

Letter to the Editor

Evidence for a MAIT-17-high phenotype in children with severe asthma

To the Editor:

Severe asthma (SA) affects 2% to 5% of the children with asthma who suffer from frequent exacerbations, leading to a reduced quality of life.¹ The mechanisms accounting for the severity of the disease are not fully elucidated. A potential implication of IL-17A (hereafter referred to as IL-17) is supported by a consistent increase in the number of IL-17-producing cells in the bronchial biopsies of adult patients with SA, as compared with patients with mild to moderate asthma or control subjects without asthma.² In the same line of evidence, it has been shown that IL-17 receptor A was overexpressed in bronchial mucosa from children with SA.³ Yet, even though CD4⁺ T cells represent the major source of IL-17 (T_H17), their frequency in the bronchial mucosa and bronchoalveolar lavage fluid (BALF) did not differ between patients with asthma and controls,^{3,4} suggesting the contribution of other sources of IL-17.

Mucosal-associated invariant T (MAIT) cells can produce several cytokines including IL-17 and IFN- γ .⁵ We have previously shown that the frequency of IL-17-producing MAIT (MAIT-17) cells in peripheral blood from children with mild asthma positively correlated with asthma symptoms.^{6,7} The aim of this study was to investigate whether circulating or bronchial MAIT-17 cells were associated with the exacerbation of symptoms in children with SA. To this end, we analyzed MAIT-17 cells in peripheral blood and BALF from these children. The percentages of IFN- γ -producing MAIT (MAIT-IFN- γ) cells and T_H17 cells were assessed in parallel. Institutional ethical approval and written informed consent were obtained.

Seventeen children with SA (median age, 9.8 years; range, 7.1-13.2 years) were included. SA was defined according to international guidelines⁸ (see this article's [Methods](#) section in the Online Repository at www.jacionline.org). Their demographic and clinical characteristics are summarized in [Table E1](#) in this article's Online Repository at www.jacionline.org. The proportions of MAIT cells in peripheral blood and BALF were similar (see [Fig E1, A](#), in this article's Online Repository at www.jacionline.org), as were the respective percentages of MAIT-IFN- γ cells (see [Fig E1, B](#)). In contrast, MAIT-17 and T_H17 cells were strikingly more frequent in BALF than in blood ([Fig 1, A and B](#)). Starting from this finding, we addressed the question whether MAIT-17 cells were related to severe exacerbation (SE). To this end, children with SA were divided into 2 groups on the basis of the number of SEs experienced in the past 12 months. Frequent SEs were defined as requiring more than 2 bursts of systemic corticosteroids in the previous year⁹ (see this article's [Methods](#) section in the Online Repository). We found similar percentages of MAIT cells in BALF from either group ([Fig 1, C](#)). However, MAIT-17 cells were more frequent in BALF from children with SA having experienced more than 2 SEs ([Fig 1, D](#)). Accordingly, the number of SEs tended to correlate with the percentage of MAIT-17 cells in the BALF although this was not statistically significant ($P = .074$; $r = 0.446$) (see [Fig E1, C](#)).

Similarly, the proportion of MAIT-17 cells was higher in BALF from children with uncontrolled SA versus those with partially

