5. Casas L, Sunyer J, Tischer C, et al. Early-life house dust mite allergens, childhood mite sensitization, and respiratory outcomes. *Allergy*. 2015;70:820-827.
6. Kantor DB, Stenquist N, McDonald MC, et al. Rhinovirus and serum IgE are associated with acute asthma exacerbation severity in children. *J Allergy Clin Immunol*. 2016;138(5):1467-1471.e9.
7. Xepapadaki P, Papadopoulos NG. Childhood asthma and infection: virus-induced exacerbations as determinants and modifiers. *Eur Respir J*. 2010;36:438-445.
8. Andreakos E, Papadopoulos NG. IL-25: the the missing link between allergy, viral infection, and asthma? *Sci Transl Med*. 2014;6:256fs38.

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Distinct patterns of CD4 T-cell phenotypes in children with severe therapy-resistant asthma

Dear Editor,

Severe therapy-resistant asthma (STRA) is characterized by frequent symptoms, asthma exacerbations, and hospitalizations, despite the use of continuous high-dose corticosteroid therapy and other controller medications. Inflammation of the airways and symptoms varies widely in children with STRA and most patients present only with mild lung function abnormalities. Low Th2 cell response,[1] low IL-10 production, and increased IL-33 have been reported in this group of patients.[2,3] Intraepithelial airway neutrophilia and IL17R expression have been associated with a better outcome of STRA in children. More diverse T-cell subsets with different functions in comparison to the so far established subsets (Th1, Th2, Th17, and Treg) have been described to play a role in the asthma immunopathology. CD4 T cell plasticity is defined as the ability of a single cell to undertake characteristics of many T-cell subsets simultaneously and can occur by co-expressing the namely lineage-specific transcriptional factors (T-bet, GATA-3, RORγt, and FoxP3). The role of CD4 T cells plasticity in children with STRA remains unclear. The aim of our study was to analyze the expression and co-expression of transcriptional factors (T-bet, GATA-3, RORγt, and FoxP3) in CD4 T cells from peripheral blood of children with STRA, compared to children with non-severe asthma.

In this cross-sectional study, we have selected children aged 8-14 years from Southern Brazil. Non-severe asthmatics (n = 118) and healthy children (n = 36) were recruited from public schools. Children with non-severe asthma were initially selected by ISAAC-based short questionnaire, with a posterior medical clinical confirmation. The diagnosis of mild and moderate asthma (non-severe) was then defined according to the Global Initiative for Asthma criteria.[4] Symptoms of allergic rhinitis, atopic dermatitis, and asthma were collected, and the healthy children recruited should not have any history of allergic symptoms or a medical diagnosis of any atopic disease. Children with STRA (n = 11) were selected from a tertiary hospital-based asthma clinic. The criteria for the diagnosis of STRA were as follows: uncontrolled disease despite treatment with high doses of inhaled corticosteroids, plus long-acting beta-agonists, and optional leukotriene receptor antagonist. Uncontrolled disease was characterized by: (a) persistent symptoms (>3 months); (b) acute asthma exacerbations—with either an ICU admission, two or more hospitalizations, or at least two courses of oral steroids over the last 12 months; (c) persistent airflow obstruction following steroid trial; or (d) the need for alternate-day or daily oral steroid to achieve control. All children had been followed in the asthma clinic for at least 6 months and underwent a detailed clinical protocol excluding other chronic lung diseases, optimizing adherence and inhaler technique, and treating or minimizing underlying factors (eg, allergic rhinitis, passive smoking, and poor allergen environmental control). Patients also underwent a protocol trial of systemic corticosteroid treatment (14 days of oral steroids with evaluation of control of disease and lung function) in order to assess corticosteroid insensitivity. Eventually, subjects with no response to the oral steroid trial were classified as STRA. Children with chronic diseases other than asthma, such as neurological disorders, congenital cardiac diseases or immunodeficiency were excluded. Ethical approval and parents written consent were obtained from Human Ethics Committee of PUCRS (CAEE #10/04978 and CONEP #16.083).

Blood samples and lung function test were collected when children were clinically stable and free from respiratory viral

Abbreviations: ERS/ATS,, European Respiratory Society and American Thoracic Society; FEF_{25-75},, forced expiratory flow at 25–75% of forced vital capacity (FVC); FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukins; ISAAC, International Study of Asthma and Allergies in Childhood; LABA, long-acting beta-2 agonist; PBMC, peripheral blood mononuclear cells; STRA, severe therapy-resistant asthma.

