Original Research

Elevated Circulating Th2 Cells in Women With Asthma and Psychological Morbidity: A New Asthma Endotype?

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ABSTRACT

Purpose: Psychological stress shifts the immune system toward the production of T-helper (Th)-2–mediated cytokines and eosinophilia, increases the risks for both asthma and depression, and can precipitate asthma exacerbations. Th2-mediated inflammation is a characteristic of allergic asthma. We have shown that the levels of $CD4^+$ Th2 cells in the peripheral blood of patients with asthma are associated with severity and/or control of the disease. To improve our understanding of the interactions between stress and asthma symptoms, we evaluated the effects of psychological comorbidity on Th2-mediated inflammation in patients with asthma.

Methods: Sixty-six asthmatic patients were recruited from the University of Alberta Asthma Clinic after they gave informed consent. Stress-related effects on asthma and psychological morbidity were assessed using the Asthma Control Questionnaire, completed by the patients at recruitment. Venous blood was collected at recruitment and Th2-mediated immunity evaluated by flow cytometry, quantitative real-time reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay.

Findings: Patients with stress-triggered asthma (n = 12) had higher percentage of $CD4^+$ T cells ($P = 0.006$) and Th2 cells ($CD4^+CRTh2^+$ T cells; $P = 0.002$) in peripheral blood compared to patients with asthma who did not experience stress-related worsening of disease (n = 54). The same was true when we analyzed patients with any form of psychological comorbidity (n = 19) compared to those without psychological comorbidities (n = 47). These differences were evident among women, but not among men. Women with psychological comorbidity also required higher doses of inhaled and oral corticosteroids compared to those without psychological comorbidity.

Implications: Asthma involving psychological morbidity associates with an elevated level of circulating Th2 cells and increased corticosteroid usage, and may be more prevalent in women. Larger-scale prospective studies are required for assessing whether these women constitute a new endotype of Th2-high asthma responsive to treatments aimed to improve psychological comorbidities. (*Clin Ther*. 2020;42:1015–1031)

Key words: asthma, CRTh2, psychological morbidity, stress, Th2 cells, women.

INTRODUCTION

Asthma is a condition recognized since antiquity, first mentioned in *The Iliad* and described medically by Hippocrates, the first to associate it with environmental exposures.[1] The term *asthma* translates from ancient Greek as "to breathe with open mouth or to pant."[2] It is considered one of the

Accepted for publication May 2, 2020
https://doi.org/10.1016/j.clinthera.2020.05.001
0149-2918/$ - see front matter

most elusive chronic disorders and has been likened to conditions such as fever, of which there are many causes. In 2006, *The Lancet* made a plea for the abandonment of asthma as a single disease concept.[3] Over the past 2 decades under the spotlight, great strides have been made in developing an asthma taxonomy based on pathobiology. As a result, asthma is now classified by inflammatory phenotype, and new therapeutic modalities are focused on treating these phenotypes.[4]

Th2-high asthma is characterized by elevated blood and airway levels of eosinophils; $CD4^+$ T-helper (Th)-2 cells; and the cytokines interleukin (IL) 4, 5, and 13.[5] Th2 cells and group 2 innate lymphoid cells (ILC2s) express CRTh2 (chemoattractant receptor–homologous molecule expressed on Th2 cells),[6,7] a receptor of the lipid mediator prostaglandin (PG)D_2,[8] which is released from mast cells following allergen–immunoglobulin (Ig) E crosslinking[9] as well as other cells.[10] PGD_2 activation of CRTh2 leads to Th2-mediated cytokine production,[11] chemotaxis,[8,12] and inhibition of apoptosis,[13] all effects that may exacerbate Th2-mediated inflammation. Therapies targeting Th2-mediated cytokines are being used in the clinic and are effective in improving the clinical course of asthma.[14–16]

Despite this progress, certain features of asthma remain elusive and are still not well incorporated into modern taxonomy. One such case is the association between psychological morbidity and asthma, which also dates back to the time of Hippocrates, who stated that "the asthmatic should guard himself against his own anger" to prevent an asthma attack.[17] The medieval physician Maimonides included a chapter in his treatise on asthma dedicated to discussing the importance of "regulating one's emotions."[18] Osler,[19] in his seminal medical textbook published in 1892, wrote that asthma was a disorder of psychogenic origin and referred to it as "asthma nervosa." Indeed, from ancient times until the middle of the 20th century, a main tenet of asthma treatment was to relieve stress and anxiety.[20] Although the potential for stress and emotion to trigger asthma symptoms and exacerbation is well recognized, the underlying pathobiology has remained less well understood.

In this study, we analyzed data from a cohort of patients with asthma that we have described in previous publications.[21,22] Here we examined the relationship between immune profile and psychological morbidity and discuss our findings in the context of previous studies regarding the effects of psychological factors, primarily stress, on Th2 cells, Th2-mediated inflammation, and the overlap with proinflammatory processes.

MATERIALS AND METHODS

Participants

Adults with asthma, as clinically diagnosed by a respiratory medicine specialist, were consented (University of Alberta Ethics Board approval number PRO1784) and recruited from the University of Alberta Asthma Clinic (Edmonton, Alberta, Canada). Data on characteristics such as age, sex, body mass index, atopy, as well as serum IgE and blood eosinophil number, were collected from the clinical charts of the patients. Atopy was determined by a skin test positive (at least 3-mm wheal) for 1 or more of a panel of 12 aero-allergens (Timothy grass, birch, poplar, cedar, cat, dog, house dust mites [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*], and/or *Alternaria, Aspergillus, Hormodendrum, Penicillium* spp).