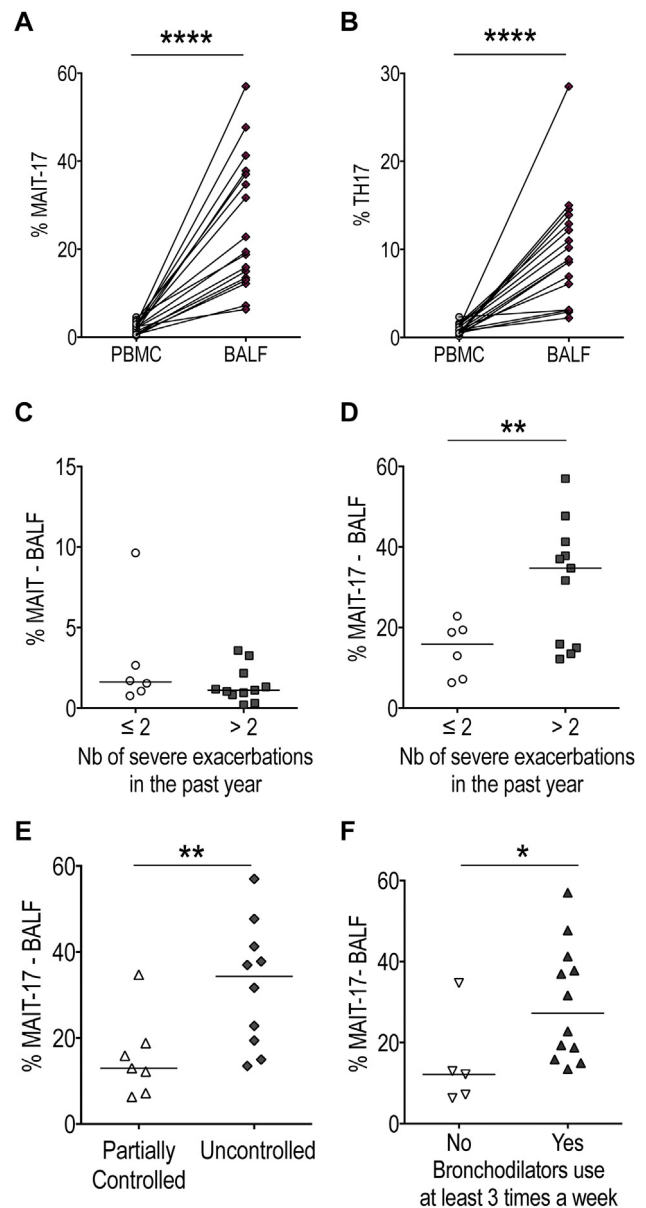


FIG 1. Percentages of IL-17⁺ cells (MAIT-17 cells; **A, D, E, F**) among gated live CD3⁺TCRV α 7.2⁺CD161⁺ MAIT cells or IL-17⁺ cells (T_H17 cells, **B**) among gated live CD3⁺CD4⁺TCRV α 7.2⁺CD161⁺ cells in PBMCs and/or BALF. Percentage of TCRV α 7.2⁺CD161⁺ MAIT cells among gated CD3⁺ cells (**C**) or of MAIT-17 cells (**D, E, F**) from BALF of patients with SA with 2 or fewer (≤ 2) versus more than 2 (> 2) exacerbations in the past year (**C, D**), with partially controlled versus uncontrolled disease (**E**) or using bronchodilators less (No) or more (Yes) than 3 times a week in the past month (**F**). * $P < .05$, ** $P < .01$, Mann-Whitney test (**C, D-F**); **** $P < .0001$, Wilcoxon test (**A and B**).

controlled SA ([Fig 1, E](#)), as well as in children requiring rescue bronchodilators at least 3 times a week in the previous month ([Fig 1, F](#)). In contrast, the frequency of MAIT-IFN- γ and T_H17 cells in BALF was similar, regardless of asthma symptoms, bronchodilator use, or lung function (see [Fig E2, A-F](#), in this article's Online Repository at www.jacionline.org).

TABLE I. Demographic and clinical characteristics of children with SA belonging to the MAIT-17–low group or the MAIT-17–high group