Araújo and Souza contributed equally to this paper.

infections in the previous 3 weeks. Peripheral blood mononuclear cells (PBMCs) were isolated for in vitro culture and analyzed using flow cytometry. Spirometry measurements were performed (Koko equipment; Ferraris Respiratory, Louisville, CO) according to the ERS/ATS criteria,[5] and the FEV_1, FEV_1/FVC ratio, FVC and $FEF_{25\%-75\%}$, pre- and post-bronchodilator (400 μg of salbutamol) were analyzed. Atopy was defined by specific IgE (ImmunoCAP; Phadia AB, Uppsala, Sweden) for *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, *Periplaneta americana*, dog and cat dander, mold, pollen, dust, and grass. Children (healthy control and non-severe) with a detection level of at least 0.35 kU/L for any of the tested allergens were considered atopic. In children with STRA, atopy was defined by skin prick tests with the same allergen panel used in the ImmunoCAP tests, and considered positive when the weal diameter was >3 mm.

Clinical characteristics of the subjects included are shown in Table 1. Age, gender, atopic status, passive smoking, and CD4 T cells were not significantly different between healthy controls, non-severe asthma, and STRA children. When analyzing lung function, we found a significant difference in forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25\%-75\%}$ *z*-score) between the groups. In addition, there were significant differences patients with STRA used more inhaled steroids and presented a higher number of exacerbations per year compared to non-severe asthmatics (Table 1).

Expression of the transcription factors (GATA-3, RORγt, T-bet, and FoxP3) were evaluated using flow cytometry in CD4 T cells from PBMCs of healthy control children (n = 35), children with non-severe asthma (n = 118) and with STRA (n = 11), and the gate analysis is demonstrated in Figure 1. We identified that the STRA group presented a lower frequency of $CD4^+GATA\text{-}3^+$ cells ($P < 0.0001$) compared to the non-severe group (Figure 2A), demonstrating that children with STRA presented a reduction in these cells in peripheral blood. This reduction is not associated with the dose of inhaled corticoid dose used by STRA (Figure S1). The percentages of $CD4^+GATA3^+$ cells are positively correlated with FEF_{25-75} *z*-score ($r = 0.785$, $P = 0.020$) in STRA patients, indicating that lower expression of $GATA3^+$ on CD4 T cells may be associated with decreased expiratory airflow (Figure 2K). The central role of GATA-3 in the regulation of Th2 response is well described,

TABLE 1 Clinical characteristics of asthmatic children and healthy controls

	Healthy controls (n = 35)	Non-severe asthma (n = 118)	STRA (n = 11)	*P* value
Age (y), mean ± SD	11.22 ± 1.17	11.19 ± 1.09	10.10 ± 3.13	0.730
Gender, male (%)	14 (40.0)	63 (53.3)	4 (36.4)	0.255
Atopy, n (%)	22 (62.9)	89 (75.4)	9 (81)	0.218
Passive smoking, n (%)[a]	11 (31.4)	53 (41.08)	5 (45.5)	0.264
CD4 T cells, mean percentage ± SD	27.31 ± 10.53	29.75 ± 11.15	27.96 ± 9.95	0.304
Lung function				
FEV_1 *z*-score, mean ± SD	0.12 ± 0.89	−0.04 ± 1.02	−0.85 ± 1.79	0.065
FVC *z*-score, mean ± SD	0.01 ± 0.94	0.28 ± 1.10	−0.17 ± 1.48	0.281
FEF_{25-75} *z*-score, mean ± SD	0.26 ± 0.80	−0.47 ± 1.00	−0.32 ± 1.13	0.001
Medications, n (%)				
Inhaled steroids[b]	—	61 (52.1)	11 (100)	0.001
LABA	—	92 (73.64)	11 (100)	0.075
Oral steroids[c]	—	—	4 (36.4)	0.171
Omalizumab	—	—	6 (54.5)	—
Exacerbations per year, n (%)				
0	35 (100)	15 (12.7)	0 (0)	0.001
1-3	0 (0)	62 (52.5)	0 (0)	
3-12	0 (0)	34 (28.8)	1 (12.5)	
>12	0 (0)	7 (6)	8 (87.5)	

FEV_1, forced expiratory volume in 1 second; FEF_{25-75}, forced expiratory flow between 25% and 75% of vital capacity; FVC, forced vital capacity; LABA: long-acting beta-2 agonist; STRA: severe therapy-resistant asthma.