Psychological Morbidity

Data on psychological comorbidities were collected from a clinic-designed questionnaire that patients completed during recruitment. Data were extracted from the following items: (1) "What irritants/allergens/triggers do you feel you are exposed to?" (free-text section) and (2) questions on comorbidities and medications. From these data we categorized patients as having stress-triggered asthma (stress, anxiety, depression, strong emotions, emotional situations; ST group) or non–stress-triggered asthma (NST group), and as having psychiatric morbidities (depression and/or taking antidepressant medications were the only psychiatric conditions identified in the questionnaire; PM group) or having no psychological effect on their asthma (NPM group).

Profiling Peripheral Blood Th2 Cells

Staining for flow cytometry was performed at room temperature, with 100 μL of well-mixed anticoagulated whole blood collected in sodium heparin tubes (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey). Fc blocker (Miltenyi Biotec, Auburn, California), mouse IgG and rat IgG (50 μg each; both, InvitroGen, Burlington, Ontario, Canada) were added to block nonspecific

binding (20 min). Cells were then incubated with biotinylated anti-CRTh2 (30 min; clone BM16; Miltenyi Biotec) or isotype control (rat IgG2a; AbD Serotech, Raleigh, North Carolina) antibodies. Red blood cell lysis buffer (BD Biosciences, Mississauga, Ontario, Canada) was added (15 min) and samples vortexed (10 s). Cells were centrifuged at 1200 rpm (5 min), and washed with 2 mL of phosphate-buffered saline:Flow Cytometry staining (0.5% bovine serum albumin, 0.1% NaN_3, 3% fetal bovine serum in phosphate-buffered saline; Sigma–Aldrich, Oakville, Ontario, Canada). For the detection of $CRTh2^+$ cells, streptavidin–Allophycocyanin (200 ng/mL; eBioscience, San Diego, California) was added. For identifying lymphocytes, anti-CD4 (Serotec, Oxford, UK), anti-CD3 and isotype control antibodies (BD Biosciences) were used. The BD CompBeads Plus reagent (anti-mouse Ig, k; BD Biosciences) was used for fluorophore compensation. Cells and compensation bead tubes were incubated in the dark (30 min), washed with 2 mL of phosphate-buffered saline:Flow Cytometry staining, centrifuged (1200 rpm, 5 min) and resuspended in 250 mL of 2% paraformaldehyde (Sigma–Aldrich).

$CD4^+$ T cells were identified by low side scatter and high CD4 staining ($SSC^{low}CD4^{high}$) and were then analyzed for CRTh2 expression. $SSC^{low}CD4^{high}$ cells were confirmed to be T lymphocytes since >99% of the cells also expressed CD3.[21] Flow cytometry data were collected on BD LSR Fortessa (BD Biosciences) using FACS Diva software and gates set in accordance with the profiles of the isotype control and/or negative control beads.

Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated from 2 mL of whole blood using the PAXgene Blood RNA Kit (PreAnalytiX, Qiagen, Mississauga, Ontario, Canada) according to the manufacturer's instructions. Reverse-transcription reactions were performed using 1 µg of total RNA, oligo-dT primers, and Superscript II reverse transcriptase (InvitroGen). TaqMan gene expression assay for *CRTH2* (alias *PTGDR2*; Hs00173717_ml; Applied Biosystems, Carlsbad, California) was used: 19 µL of TaqMan gene expression master mix and 1 µL of cDNA. *GAPDH* mRNA was used as an internal control and was quantified by a custom FAM-labeled TAMRA probe (5′-AAA TCC CAT CAC CAT CTT CCA GGA GCG A-3′) (Applied Biosystems) and the following primers: forward, *GAPDH*-F (5′-CAA GGCT GAG AAC GGG AAG-3′); and reverse, *GAPDH*-R (5′-GCA AAT GAG CCC CAG CCT T-3′). The polymerase chain reaction protocol consisted of 10 min at 95 °C followed by 40 cycles of 30 s at 95 °C and 60 s at 60 °C. All samples were run in triplicate. *CRTH2* mRNA was analyzed using the ΔΔCycle threshold (C_τ) compared to GAPDH method. Briefly, the ΔC_τ was determined by subtracting the C_τ of GAPDH from the C_τ of CRTh2. All ΔC_τ values were subtracted from one patient's ΔC_τ to calculate $\Delta\Delta C_\tau$. The fold-increase was then calculated using the $\Delta\Delta C_\tau$ as a negative exponent to the base of 2 ($2^{-\Delta\Delta C\tau}$).

Enzyme-Linked Immunosorbent Assay

Venous blood was obtained (BD Vacutainer blood collection tubes) and serum collected following centrifugation at 1200 rpm for 10 min. Serum was stored at –20 °C until use. IL-13 was detected using a commercially available ELISA kit (Diaclone Research, Besancon, France; detection limit, 3.12 pg/mL).

Statistical Analysis

Differences in demographic and phenotypic clinical characteristics between the groups were assessed using the Mann–Whitney *U* test for continuous variables. The Fisher exact test was used for categorical variables. Statistical analyses were performed using SPSS version 21.1 (IBM, Chicago, Illinois) and graphs generated using R software version 3.2.1 (The R Foundation for Statistical Computing, www.r-project.org). *P* values of <0.05 were considered significant.

RESULTS

Demographic and Clinical Characteristics

Sixty-six patients with asthma were recruited between March 2012 and November 2013 for a previously published study of differences in Th2-mediated inflammation between patients with mild/moderate and severe asthma.[21,22] Patients gave informed consent, filled out questionnaires, and gave blood for analysis of Th2 parameters. On the questionnaires they filled out were questions on psychological morbidity and use of medications. When we analyzed the data on psychological

morbidity we noticed that 18% of the population (n = 12) reported asthma symptoms worsening due to stress (n = 7) or stress-related circumstances (strong emotions, anxiety, or depression; n = 5). The demographic and clinical parameters of the patients with ST asthma showed no differences in age, sex, body mass index, serum IgE, blood eosinophils, smoking history, lung function, symptoms as determined by the Asthma Control Questionnaire, inhaled corticosteroid (ICS) or oral corticosteroid (OCS) use compared to patients whose asthma was not stress triggered (NST; n = 54) (Table I).