Characteristic	Group		P value
	MAIT-17–low	MAIT-17–high	
Study participants, n	10	7	
Age (y)	10.8 (7-14.6)	9.8 (7.3-11.3)	.65
Sex: female/male	4/10	2/7	>.99
BMI (kg/m ²)	19.3 (15.2-25.9)	16.3 (15.3-20.6)	.63
Post-BD FEV ₁ (%)	101 (42-116)	113 (101-116.3)	.67
Post-BD FEV ₁ /FVC (%)	86 (36.5-93.5)	87.5 (79.5-91.2)	.75
SEs in the past year, n	2 (1-6)	7 (5-12)	.03
Uncontrolled asthma	4/10	6/7	.13
Partially controlled	6/10	1/7	.13
Allergic rhinitis	8/10	5/7	>.99
Atopic dermatitis	7/10	0/7	.01
ICS (μg/d equivalent fluticasone)	625 (500-1000)	500 (500-500)	.07
Bronchodilator use at least 3 times a week in the past month	6/10	6/7	.31
Circulating total IgE levels (ku/L)	1054 (537-1432)	801 (229-1016)	.13
Blood leukocytes (× 10 ⁻³ /μL)	6.6 (4.6-8.2)	6.8 (5.8-7.4)	.98
Blood eosinophils (× 10 ⁻³ /μL)	0.4 (0.3-0.8)	0.6 (0.3-1.2)	.33
Blood neutrophils (× 10 ⁻³ /μL)	2.9 (1.9-4.1)	2.6 (1.9-4.1)	.39
Blood lymphocytes (× 10 ⁻³ /μL)	2.2 (1.6-2.9)	2.6 (2.2-2.9)	.48
BALF leukocytes (× 10 ⁻² /μL)	1.5 (0.9-1.9)	1.6 (1.0-1.4)	.34
BALF eosinophils (× 10 ⁻² /μL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.99
BALF neutrophils (× 10 ⁻² /μL)	0.1 (0.1-0.1)	0.1 (0.0-0.3)	.92
BALF lymphocytes (× 10 ⁻² /μL)	0.1 (0.0-0.1)	0.1 (0.0-0.1)	.79
Positive BALF bacteriology, n	3/10	1/7	.60
Positive BALF virology, n	2/10	1/7	>.99

Data are presented as the median (interquartile range). Boldface values are statistically significant ($P < .05$).

BMI, Body mass index; BD, bronchodilator; FVC, forced vital capacity; ICS, inhaled corticosteroid.

It is generally acknowledged that, in physiological conditions, MAIT-IFN- γ -cell subset is more frequent than its MAIT-17–cell counterpart.⁵ This was effectively the case in peripheral blood but not in BALF from children with SA in which the MAIT-17/MAIT-IFN- γ cell ratios were elevated in BALF relative to blood (see Fig E3, A, in this article's Online Repository at www.jacionline.org). A more detailed analysis of MAIT-17 cells in BALF highlighted the presence of 2 groups of children with SA according to the percentage of MAIT-17 cells: MAIT-17–low and MAIT-17–high. These groups had respectively less or more than 25.4% (mean value) of MAIT-17 cells (see Fig E3, B). These 2 groups presented no major differences in terms of demographic features, such as age, sex, or body mass index (Table I). Children in the MAIT-17–high group experienced more SEs in the past year (Table I). No significant difference was observed in terms of allergic rhinitis in both groups (Table I). In contrast, 70% children of the MAIT-17–low group presented atopic dermatitis versus 0% of the MAIT-17–high group (Table I).

Previous studies reported that the frequency of MAIT in the bronchial mucosa was reduced in adults with asthma as compared with healthy controls and that the frequency of circulating MAIT cells could be decreased in these patients by a 1-week treatment with oral prednisolone.⁴ Here, the doses of inhaled corticosteroids in children of the MAIT-17–high group tended to be lower than those used in their MAIT-17–low counterpart, although this difference was not statistically significant (Table I). This observation indicates that inhaled corticosteroids may decrease the frequency of BALF MAIT-17 cells. However, no significant correlation was observed between the frequency of BALF MAIT-17 cells and inhaled corticosteroid use (see Fig E3, C). Because MAIT cells are thought to be involved in host defense against pathogens,

we further analyzed bacterial and viral loads in BALF and found no significant differences between the groups of MAIT-17–low and MAIT-17–high cells regarding the presence of these pathogens (Table I).

Our study has several limitations. For ethical reasons, we could not include BALF from children without SA or healthy children. Consequently, we cannot assert that the observed differences in BALF are restricted to SA disease. Furthermore, because of the small size of our cohort, results need to be confirmed in larger series.