[a]Passive smoking was assessed by parent report.

[b]Range of inhaled corticosteroid dose for STRA between 400 and 1000 mg/d.

[c]Range of oral steroids dose between 5 and 10 mg in alternative days

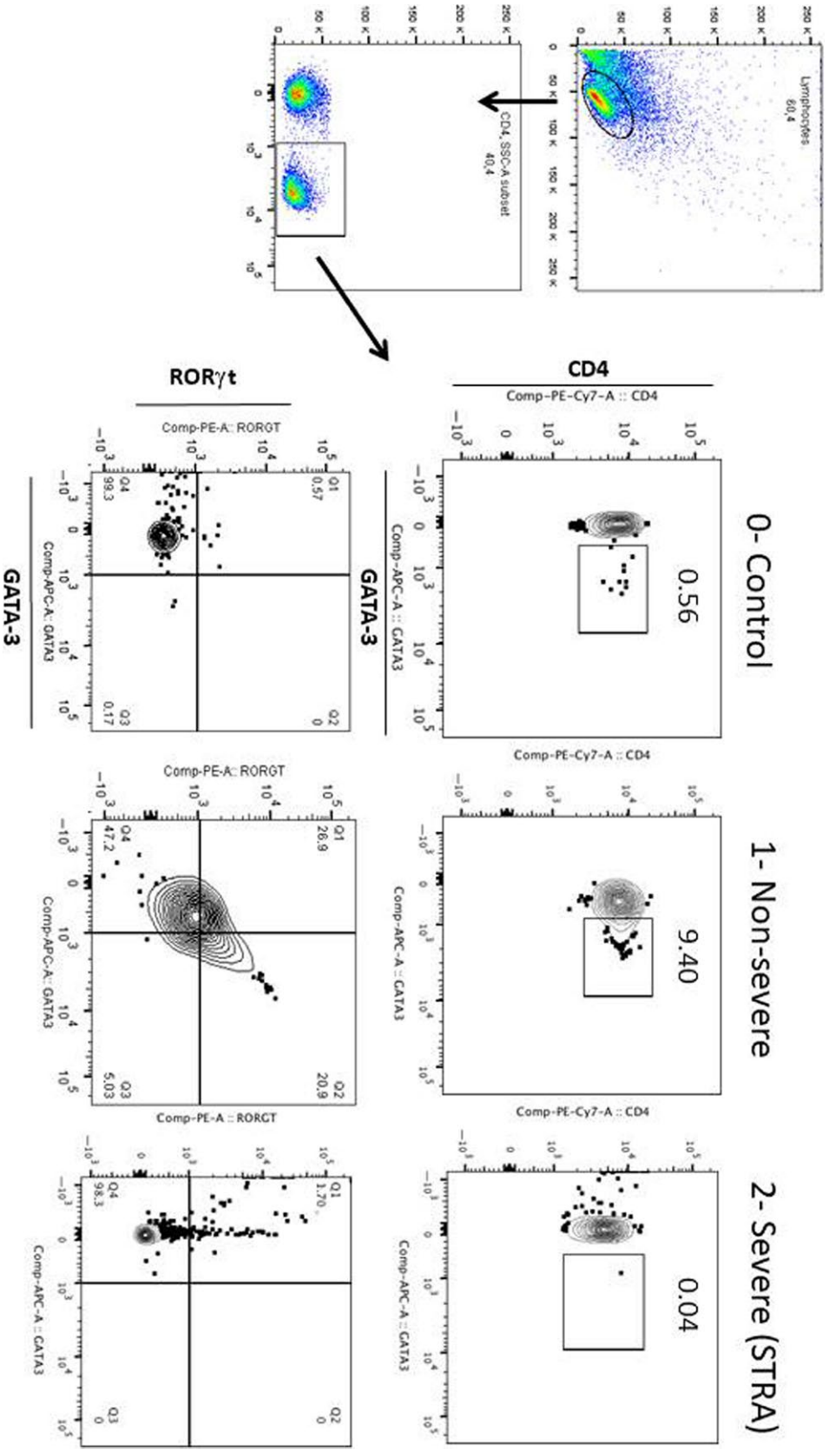

FIGURE 1 Representative plots of gate strategy for analysis of $CD4^+GATA\text{-}3^+$ and $CD4^+GATA\text{-}3^+ROR\gamma t^+$ cells from a sample of a healthy control child, a child with non-severe asthma and a child with STRA [Colour figure can be viewed at wileyonlinelibrary.com]