Association Between Stress-Triggered Asthma and Circulating Th2 Cells

Using flow cytometry (gating strategy described in *Materials and Methods* and shown in Fig. 1), we assessed the percentage of $CD4^+$ T cells and Th2 cells ($CD4^+CRTh2^+$ T cells) in peripheral blood. Patients with ST asthma had significantly higher percentage of $CD4^+$ T cells ($P = 0.006$) and Th2 cells ($P = 0.002$) in peripheral blood compared to those in patients with NST asthma (Table II and Fig. 2). Among patients with ST asthma, women tended to have more Th2 cells in total peripheral blood compared to men ($P = 0.061$; data not shown). Since 66% of patients with ST asthma were women, we performed a sex-stratified analysis. We found that women with ST asthma had higher percentage of $CD4^+$ T cells ($P = 0.005$) and Th2 cells ($P = 0.003$) in peripheral blood compared to those in women with NST asthma, while this was not the case in men (Table III). Women with ST asthma were also taking higher daily doses of ICS than were women with NST asthma ($P = 0.020$), indicating that increased inflammation may necessitate increased anti-inflammatory medications for effective treatment. Interestingly, men with ST asthma did exhibit lower number of eosinophils ($P = 0.045$) and *CRTH2* mRNA in whole blood ($P = 0.040$) compared to those in men with NST asthma, something that was not evident in women (Table III).

Th2 Cells and Th2-Mediated Inflammation in Asthmatic Patients With Psychological Morbidity

Data on other psychological-related morbidity in our population indicated that 7 patients reported being treated with antidepressant medications and 1 more reported having depression; of this group, 1 also experienced ST asthma. Comparing these 8 patients with the rest of the population, we saw a trend toward increased $CD4^+$ T cells and Th2 cells, similar to that seen in ST asthma (P = NS; data not shown). This was a very small group of patients,

Table I. Demographic and clinical characteristics of patients with NST and ST asthma.

Characteristic	NST (n = 54)	ST (n = 12)	*P*
Age, median (IQR), y	40 (31.0–57.0)	46.5 (28.5–58.7)	0.861
Female sex, no. (%)	29 (54)	8 (66.7)	0.527
BMI, median (IQR), kg/m^2	32.9 (23.3–35.4)	28.7 (27.3–35.2)	0.435
Atopy, no. (%)	39/49 (80)*	8/11 (73)†	0.690
Serum IgE, median (IQR), kU/L	1.8 (1.4–2.0)‡	2.1 (1.3–2.5)	0.618
Smoking habit			
History of smoking, no. (%)	25 (46)	4 (33)	0.527
Current smoking, no. (%)	6 (11)	0	0.582
Number of pack-years, median (IQR)	0.0 (0.0–10.5)	0.0 (0.0–9.2)	0.533
FEV_1, median (IQR), % predicted	84.0 (69.5–91.0)	77.5 (53.5–90.0)	0.378
FEV_1/FVC, median (IQR), %	68.5 (58.0–76.0)	65.5 (45.2–76.0)	0.302
ACQ score, median (IQR)	1.7 (0.8–2.5)§	2.1 (0.5–2.4)‖	0.759

ACQ = Asthma Control Questionnaire; BMI = body mass index; FEV_1 = forced expiratory volume in 1 s; FVC = forced vital capacity; IgE = immunoglobulin E; IQR = interquartile range; NST = non stress triggered; ST = stress triggered.
n = *49, †11, ‡45, §25, and ‖5 due to missing data in some patients.

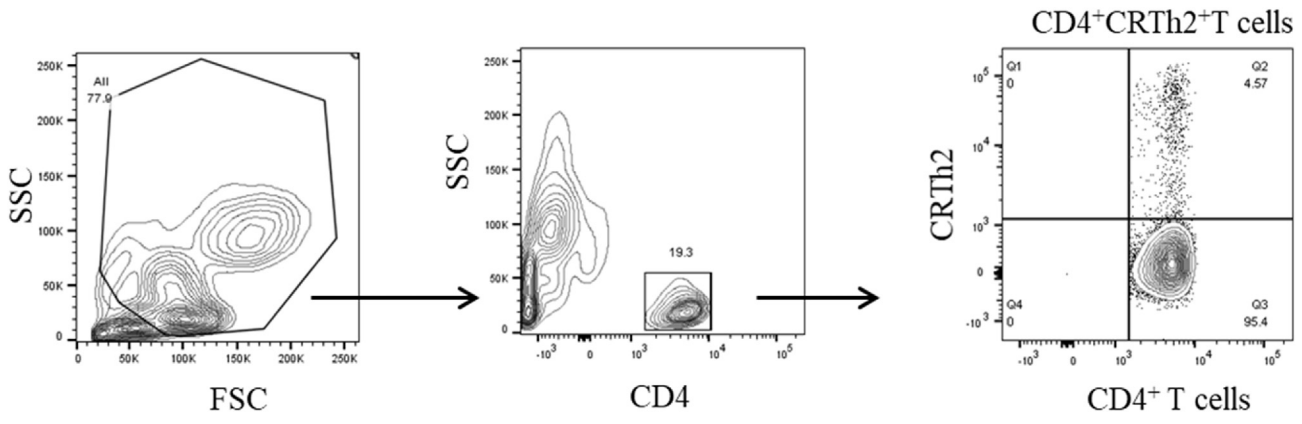

Figure 1. Flow cytometry—gating strategy to analyze CRTh2 (chemoattractant receptor—homologous molecule expressed on Th2 cells) expression on peripheral blood $CD4^+$ T cells. FSC = forward scatter; SCC = Side scatter.