Overall, this study (a) provides the first evidence for the presence of MAIT-17 cells in BALF from children with SA, (b) shows that MAIT-17 cells are overrepresented in BALF relative to peripheral blood, and (c) suggests that children with SA can be divided into MAIT-17–low and MAIT-17–high phenotypes. Children in the MAIT-17–high group presented more SE than did children in the MAIT-17–low group. We suggest that the analysis of BALF MAIT-17 cells should be taken into consideration for a better characterization of new phenotypes of children with SA in future studies. Even though our study remains descriptive, the potential implication of bronchial MAIT-17 cells in asthma exacerbations in children with SA deserves further notice for a better understanding of the mechanisms underlying this process.

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REFERENCES

1. Fleming L, Murray C, Bansal AT, Hashimoto S, Bisgaard H, Bush A, et al. The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts. *Eur Respir J* 2015;46:1322-33.
2. Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemiere C, et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009;123:1185-7.
3. Andersson CK, Adams A, Nagakumar P, Bossley C, Gupta A, De Vries D, et al. Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. *J Allergy Clin Immunol* 2017;139:1819-29.e11.
4. 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;136:323-33.
5. Le Bourhis L, Guerri L, Dusseaux M, Martin E, Soudais C, Lantz O. Mucosal-associated invariant T cells: unconventional development and function. *Trends Immunol* 2011;32:212-8.
6. Lezmi G, Abou Taam R, Dietrich C, Chatenoud L, de Blic J, Leite-de-Moraes M. Circulating IL-17-producing mucosal-associated invariant T cells (MAIT) are associated with symptoms in children with asthma. *Clin Immunol* 2018;188:7-11.
7. Lezmi G, Leite-de-Moraes M. Invariant natural killer T and mucosal-associated invariant T cells in asthmatic patients. *Front Immunol* 2018;9:1766.
8. 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;43:343-73.
9. Lezmi G, Galmiche-Rolland L, Rioux S, Jaubert F, Tillie-Leblond I, Scheinmann P, et al. Mast cells are associated with exacerbations and eosinophilia in children with severe asthma. *Eur Respir J* 2016;48:1320-8.

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METHODS

Subjects

Children aged 6 to 18 years with difficult-to-treat asthma were referred to Necker Hospital. Those with persistent symptoms and/or airway limitation under high doses of inhaled corticosteroids, despite good adherence and inhalation technique, underwent further investigations. Investigations included a chest computed tomographic angioscan, a 24-hour pH study, echocardiography, sweat test, and allergy testing for current inhalant and food allergens. Flexible bronchial endoscopy with bronchoalveolar lavage and endobronchial biopsy were performed to assess airway inflammation and remodeling and exclude differential diagnosis. Adherence was assessed; underlying modifiable factors including gastroesophageal reflux, rhinitis, sinusitis, and nasal polyps were treated; and environmental factors (allergens, smoke exposure) were controlled before the diagnosis of SA was made. Children with SA were defined according to international guidelines, as those with persistent asthma symptoms and/or persistent airflow limitation, despite being prescribed high-dose inhaled corticosteroid and another controller medication.^{E1} SEs were defined as exacerbations requiring bursts of systemic corticosteroids (≥ 3 days).^{E1,E2} Frequent SEs are usually defined as requiring 2 or more bursts of systemic corticosteroids in the previous year.^{E2-E5} However, in our population, 14 children had 2 or more SEs in the past year and only 3 children had fewer than 2 SEs. To avoid describing unbalanced groups, we defined frequent exacerbators as having more than 2 SEs in the past year.

Asthma control was assessed according to the Global Initiative for Asthma guidelines.^{E3} Children with uncontrolled asthma were compared with those having a partially controlled asthma.

Nonadherence to treatment and poor inhaler technique were assessed in each patient during the evaluation in our department. Patients were not included if their adherence or technique was considered poor by medical doctors, on the basis of both their history and how parents and children used the inhaler. The diagnosis of SA was made after adherence and inhaler technique were assessed. All the children included in this study were considered adherent to the treatment.