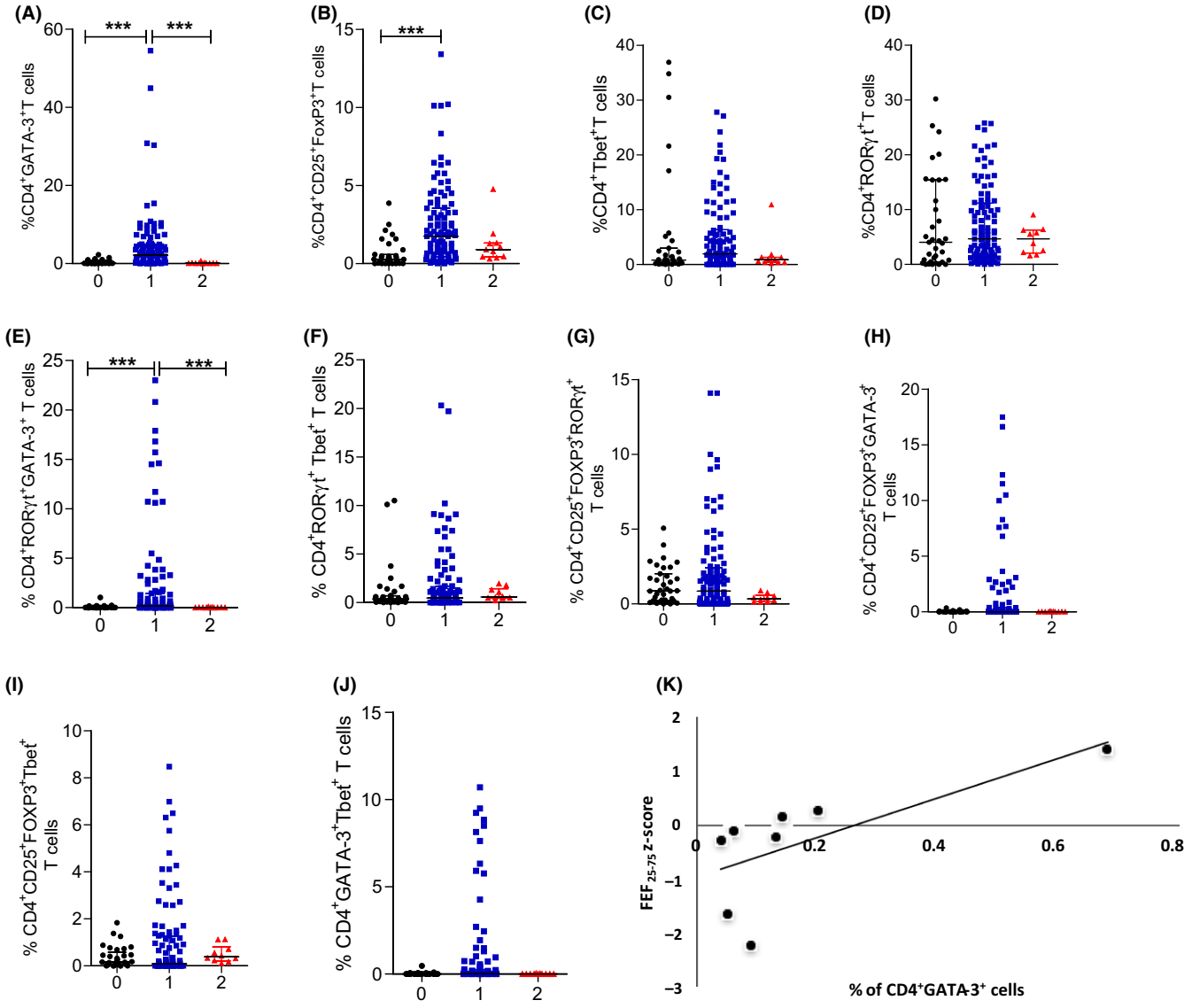

FIGURE 2 Children with STRA present a lower percentage of $CD4^+GATA\text{-}3^+$ cells. PBMC were collected from 35 healthy controls children (0), 118 children with non-severe asthma (1) and 11 children with STRA (2). The expression of master regulator transcriptional factors (MRTF), GATA-3, T-bet, RORγt, and FoxP3, was analyzed in CD4 T cells by flow cytometry. Graphs show the median percentage and the interquartile range of (A) $CD4^+GATA\text{-}3^+$ ($P < 0.0001$***); B, $CD4^+CD25^+FoxP3^+$ ($P < 0.0001$***); C, $CD4^+T\text{-}bet^+$; D, $CD4^+ROR\gamma t^+$; E, $CD4^+GATA\text{-}3^+ROR\gamma t^+$ ($P < 0.0001$***); F, $CD4^+ROR\gamma t^+T\text{-}bet^+$cells; G, $CD4^+CD25^+FoxP3^+ROR\gamma t^+$; H, $CD4^+CD25^+FoxP3^+GATA\text{-}3^+$; I, $CD4^+CD25^+FoxP3^+T\text{-}bet^+$; J, $CD4^+GATA\text{-}3^+T\text{-}bet^+$cells. Kruskal-Wallis test was used followed by Dunn post-test for comparing the groups. K, Correlation between $FEF_{25\%\text{-}75\%}$ and the frequency of $CD4^+GATA3^+$ cells in STRA children (Spearman) [Colour figure can be viewed at wileyonlinelibrary.com]

