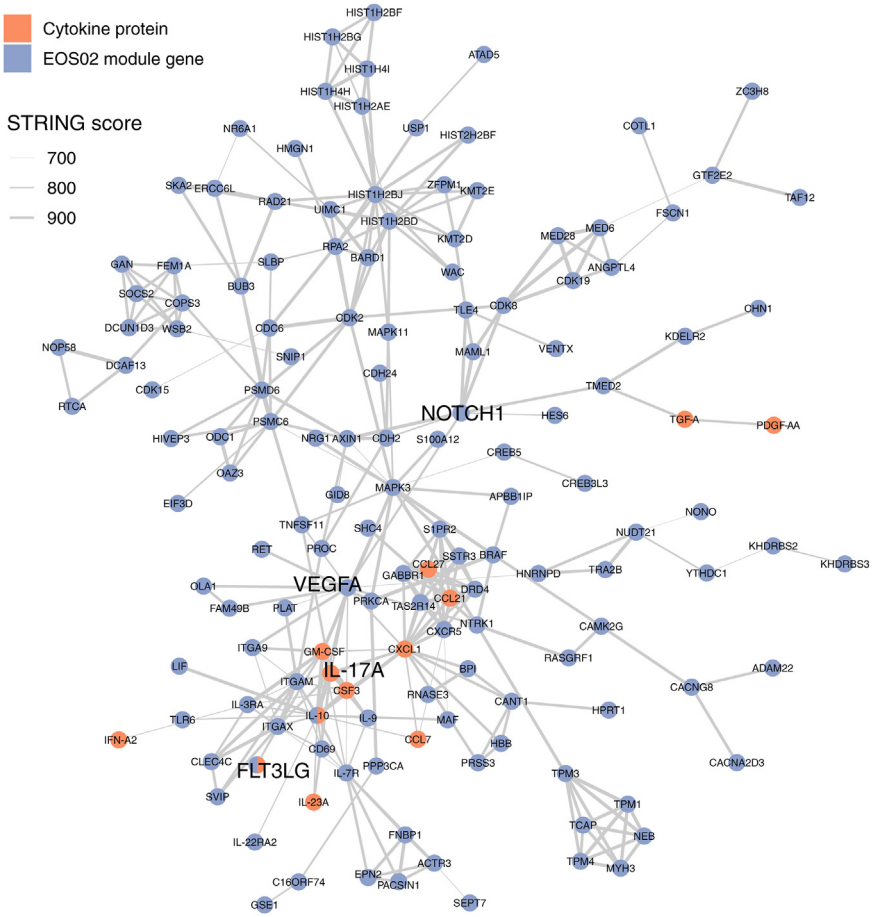

FIG 3. STRING network of sPLS-selected genes and proteins associated with neuronal responses to SBP-Ag. Color indicates EOS02 module genes (*purple*) and protein cytokines (*orange*) significantly associated with 1 or more fMRI outcome. Edges with STRING combined score greater than 700 are shown in *gray* with width corresponding to score. The largest cluster of 147 nodes is shown. A small cluster (N = 49) and isolated nodes (N = 196) are not shown.

central to this module were linked more to a T_H17 response with mixed inflammatory and proliferative pathways rather than to the canonical type 2 inflammatory pathways typically associated with eosinophils and asthma.[54] Indeed, type 2 pathways (ie, EOS01 module and IL-4, IL-5, and IL-13 proteins) were not strongly associated with increased activation in the SN (Table E3) in the present study. We similarly did not detect significant associations with other aspects of asthma-related inflammation, including mast cell genes or IL-6 signaling, although these also increased following SBP-Ag. The absence of a mast cell signal may relate to the timing of sample collection at 48 hours postchallenge.[55] T_H17 inflammation has been variably associated with eosinophilic or neutrophilic asthma[56,57] and can be coincident with type 2 inflammation.[58-60] In a previous work,[61] we showed that eosinophils spontaneously release IL-1β to increase the production of IL-17A by activated memory $CD4^+$ T cells, which is consistent with our current data showing that the IL-17A signal is closely linked with an eosinophilic response rather than with neutrophils. The baseline percentage of T_H17 cells in circulation has been shown to be elevated, even in mild asthma, and to increase, together with IL-17 expression, following allergen challenge.[62] Interestingly, during stress[16] or episodes of inflammation,[63,64] T_H17 cells can accumulate in the brain. This is noteworthy because systemic administration of IL-17 in animal models evokes depressive-like behavior[65] and anti–IL-17 confers resistance.[16] Consistent with its effect in animal models, IL-17 expression is elevated in depression in humans[66,67] and is associated with depression that is treatment-resistant. Taken together, our results provide a potential direct link between asthma and depression through T_H17 rather than through canonical type 2 inflammation.