which may have contributed to the lack of statistical significance. Therefore, we combined the data from these patients with those from ST asthmatic patients and analyzed the data from those with psychological morbidity (PM group, n = 19; 12 with ST asthma plus 8 with depression, minus 1 individual who belonged to both groups) and those without psychological morbidity (NPM asthma, n = 47). We found no demographic or clinical differences (Table IV). Patients with PM asthma exhibited a strong Th2 phenotype, with higher percentage of $CD4^+$ T cells ($P = 0.001$) and Th2 cells ($P = 0.001$) in peripheral blood and a higher level of serum IL-13 ($P = 0.034$) compared to the percentage in those with NPM asthma (Table V). Those with PM asthma used a similar amount of ICSs compared to those with NPM asthma (1000 vs 586 μg/d; $P = 0.077$). Similarly, they required similar amounts of steroid burst ($P = 0.059$) and hospitalization ($P = 0.069$) (Table V) during the previous year, compared to those with NPM asthma.

Among patients with PM asthma, 74% were women and a sex-stratified analysis was also performed. Similar to those with ST asthma, women with PM asthma had more $CD4^+$ T cells ($P = 0.003$) and Th2 cells ($P = 0.004$), were taking more ICSs (μg/d) ($P = 0.002$), and were more likely to have taken an OCS in the previous year ($P = 0.021$) compared to women with NPM asthma, while these differences were not evident in men (Table VI). On the other hand, men with PM did have lower number of eosinophils ($P = 0.010$) and *CRTH2* mRNA in whole blood ($P = 0.008$) compared to men with NPM asthma (Table V), as was also shown in men with ST asthma.

DISCUSSION

Psychological stress influences the risk for developing, and the trajectories of, many chronic inflammatory diseases,[23] including asthma.[24,25] Demystifying the pathobiology underlying this association is an active area of investigation. In this study, we found that the prevalence of psychological morbidity such as stress-triggered asthma or depression was relatively high, associated with the presence of increased numbers of Th2 cells in peripheral blood, and was more common in women. Efforts to improve asthma classification have moved toward identifying endotypes—distinct pathophysiologic mechanisms driving disease at the cellular and molecular levels. Our data suggest that asthma with psychological morbidity may constitute a new endotype of Th2-high asthma that primarily occurs in women. Efforts should be focused on validating these results, better defining this endotype, and identifying the most effective treatment in these patients.

Our first analysis was based on patients reporting stress and related conditions as a trigger of asthma.

Table II. Immunologic and clinical characteristics of patients with NST and ST asthma.

Characteristic	NST (n = 54)	ST (n = 12)	*P*
$CD4^+$ T cells in PB, median (IQR), %	5.85 (3.92–8.51)	10.13 (6.50–14.0)	**0.006**
$CD4^+/CRTh2^+$ T cells in PB, median (IQR), %	0.22 (0.14–0.32)	0.45 (0.25–0.59)	**0.002**
CRTH2 mRNA in WB, median (IQR), %	3.68 (1.82–6.10)*	3.89 (1.94–6.10)†	0.946
Eosinophils, median (IQR), cells/μL	200 (100–475)‡	200 (100–300)	0.507
Total dose of inhaled corticosteroid, median (IQR), μg/d	586 (293–1000)	1000 (521–1169)	0.104
IL-13, median (IQR), pg/mL	3.12 (3.12–15.4)§	19.9 (3.12–35.1)	0.475
Patients receiving at least 1 steroid burst during the previous year, no. (%)	19 (35.2)	7 (58.3)	0.193
Patients hospitalized in the previous year, no. (%)	3 (5.6)	1 (8.3)	0.561

IL = interleukin; IQR = interquartile range; NST = non stress triggered; PB = peripheral blood; ST = stress triggered; Th = T-helper type-2 cells; WB = whole blood.
n = *46, †9, ‡52, and §53 due to missing data in some patients.

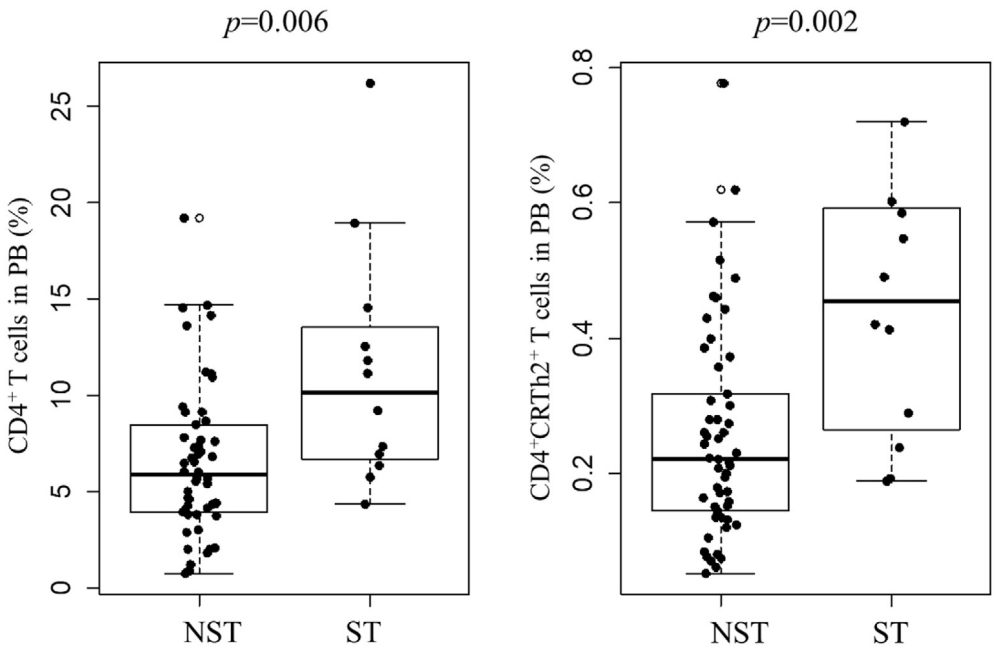

Figure 2. Percentages of $CD4^+$ T cells (A) and $CD4^+/CRTh2^+$ T cells (B) in the peripheral blood (PB) of patients with stress-triggered and non–stress-triggered asthma.