although we did not measure the cytokine production of the GATA3 positive cells in our study. One previous study already showed a reduction in Th2 cytokine in children with STRA, with lower $IL\text{-}5^+$ cell counts in bronchial biopsies, when compared to healthy controls.[1] Those data confirmed the heterogeneity of STRA in children, considering that Th2 immune response has been described as one of the major severity risk factors for asthma. In addition, GATA-3 has been proposed as a therapeutic target to asthma,[6] however our results suggest that this target could not be effective in children with STRA.

In addition, we found that children with non-severe asthma showed an increased percentage of $CD4^+CD25^+FoxP3^+$ cells compared to healthy controls (Figure 2B). This is in contrast with other studies, which showed lower percentage of Treg cells in children with asthma.[7,8] However, in line with our results, a few studies observed an increased frequency of $CD4^+FoxP3^+$ cells in patients with asthma compared to controls.[9,10]

Given the fact that the co-expression of key lineage-specifying transcription factor affects the functional capabilities and flexibility of CD4 T cells, we further evaluated the cells that double co-express RORγt, GATA-3, T-bet, and FoxP3. Intriguingly, children with STRA presented a significant reduction in $CD4^+GATA3^+ROR\gamma t^+$ cells compared to non-severe asthma (Figure 2E). Cells that co-express the