In concert with the SBP-Ag–induced upregulation of genes in the T_H17 inflammatory pathway, *NOTCH1* and *VEGFA* were central to the STRING network of proteins and genes in the EOS02 module that was correlated with SN responses to allergen (Fig 2). These genes and their associated pathways have important implications for both asthma and neuronal function. In asthma, NOTCH1 is involved in multiple aspects of pathophysiology,[68] drives differentiation of T_H17 cells,[69,70] and is essential for eosinophil chemotaxis and transendothelial migration.[71] VEGFA plays an important role in airway blood vessel growth in asthma and induces vascular permeability and leakage, in addition to its participation in allergic sensitization and type 2 inflammation.[72] In the brain, IL-17 activates NOTCH1,[73] which regulates differentiation of neural stem cells[52,74] and promotes neuroinflammation during neural injury or neurodegeneration via regulation of astrocyte

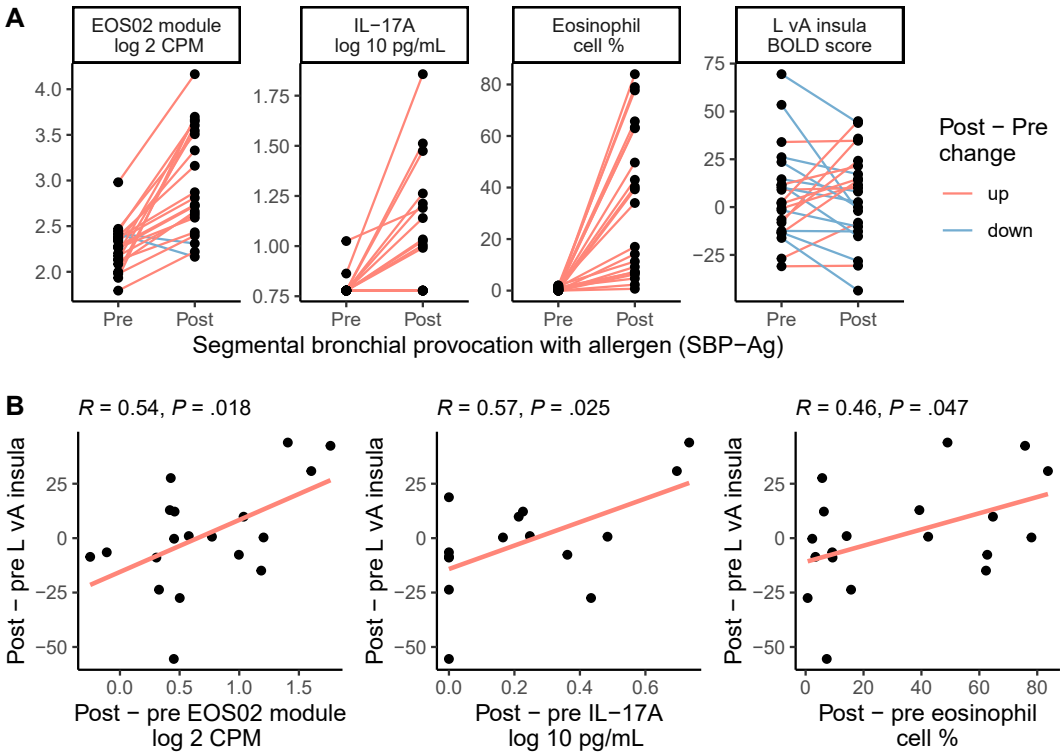

FIG 4. EOS02 module genes and IL-17A protein associated with L Va insula response to SBP-Ag. **A,** Eosinophil cell percentage, mean $\log_2$ CPM expression of EOS02 module genes, $\log_{10}$ pg/mL abundance of IL-17A protein, and L vA insula pre- and postsegmental bronchial provocation with allergen (SBP-Ag). Paired samples from the same individual are connected by a line colored by post- pre changes up (*red*) or down (*blue*). **B,** Pearson correlation (*R*) of L vA insula with eosinophil percentage, EOS02 module, and IL-17A protein. Trend lines represent a best fit linear model. *L vA,* left ventral anterior.