Children (6-18 years) with SA were included after these investigations in the CLASSE (Cellules Lymphoïdes Innées dans l'ASThme Sévère de l'Enfant) cohort. This cohort was designed to assess innate immunity in children with SA.

Flow cytometry

Blood and BALF were obtained from each patient on the same day. Freshly isolated PBMCs were obtained by using Ficoll-Paque density centrifugation (1.077 g/mL; PAA Laboratories GmbH, Velizy Villacoublay, France). BALF was immediately filtered through MACS SmartStrainers (100 μ m) (Miltenyi Biotec, Paris, France) to remove mucus. BALF was centrifuged to separate cells from fluids.

Blood and BALF cells were then washed in fluorescence-activated cell sorting buffer (2% w/v BSA, 0.1% NaN₃ sodium azide in PBS) and then stained with eFluor 506–fixable viability dye (ThermoFisher Scientific, Illkirch, France) for 10 minutes at 4°C. Next, cells were stained with

fluorochrome-conjugated antibodies for 30 minutes at 4°C. Dead cells were excluded when positive for the eFluor 506–fixable viability dye and doublets were excluded using pulse-width and pulse-area and side-scatter properties. MAIT cells (CD3⁺TCRV α 7.2⁺CD161⁺) were identified by the following mAbs in combination with anti-CD3, anti-CD4, and anti-CD8 mAbs (BD Biosciences, Le Pont de Claix, France): fluorescein isothiocyanate-conjugated anti-V α 7.2 (clone 3C10, Biolegend, Paris, France) and allophycocyanin-conjugated anti-CD161 mAb (clone 3G10, eBioscience, Illkirch, France).

For intracellular cytokine analysis, freshly isolated PBMCs and BALF cells were cultured for 6 hours with 25 ng/mL phorbol 12-myristate 13-acetate and 10⁻⁶ M ionomycin, in the presence of 10 ng/mL Brefeldin A (all from Sigma-Aldrich, Saint-Quentin Fallavier, France), as previously described.^{E4,E5} IL-17A and IFN- γ -producing T cells were identified by intracellular staining for 45 minutes with anti-IL-17A-PerCP-Cy5.5 (clone eBio64DEC17, eBioscience) and anti-IFN- γ -PE-Cy7 (clone 4SB3). The frequency of MAIT-17 and of MAIT-IFN- γ cells was expressed as the percentage of IL-17⁺ and IFN- γ ⁺ cells among gated MAIT cells. Similarly, the percentage of T_H17 cells was defined as the frequency of IL-17–producing cells gated among the CD3⁺iNKT[−]MAIT[−]CD4⁺ subset (Fig E4). Cells were identified by flow cytometry using a LSRFortessa (BD Biosciences) and analyzed with FlowJo 10.0.8r1 software (FlowJo, Ashland, Ore).

Statistical analysis

Data are expressed as the median (interquartile range). Differences between groups were assessed with the Mann-Whitney or Wilcoxon test. Correlation analyses were made using Spearman rank correlation analysis. *P* value of less than .05 was considered to be significant. Data were analyzed using GraphPad Prism version 5.03 (GraphPad Software, Inc, San Diego, Calif).

REFERENCES

- E1. 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;43:343-73.
- E2. Bush A, Saglani S. Management of severe asthma in children. *Lancet* 2010;376:814-25.
- E3. Global Strategy for Asthma Management and Prevention. 2016 GINA report. Available at: <https://ginasthma.org/archived-reports/>. Accessed August 30, 2019.
- E4. Bel EH, Sousa A, Fleming L, Bush A, Chung KF, Versnel J, et al. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax* 2011;66:910-7.
- E5. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. *Lancet* 2018;391:350-400.
- E6. Lezmi G, Abou Taam R, Dietrich C, Chatenoud L, de Blic J, Leite-de-Moraes M. Circulating IL-17-producing mucosal-associated invariant T cells (MAIT) are associated with symptoms in children with asthma. *Clin Immunol* 2018;188:7-11.
- E7. Allali S, Dietrich C, Machavoine F, Rignault-Bricard R, Brousse V, de Montalembert M, et al. Innate-like T cells in children with sickle cell disease. *PLoS One* 2019;14:e0219047.