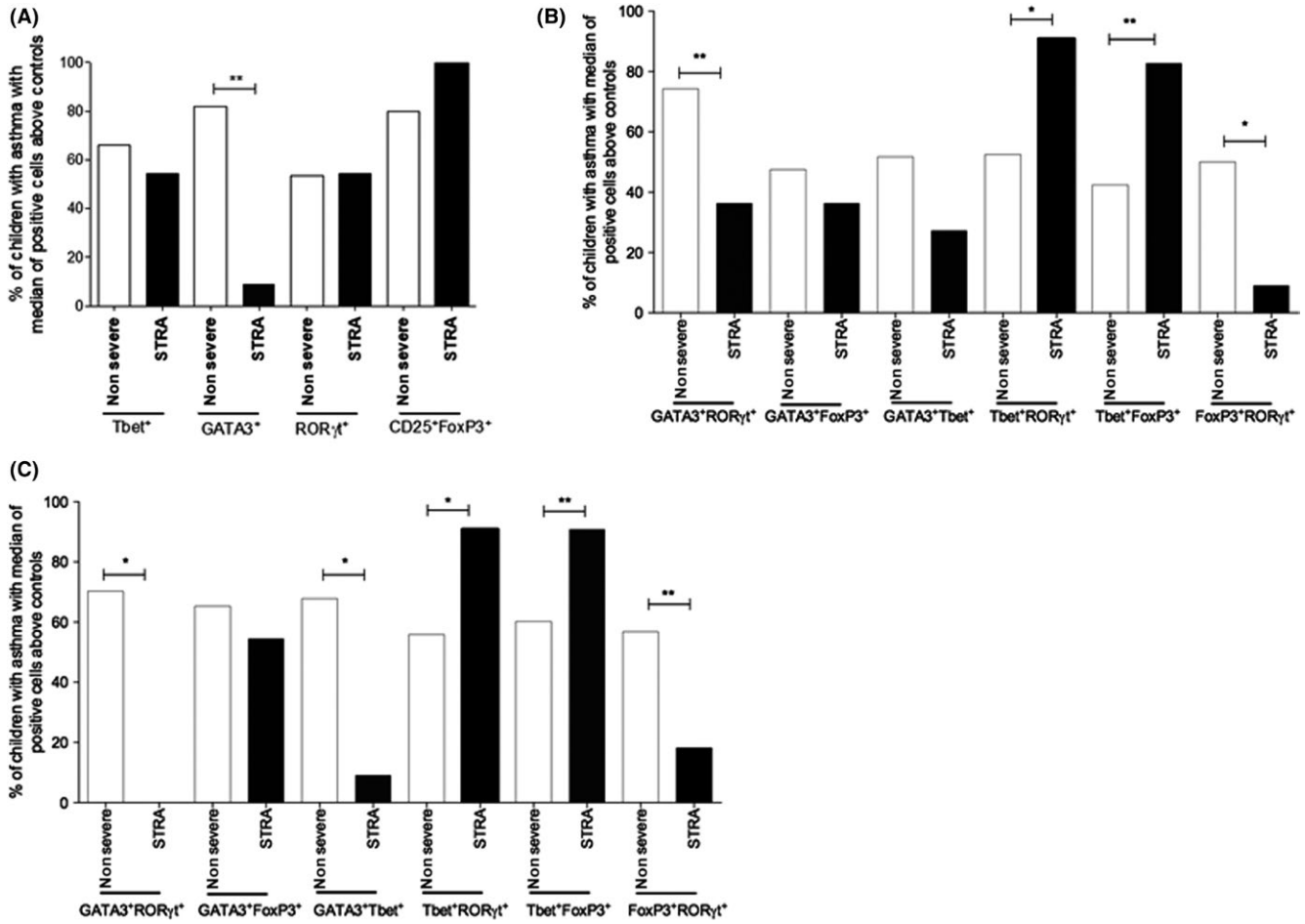

FIGURE 3 Children with STRA present a distinct expression of major transcription factor comparing to non-severe asthma. PBMC were collected from 35 healthy children, 118 children with non-severe asthma and 11 children with STRA. The expression of lineage specific transcriptional factors (GATA-3, T-bet, RORγt and FoxP3) was analyzed in CD4 T cells by flow cytometry. To compare non-severe and STRA children, data from the transcriptional factor analysis were categorized in above or below the median of healthy controls. A, Graph shows the percentage of children with asthma with median of the cells above healthy controls (Qui-square test was used for comparing the groups). Graph shows the percentage of children with median of (B) un-stimulated cells, and (C) stimulated cells with anti-CD3 and anti-CD28 during 24 hours above healthy controls (Qui-square test was used for comparing the groups)

transcription factors GATA3 and RORγt and co-produce Th17 and Th2 cytokines promote exacerbation of chronic allergic asthma in a murine model.[11] In contrast to our results, cells expressing GATA-3 and RORγt$^+$, have been associated with disease severity in adults with asthma.[12] These findings indicate that the severity markers of asthma in children are different from the disease in adults.

We also performed a different set of analysis to compare non-severe and STRA patients; data from the transcriptional factor analysis were categorized in above or below the median of healthy controls. Our data show that only 9.1% of STRA children showed the median of CD4$^+$GATA3$^+$ cells above healthy controls, compared to 82% of children with non-severe asthma ($P = 0.001$) (Figure 3A). We also found that less than 10% of STRA children presented a median of CD4$^+$CD25$^+$FoxP3$^+$ RORγt$^+$ cells above healthy control children, indicating a reduced proportion of this cell phenotype in this group of patients ($P = 0.009$; Figure 3 B). In human cells, it was described that cells co-expressing FoxP3 and RORγt retain suppressive function.[13] The reduction in FoxP3 and RORγt co-expressing cells in STRA might be associated with less IL-10 production and asthma regulation. In contrast, the majority of STRA children, 82.8%, showed the median of CD4$^+$T-bet$^+$FoxP3$^+$ above healthy controls, compared to 42.4% of children with non-severe asthma (Figure 3B; $P = 0.012$). Cells co-expressing FoxP3 and T-bet are a stable population responsible for inhibiting Th1 response.[14] The present study is the first to describe the presence of this population of cells in peripheral blood of children with STRA.