Stress was first described by Selye and defined as "the state of altered homeostasis due to an internal or external stimulus."[26,27] A *stressor* is anything that is perceived as stressful by the individual and causes, according to Selye, "distress."[28] Stress activates the hypothalamic-pituitary-adrenal axis, leading to the release of, among others, corticotropin-releasing hormone and cortisol.[24,29] Stress also leads, in most

Table III. Immunologic and clinical characteristics of men and women with NST and ST asthma.

Characteristic	Men			Women		
	NST (n = 25)	ST (n = 4)	*P*	NST (n = 29)	ST (n = 8)	*P*
$CD4^+$ T cells in PB, median (IQR), %	6.56 (4.32–9.14)	8.72 (4.83–11.62)	0.507	5.54 (3.75–7.69)	10.8 (7.0–17.8)	**0.005**
$CD4^+/CRTh2^+$ T cells in PB, median (IQR), %	0.25 (0.14–0.33)	0.32 (0.19–0.42)	0.376	0.21 (0.14–0.33)	0.56 (0.34–0.69)	**0.003**
CRTH2 mRNA in WB, median (IQR), %	4.32* (2.6–5.8)	1.67† (1.53–NA)	**0.040**	3.48‡ (1.63–6.52)	4.96§ (3.63–6.62)	0.230
Eosinophils, median (IQR), cells/μL	250‖ (100–650)	50 (0.0–175)	**0.045**	200‖ (100–400)	250 (200–525)	0.413
Total dose of inhaled corticosteroid, median (IQR), μg/d	1000 (500–1000)	646 (73–1129)	0.563	586 (216–1000)	1068 (689–1479)	**0.020**
IL-13, median (IQR), pg/mL	3.12# (3.12–20.86)	24.87 (16.02–35.12)	0.058	3.12 (3.12–15.45)	11.92 (3.12–35.87)	0.475
Patients receiving at least 1 steroid burst during the previous year, no. (%)	8 (32)	1 (25)	1	11 (37.9)	6 (75)	0.109
Patients hospitalized in the previous year, no. (%)	0	0	0	3 (10.3)	1 (12.5)	1

IL = interleukin; IQR = interquartile range; NST = non stress triggered; PB = peripheral blood; ST = stress triggered; Th = T-helper type-2 cells; WB = whole blood.
n = *21, †3, ‡25, §6, ‖24, ¶28, and #24 due to missing data in some patients.

Table IV. Demographic and clinical characteristics of patients with NPM and PM asthma.

Characteristic	NPM (n = 47)	PM (n = 19)	*P*
Age, median (IQR), y	40 (31.0–55.0)	45.0 (31.0–60.0)	0.353
Female sex, no. (%)	23 (49)	14 (74)	1
BMI, median (IQR), kg/m^2	27.0 (23.2–35.0)	29.3 (27.2–37.7)	0.169
Atopy, no. (%)	32/42 (76)*	15/18 (83)[†d]	0.736
Serum IgE, median (IQR), kU/L	2.0 (1.5–2.3)[‡]	1.8 (1.0–2.4)	0.354
Smoking habit			
History of smoking, no. (%)	21 (45)	8 (42)	1
Current smoking, no. (%)	5 (11)	1 (5)	0.582
Number of pack-years, median (IQR)	0.0 (0.0–10.0)	0.0 (0.0–12.5)	0.969
FEV_1, median (IQR), % predicted	88.0 (68.0–91.0)	78.0 (55.0–86.0)	0.165
FEV_1/FVC, median (IQR), %	83.0 (73.0–91.0)	83.0 (67.0–92.0)	0.314
ACQ score, median (IQR)	1.3 (0.8–2.5)[§]	2.1 (1.1–2.4)[‖]	0.725

ACQ = Asthma Control Questionnaire; BMI = body mass index; FEV_1 = forced expiratory volume in 1 s; FVC = forced vital capacity; IgE = immunoglobulin E; IQR = interquartile range; NPM = non psychological morbidity; PM = psychological morbidity.
n = *42, [†]18, [‡]39, [§]22, and [‖]8 due to missing data in some patients.

Table V. Immunologic and clinical characteristics of patients with NPM and PM asthma.