differentiation and proliferation and microglial activation.[52] VEGFA is integrally involved in angiogenesis in the brain. It is also produced by reactive astrocytes, leading to increased blood-brain barrier permeability and degradation of barrier function, which is a major contributor to neuroinflammation,[75] an important factor in stress-related vulnerability versus resilience to depression.[76] Thus, the implication of NOTCH1 and VEGFA in the relationship between SBP-Ag–induced airway changes and neural function suggests that airway inflammation may influence neuronal activity, at least in part, via neuroinflammatory pathways, contributing to negative emotional and cognitive outcomes. Indeed, NOTCH1 has previously been implicated in vulnerability to psychopathology.[77]

Collectively, our data support previous observations that allergen-driven eosinophilic inflammation is associated with enhanced reactivity in brain networks important in regulating emotion.[4,5] Here, we further provide a proposed mechanism of action wherein T_H17 inflammation, linked to expression of neuronal and vascular signaling molecules in the lung, promotes neuroinflammation, and ultimately emotional and cognitive dysfunction. There are multiple potential ways through which changes in gene and protein expression in the lung could influence neuronal function, resulting in the pattern of associations reported here. It is possible that T_H17 cells in the lung migrate to the brain, where they promote neuroinflammation through astrocyte and microglial differentiation and activation. Glia are also intimately involved in regulating neurotransmission and neuronal activity by shaping synaptic architecture and function[78] and through regulating the amount of glutamate in the synapse.[79] This mechanistic hypothesis is supported by recent work in which we found that the concentration of a blood-based marker of reactive astrocytes in asthma was highly related to changes in brain microstructure, indicating the presence of neuroinflammation.[80] Our hypothesis is further supported by a rodent model of mixed T_H17 and type 2 asthma, wherein allergen exposure selectively increased the number of T_H17 cells in the brain. This increase was associated with morphologically activated microglia in the subfornical organ, which projects to the SN, as well as changes in neuronal activity in SN nodes.[81]

It is important to note that this study was limited to measuring changes in gene expression in BAL fluid cells and not directly in brain cells. However, there is evidence that differential expression of genes in peripheral immune cells is mirrored in neurons and brain immune cells.[82] For example, genes differentially expressed in lymphocytes from patients with schizophrenia were found to have concordant differential expression in postmortem brain samples (both neurons and immune cells).[83] Furthermore, the evaluation of gene expression changes, including NOTCH1 and VEGFA, in cells drawn from peripheral samples shows

neuropathophysiological predictive value and is being explored as a noninvasive approach in determining the clinical trajectory of cognitive decline.[84] Nonetheless, this is a known limitation of the present study and will require confirmation in an animal model. A further limitation of the study was the absence of a control or placebo challenge. In a previous work,[4] we compared the neural response to WLAC to that of inhaled saline, which revealed no significant changes, obviating the need to understand immune signaling pathways underlying this effect in the present study. Finally, given the relative homogeneity of our sample (83% non-Hispanic White), our results may not generalize to a more diverse sample and should be validated in a larger, more representative sample.

Our results identify the importance of T_H17 inflammation and associated NOTCH1 and VEGFA signaling in the interaction between airway inflammation and emotion-related SN activity. More broadly, our data emphasize that asthma-related inflammation has a systemic component with potentially profound effects outside the lung. The connection of T_H17 activity with expression of genes important in neuroinflammation raises new hypotheses regarding the scope of the impact of airway inflammation on the brain. We speculate that targeted control of specific aspects of airway inflammation in asthma is an important step to prevent or reduce comorbid conditions including depression and cognitive dysfunction as well as deterioration in brain health more generally.

DISCLOSURE STATEMENT

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Key messages

- **Airway inflammation has an impact on how the brain responds to emotional information.**
- **Changes in T_H17-related inflammation are associated with brain impacts of asthma.**
- **T_H17 signaling pathways may contribute to psychological and neurologic sequelae of asthma.**

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