In addition, 90.9% of STRA children showed the median of CD4$^+$RORγt$^+$T-bet$^+$ cells above healthy controls, compared to 54.5% of children with non-severe asthma ($P = 0.014$; Figure 3B). CD4$^+$T-bet$^+$RORγt$^+$ cells are generated in vivo during experimental allergic encephalomyelitis.[15] We suggest that these cells may also be involved in the allergic immunopathogenesis of STRA in children, given that the majority of these children are atopic.

Finally, we next evaluated whether the co-expressing two-lineage specific transcription factor would change after a polyclonal T-cell

activation in vitro. When the cells were stimulated with anti-CD3/anti-CD28, children with STRA continued to present similar results, but also showed a significant decrease in the percentage of $CD4^{+}$T-$bet^{+}GATA\text{-}3^{+}$ cells, given that only 9.1% of children with STRA presented the median of this phenotype above healthy controls, compared to 67.8% of children with non-severe asthma ($P = 0.001$; Figure 3C). These cells are known to maintain Th2 and Th1 functions.[16] Larger studies are required for better understanding the clinical impact of both distinct immune expressions in children with STRA.

One limitation of our study is that all the analysis for the immune response patterns were performed in peripheral blood samples. Asthma is an airway disease in which, ideally, biological samples should be collected and studied directly from the airways. However, this approach requires more invasive procedures, which are ethically difficult to approve in studies of children in many research centers.

In conclusion, our data demonstrated that GATA3 expression on T cells is decreased in STRA children and seems to be associated with lower small airway lung function. In addition, we found a significant reduction in cells that double expressed GATA-3 and RORγt. Our results add important information on T-cell markers in children with severe asthma, and may contribute to treatment decision and further mechanistic studies in this group of patients.

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CONFLICT OF INTEREST

All authors have no conflict of interest to declare

AUTHOR CONTRIBUTION

PDA, APDS, BNP, LA, RG, SPM, RM, EES, and GS were involved in acquisition and analysis of data, interpretation of data, and revising the work critically. PMP, MHJ, CB, and RTS were involved in interpretation of data, drafting the work, and revising it critically. APDS and PDA contribute equaly for this work

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REFERENCES

1. Bossley CJ, Fleming L, Gupta A, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without TH2 cytokines. *J Allergy Clin Immunol*. 2012;129:974-982.e13.
2. Saglani S, Lui S, Ullmann N, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol*. 2013;132:676–685.e13.
3. Gupta A, Dimeloe S, Richards DF, et al. Defective IL-10 expression and in vitro steroid-induced IL-17A in paediatric severe therapy-resistant asthma. *Thorax*. 2014;69:508–515.
4. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2017.
5. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319–338.
6. Krug N, Hohlfeld JM, Kirsten A-M, et al. Allergen-induced asthmatic responses modified by a GATA3-specific DNAzyme. *N Engl J Med*. 2015;372:1987–1995.
7. Agarwal A, Singh M, Chatterjee BP, Chauhan A. Interplay of T helper 17 cells with CD4(+)CD25(high)FOXP3(+) Tregs in regulation of allergic allergic asthma in pediatric patients. *Int J Pediatr*. 2014;2014:636238.
8. Stelmaszczyk-Emmel A, Zawadzka-Krajewska A, Kopatys A. Th1, Th2, Th17, and regulatory cytokines in children with different clinical forms of allergy. *Adv Exp Med Bbology*. 2013;788:321–328.
9. Meszaros G, Szalay B, Toldi G, et al. FoxP3+ regulatory T cells in childhood allergic rhinitis and asthma. *J Investig Allergol. Clin. Immunol*. 2009;19:238–240.