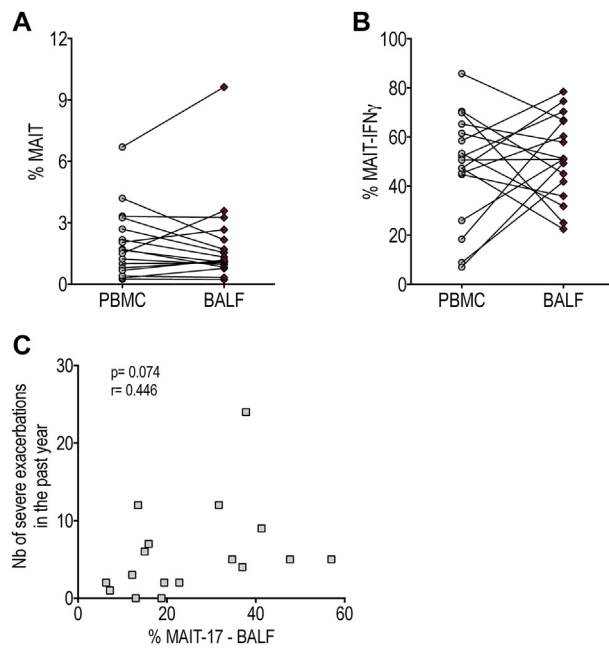


FIG E1. Percentages of TCRV α 7.2⁺CD161⁺ MAIT cells among gated live CD3⁺ cells (**A**) and percentages of IFN- γ ⁺ cells (MAIT-IFN- γ ; **B**) among gated MAIT cells in PBMCs and BALF. **C**, Correlation between the percentage of MAIT-17 cells in BALF and the number of SEs in the past year.