Characteristic	NPM (n = 47)	PM (n = 19)	*P*
$CD4^+$ T cells in PB, median (IQR), %	5.54 (3.80–7.81)	9.17 (6.35–12.50)	**0.001**
$CD4^+$/$CRTh2^+$ T cells in PB, median (IQR), %	0.22 (0.13–0.31)	0.42 (0.22–0.58)	**0.001**
CRTH2 mRNA in WB, median (IQR), %	3.71 (1.79–6.02)*	3.68 (2.09–6.21)[†]	0.941
Eosinophils, median (IQR), cells/μL	200 (100–400)[‡]	200 (100–500)	0.958
Total dose of inhaled corticosteroid, median (IQR), μg/d	586 (293–1000)	1000 (500–1173)	0.077
IL-13, median (IQR), pg/mL	3.12 (3.12–13.5)[§]	19.1 (3.12–40.5)	**0.034**
Patients receiving at least 1 steroid burst during the previous year, no. (%)	15 (31.9)	11 (57.9)	0.059
Patients hospitalized in the previous year, no. (%)	1 (2.1)	3 (15.8)	0.069

IL = interleukin; IQR = interquartile range; NPM = non psychological morbidity; PB = peripheral blood; PM = psychological morbidity; Th = T-helper type-2 cells; WB = whole blood.
n = *39, [†]16, [‡]45, and [§]46 due to missing data in some patients.

cases, to the development of a "fight or flight" response, characterized by the activation of the autonomic nervous system, with the release of neurotransmitters and neuropeptides that can increase bronchial tone and hyper-responsiveness associated with asthma symptomatology.[30] Stress–immune system interactions are complex, but many studies suggest that chronic stress leads to a shift toward Th2-mediated cytokine production and humoral immunity.[31–36] There is also much support for a link between stressful situations and asthma prevalence and morbidity in humans.23 Chronic stress stemming from family situations has been associated with asthma attack.[37] Domestic violence[38]

Table VI. Immunologic and clinical characteristics of men and women with NPM and PM asthma.

Characteristic	Men			Women		
	NPM (n = 24)	PM (n = 5)	*P*	NPM (n = 23)	PM (n = 14)	*P*
$CD4^+$ T cells in PB, median (IQR), %	6.29 (4.29–9.0)	11.1 (5.34–13.32)	0.157	4.44 (3.01–7.25)	8.37 (6.5–13.0)	**0.003**
$CD4^+/CRTh2^+$ T cells in PB, median (IQR), %	0.24 (0.13–0.31)	0.41 (0.21–0.44)	0.119	0.20 (0.13–0.28)	0.48 (0.21–0.60)	**0.004**
CRTH2 mRNA in WB, median (IQR), %	4.39* (2.7–5.9)	1.60† (1.50–2.08)	**0.008**	2.38‡ (1.37–6.36)	4.58§ (3.0–7.17)	0.114
Eosinophils, median (IQR), cells/μL	300‖ (100–700)	0 (0.0–150)	**0.010**	200¶ (100–300)	300 (200–525)	0.058
Total dose of inhaled corticosteroid, median (IQR), μg/d	1000 (500–1000)	293 (0.0–1086)	0.196	500 (137–880)	1068 (586–1198)	**0.002**
IL-13, median (IQR), pg/mL	3.12# (3.12–12.82)	30.6 (17.05–40.95)	**0.014**	3.12 (3.12–13.67)	9.60 (3.12–41.51)	0.252
Patients receiving at least 1 steroid burst during the previous year, no. (%)	8 (33.3)	1 (20.0)	1	7 (30.4)	10 (71.4)	**0.021**
Patients hospitalized in the previous year, no. (%)	0	0	0	1 (4.3)	3 (21.4)	0.142

IL = interleukin; IQR = interquartile range; NPM = non psychological morbidity; PB = peripheral blood; PM = psychological morbidity; Th = T-helper type-2 cells; WB = whole blood.
n = *20, †4, ‡19, §12, ‖23, ¶22, and #23 due to missing data in some patients.

and post-traumatic stress disorder have been shown to influence the risk for asthma.[39] In children with asthma, lower socioeconomic status has been associated with higher levels of IL-5, IL-13, and eosinophils,[40] and young adolescents were reported to have higher IL-5 levels during examination periods than midsemester.[41] Marin et al[42] showed that acute stress in asthmatic children was associated with enhanced Th2-mediated inflammation and asthma symptoms, but only if the children were living with higher levels of chronic family stress. The effects of stress on Th2-mediated inflammation may be generational, since prenatal stress has been found to lead to increased Th2-mediated cytokines in teenaged children of these mothers.[43] Mechanistically, the influence of psychological stress on Th2-mediated inflammation and asthma is not well understood, though studies have shown durable changes in glucocorticoid receptor sensitivity and ensuing inflammation in patients with post-traumatic stress disorder.[44,45] On the other hand, the impact of early-life psychological stress on asthma has also been reported to be associated with a hyporesponsive hypothalamic-pituitary-adrenal axis, independent of glucocorticoid receptor activity.[46] Further research is needed to fully delineate the mechanism(s) underlying stress-related asthma symptoms.

We report here that patients with stress-triggered asthma had more circulating Th2 cells. The majority of Th2 cells within the blood are of central memory phenotype,[47] cells tasked with circulating throughout the body as sentinels ready to deploy to sites of insult and barrier disruption.[48,49] Previously we showed that the proportion of circulating Th2 cells was associated with asthma severity and was a highly sensitive classifier in patients who had experienced exacerbation in the last year.[21] Therefore, patients with stress-triggered asthma may be more susceptible to reacting to triggers and/or primed for higher production of inflammatory mediators. This would be in line with studies both in humans and animal models showing that students with mild asthma submitted to allergen challenge exhibited higher sputum IL-5 and eosinophils when challenged during examination periods compared to midsemester,[50] and that in mouse models of allergic airway inflammation, acute stress enhanced the production IL-4,[51] -6, and -9[33] in the airways. Chronic psychological stress has been associated with increased risk for depression.[52,53] Depression and anxiety are higher in those with uncontrolled compared to controlled asthma,[54] with studies reporting 2- to 8-fold higher prevalences.[55,56] Our observation that asthmatic patients with depression as a comorbidity and those with stress-triggered asthma had a similar profile of higher circulating Th2 cells would support the evidence that overlapping inflammatory pathways are mediating these diseases. For instance, depression scores correlate with asthma-related quality of life,[57] and studies in twins suggest that there may be shared genetics between asthma and depression.[58] Interestingly, chronic stress, depression, and some asthma phenotypes have been associated with elevated levels of the proinflammatory mediators IL-1β, tumor necrosis factor α, IL-6, and interferon γ,[59–66] and antidepressant medications reduce the level of these cytokines.[67–69]