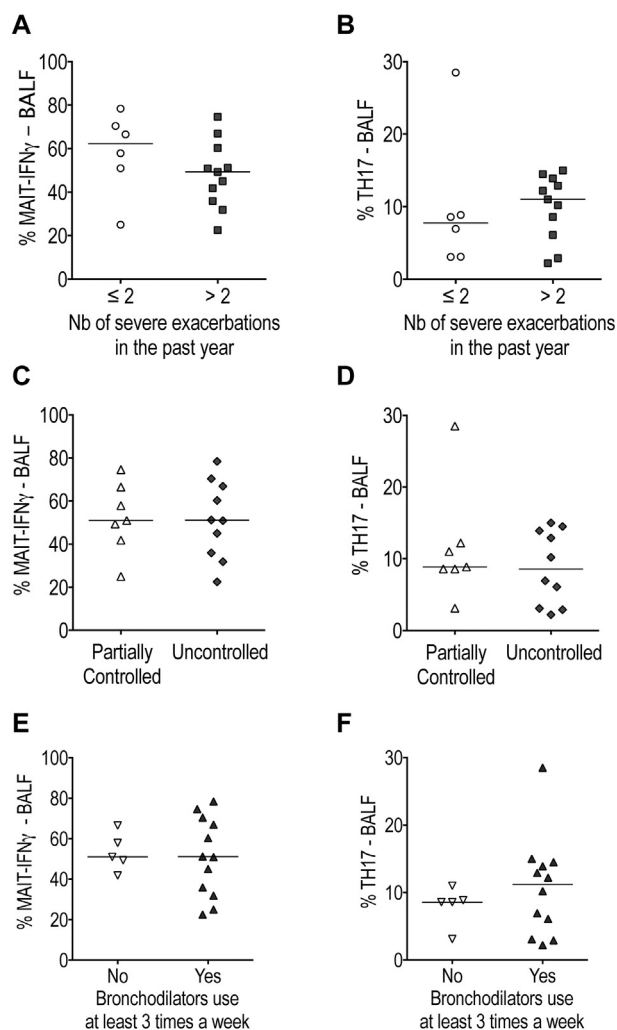


FIG E2. Percentages of MAIT-IFN- γ cells (**A, C, E**) among gated MAIT cells or IL-17 $^{+}$ cells (TH17 cells, **B, D, F**) among gated CD3 $^{+}$ CD4 $^{+}$ TCRV α 7.2 $^{-}$ CD161 $^{-}$ live cells in BALF from patients with SA with 2 or fewer (≤ 2) vs more than 2 (> 2) SEs in the past year (Fig E2, A and B), with partially controlled vs uncontrolled disease (Fig E2, C and D) or using bronchodilators less (no) or more (yes) than 3 times a week (Fig E2, E and F).

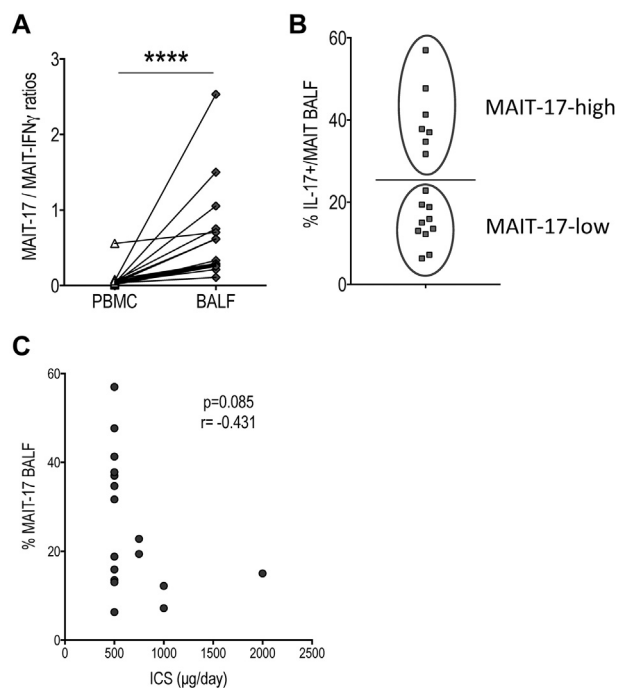


FIG E3. A, MAIT-17/MAIT-IFN- γ cell ratios in blood versus BALF of patients with SA. **** $P < .0001$, Wilcoxon test. **B,** Patients with SA were divided into 2 groups (MAIT-17-high and MAIT-17-low) according to their percentage of MAIT-17 cells in BALF. The horizontal line represents the mean. **C,** Correlation between the percentage of MAIT-17 cells in BALF and inhaled corticosteroid use.

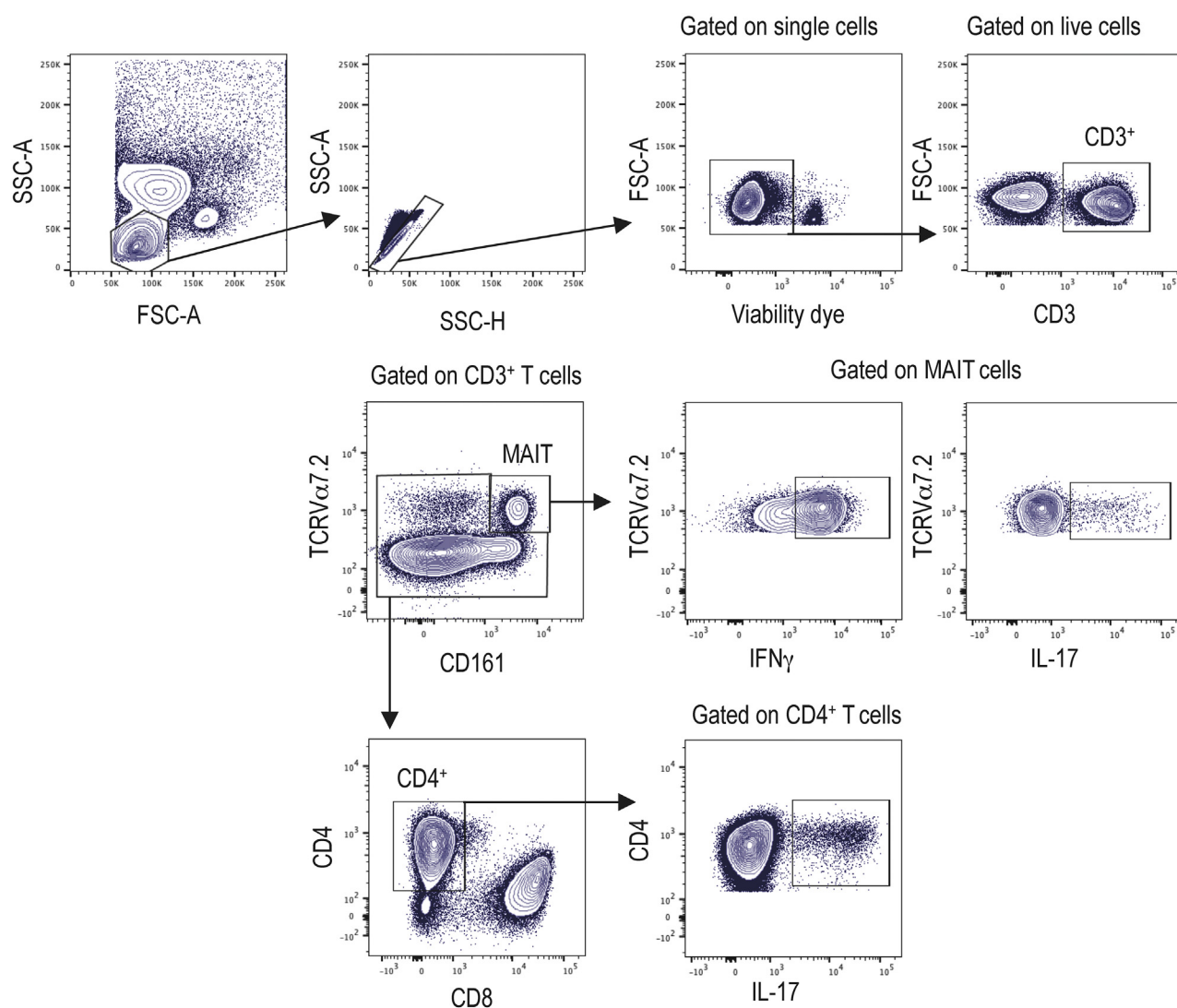


FIG E4. Flow cytometric gating strategy used to identify MAIT (CD3⁺ CD161⁺ TCRV α 7.2⁺) and CD4⁺ (CD3⁺ CD161⁻ TCRV α 7.2⁻ CD8⁻ CD4⁺) cells and their ability to produce IL-17 and IFN- γ . FSC-A, Forward scatter-area; SSC-A, side scatter-area; SSC-H, side scatter-height.

TABLE E1. General characteristics of the children with SA

Characteristic	Value
N	17
Age (y)	9.8 (7.1-13.2)
Sex: female/male	7/10
Body mass index (kg/m ²)	17.1 (15.4-25.6)
Regular ICS, n	17/17
ICS (μg/d equivalent fluticasone)	500 (500-750)
Total IgE (Ku/L)	801 (222-1054)
Blood eosinophil counts ($\times 10^{-3}/\mu\text{L}$)	0.5 (0.3-1.07)
Atopic dermatitis, n	7/17
IgE-mediated food allergy, n	5/17
Allergic rhinitis, n	13/17
SEs past 12 mo, n	5 (2-8)
ACT score	17 (12-22)
Post-BD FEV ₁ , %	113 (93-116)
Post-BD FEV ₁ /FVC, %	87 (73-92)

Data are presented as the median (interquartile range).

ACT, Asthma Control Test; BD, bronchodilator; FVC, forced vital capacity; ICS, inhaled corticosteroid.