Women are twice as likely to have asthma or severe asthma and to relapse following asthma exacerbation.[70–72] Stress- and depression-related asthma symptoms are also reported to be higher in women than men.[73] We found that women with asthma and psychological morbidity had more circulating Th2 cells, were taking higher daily doses of ICSs, and were also more likely to have needed OCSs in the previous year. While men showed a nonsignificant trend toward having more Th2 cells, they were different in other aspects of Th2-mediated inflammation. Men with psychological morbidity had significantly more serum IL-13, but fewer eosinophils and *CRTH2* mRNA. These data must be considered with caution, as the numbers were quite small, but do suggest that there may be differences in the influence of psychological morbidity on Th2-mediated inflammation between men and women. A murine model of combined social disruption–related stress and allergic sensitization found a reduction in the anti-inflammatory effect of corticosteroids and prolonged airway inflammation,[74] indicating that stress may play a role in corticosteroid response and asthma severity. Our findings show that despite taking more corticosteroids, women with asthma and PM showed no reduction in *CRTH2* mRNA or eosinophils compared to women with NPM. These immune markers, however, were significantly reduced in men with psychological morbidity. Collectively, these findings could suggest that women with psychological morbidity experience less benefit from glucocorticoid therapy than do men. Indeed, a

number of studies have suggested that there may be a sex difference in corticosteroid response (reviewed by Hunninghake and Gold[75]), but have conceded that more study is needed. Nonetheless, our data suggest that asthma with psychological morbidity should be examined in a sex-specific manner.

Historically, Th2-mediated inflammation was considered to arise through adaptive immune

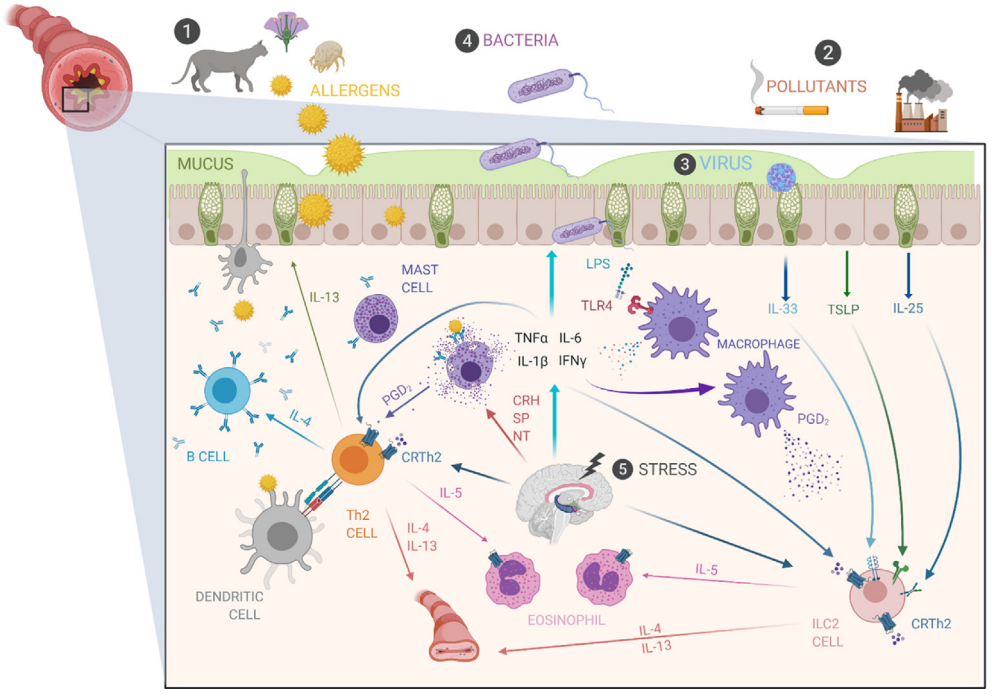

Figure 3. T-helper (Th)-2 inflammation is mediated by multiple environmental exposures. 1. Allergens are picked up by dendritic cells and presented to naïve $CD4^+$ T cells which differentiate into Th2 cells, produce interleukin (IL)-4, IL-5 and IL-13 and mediate immunoglobulin E production from B cells leading to among other effects eosinophilia. Th2 cells express CRTh2 (chemoattractant receptor—homologous molecule expressed on Th2 cells),[6] a receptor for prostaglandin (PG)-D_2,[8] a lipid mediator released from mast cells following allergen-IgE crosslinking.[9] PGD_2 activation of CRTh2 mediates further type 2 cytokine production,[11] as well as chemotaxis[8] and inhibition of apoptosis.[13] 2. Pollutant exposure: cigarette smoke and particulate air pollution increase epithelial production of the alarmins TSLP,[76] IL-33,[76] and IL-25,[77] cytokines that drive group 2 innate lymphoid cells (ILC2) to produce type 2 cytokines.[7,79] 3. Rhinovirus infection also induces epithelial production of IL-25 and IL-33,[79,80] having effects with pollutant exposure similar to those described in step 2.4. Bacterial exposures like LPS induce macrophages to release PGD_2[9] and potentiate the type 2 response by producing the proinflammatory cytokines IL-1β, tumor necrosis factor (TNF)-α, IL-6, and interferon (IFN)-γ.[82–84] IFNγ treated macrophages produce PGD_2[85] and together with TNFα to increase CRTh2 expression and response to PGD_2.[86] 5. Stress may affect various steps of all these pathways. Stress may regulate type 2 inflammation by causing release of neuropeptides (corticotropin-releasing hormone [CRH], neurotensin [NT], and substance P [SP]) which activate mast cells[87] and macrophages[100] to release of TNFα, IL-1β, IL-6, and IFNγ,[59–61] which both enhance CRTh2 expression[86] and increase epithelial permeability.[92] (Artwork created with Biorender.com.) LPS = lipopolysaccharide.

responses involving allergic sensitization and development of Th2 cells; there is now an abundance of data showing that Th2-mediated inflammation also develops in response to environmental exposures activating resident airway cells, such as the epithelium, ILC2, and macrophages.[5,52] For instance, exposure to particles such as cigarette smoke and air pollution has been associated with elevated expression of Thymic stromal lymphopoietin (TSLP),[76] IL-33,[77] and IL-25,[78] cytokines that drive ILC2 production of Th2-mediated cytokines.[7,79] Exposure to microbials such as lipopolysaccharides induces macrophages to release PGD_2,[10] and epithelial cells infected with rhinovirus produce IL-25 and -33.[80,81] Like stress and depression, microbial exposures also induce the expression of proinflammatory cytokines such as IL-1β, tumor necrosis factor α, IL-6, and interferon γ.[82–84] These cytokines enhance macrophage production of PGD_2,[85] CRTh2 expression, and response to PGD_2,[86] and so are considered to potentiate Th2-mediated inflammation. Another important interaction between stress and asthma may relate to the effects of stress on mast cells.[87] Indeed, there is a plethora of data demonstrating how stress activates mast cells through various mechanisms that depend on mast cell localization in specific microenvironments and the prevailing local stress-induced mediators, such as neurotensin,[88,89] corticotropin-releasing hormone, and substance P.[90] Stress also increases permeability of gut[91] and tracheal[92] epithelium. In many cases this effect is dependent on corticotropin-releasing hormone–induced mast cell activation and the release of mediators that affect epithelial permeability.[93–95] In other cases mast cell activation makes the epithelium more sensitive to the effects of substance P released locally by sensory nerves.[92] Stress also decreases the development of tolerogenic dendritic cells and T-regulatory cells, tipping the immune system away from antigenic tolerance and increasing the susceptibility to asthma.[96] Therefore, psychological stress generates immune responses and structural changes within the airways similar to those induced by allergens, pollution, and/or microbes (Fig. 3). The direction of causality, however, is an important question. It may be that inflammatory cytokines themselves drive depression. For instance, recently a large-scale cross-sectional study showed that the risk for depression was 42% higher in patients with rhinitis[97]; others have reported higher levels of Th2-mediated cytokines in patients with major depressive disorder,[98] and environmental factors that drive Th2-mediated inflammation, such as air pollution, were risk factors for depression.[99]

Our study had some limitations, the main one being the relatively small number of individuals with psychological disease. As such, these findings need to be validated in larger-scale prospective studies. We also did not have data for delineating the role of patients' stress burden or degree of depression symptoms during the period of evaluation, and so we do not know whether the differences we describe were the result of acute and/or chronic stress exposure or uncontrolled depression. Since patients with stress-related disease and depression have been reported to be less compliant in taking medication,[101] we cannot rule out the possibility that the differences we observed were related to these patients not taking their medication. This seems less likely, though, as women with asthma and PM were prescribed more ICSs and OCSs, while men were not. Future studies will need to address these issues. Importantly, more work is needed for validating the intriguing possibility that asthma with PM may be a newly identified endotype of Th2-high asthma, possibly more common in women. The correlation between depression scores and asthma-related quality of life[57] leads one to consider whether the treatment of psychological comorbidities would improve inflammatory indices and asthma trajectory. Randomized controlled trials have shown that antidepressants can be effective in treating asthma and that improvements in asthma and depression following antidepressant therapy were significantly correlated.[102–105] Despite these findings, the benefit of treating depression in asthmatic patients has not been fully investigated. Another approach may be to address how people deal with stress directly. For instance, techniques involving mindfulness and focusing on gratitude have been reported to downregulate NF-κB,[106] to reduce levels of inflammatory cytokines,[107] and to be associated with improved asthma control.[108]

CONCLUSIONS

Psychological distress may result in Th2-mediated inflammation in a fashion similar to external triggers,

such as allergens, damaging environmental agents, and/or microbes. A more thorough investigation of psychological morbidity in asthma classification seems warranted, as presumably this endotype would respond to current anti-Th2 therapy and may be more common in women. It is tempting to speculate, however, that treating the psychological morbidity itself could target the mechanism that regulates Th2-mediated inflammation in these patients, reducing the need for further therapy.

FUNDING SUPPORT

This work was supported by Canadian Institutes of Health Research (CIHR) and GSK/CIHR Chair in Airway Inflammation grants (H.V.) and by a CIHR and Western University grant (L.C.).

AUTHOR CONTRIBUTIONS

L.C. contributed study design and data analysis, supervised data collection, and authored and reviewed the final draft of the manuscript. N.S.P. participated in study design, performed flow cytometry experiments and data analysis, and authored for the first draft of the manuscript. C.L. designed the database used for collecting clinical data and participated in clinical data collection. H.V. contributed study design, supervised data collection, and authored the final draft of the manuscript. All of the authors read and approved the final manuscript.

DISCLOSURES

The authors have indicated that they have no conflicts of interest with regard to the content of this article.

ACKNOWLEDGMENTS

The authors thank Jenna Fortunato, Western University (London, Ontario, Canada), for generating the artwork for Fig. 3.

Flow cytometry facility experiments were performed at the University of Alberta, Faculty of Medicine & Dentistry Flow Cytometry Facility, which receives financial support from Faculty of Medicine & Dentistry and Canada Foundation for Innovation awards to contributing investigators.

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