10. Raedler D, Ballenberger N, Klucker E, et al. Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allergy Clin Immunol*. 2015;135:81–91.
11. Wang Y-H, Voo KS, Liu B, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med*. 2010;207:2479–2491.
12. Irvin C, Zafar I, Good J, et al. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. *J Allergy Clin Immunol*. 2014;134:1175–1186.e7.
13. Voo KS, Wang Y-H, Santori FR, et al. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci USA*. 2009;106:4793–4798.
14. Levine AG, Medoza A, Hemmers S, et al. Stability and function of regulatory T cells expressing the transcription factor T-bet. *Nature*. 2017;546:421–425.
15. Ronchi F, Basso C, Preite S, et al. Experimental priming of encephalitogenic Th1/Th17 cells requires pertussis toxin-driven IL-1β production by myeloid cells. *Nat Commun*. 2016;7:11541.
16. Hegazy AN, Peine M, Helmstetter C, et al. Interferons direct Th2 cell reprogramming to generate a stable GATA-3+T-bet+ cell subset with combined Th2 and Th1 cell functions. *Immunity*. 2010;32:116–128.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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CD18 is redundant for the response to multiple vaccines: A case study

Dear Editor,

A first-born boy to unrelated healthy Danish parents was presented with marked neutro- and lymphocytosis after birth (Table 1). His umbilical cord did not separate until age of five weeks. Due to chronic skin infection (without pus formation) and protracted leukocytosis (hematological malignancy was excluded), he was referred to our university hospital at the age of 11 months. We found concentrations of elevated $CD19^+$ B cells, and $CD4^+$ and $CD8^+$ T cells (Table 1) and normal concentrations of IgA, IgM, and IgG (data not shown), but flow cytometry revealed total absence (0%) of neutrophil-expressed CD18 (integrin beta chain-2) consistent with severe leukocyte adhesion deficiency type 1 (LAD-1). The functional relevance of his complete CD18 deficiency was confirmed by observing that his neutrophils failed to generate neutrophil extracellular traps (NETS) in response to *Staphylococcus aureus*,[1] a process which depends upon functional MAC-1 and hence functional CD18. Microarray analysis and Sanger sequencing revealed a missense mutation (*ITGB2*: c.817G>A; pGly273Arg) on the paternal allele, known to prevent CD11a/CD18 heterodimerization[2] and a novel 1-Mb deletion (arr [hg19] 21q22.3 (45 441 009-46 511 411)), encompassing the maternal *ITGB2* gene. Having received standard childhood multiple vaccinations at the age of 3 and 5 months respectively, our patient, now aged 12 months, received his third standard childhood multiple vaccination comprising: (a) *Haemophilus influenzae* type B conjugate, (b) (tridecavalent) pneumococcal conjugate vaccine (PCV), (c) toxoids (tetanus, diphtheria, and pertussis), and (d) inactivated poliovirus. This standard regimen was supplemented with: (e) a live attenuated varicella-zoster vaccine (VZV). The decision to include the VZV vaccine relied on the following considerations: (a) MMR infections being very rare in Denmark due to reasonably high vaccination coverage, VZV infection was considered a greater risk for the patient; (b) adequate antibody reactions were shown for the preceding immunizations indicating sound T-cell function; (c) acyclovir was available in case of post-vaccination disease; and (d) parents received detailed instructions on how to observe any untoward events following vaccination.

Table 1 displays the PCV vaccination kinetics and the VZV, *Haemophilus influenzae*, *Clostridium tetani and Clostridium diphtheria* post-vaccination (21 days) titers (pre-transplantation determination of polio titers unfortunately failed due to technical reasons and the response to pertussis toxin is not used for assessing vaccination responses, sources: Statens Serum Institut, Copenhagen, Denmark). At the age of 24 months (52 weeks post-vaccination), our patient received an allogeneic bone marrow transplantation. His infectious history until then was characterized solely by recurrent bacterial skin infections amenable to treatment with systemic antibiotics. Patient antibody levels remained protective 52 weeks post-vaccination except for antibodies against *H. influenzae* type B, which had dropped below the long-term protective level (≥1 μg/mL) 20 weeks post-vaccination. In agreement with informed parental consent and with protocol: S-20150176 (The Regional Committees on Health Research Ethics for Southern Denmark), further immunologic workup was initiated. Patient $CD4^+CD45RA^-CXCR5^+CCR7^{lo}$ $PD\text{-}1^{hi}$ peripheral T follicular helper cell (pT_{FH}) frequencies displayed the characteristic expansion 7 days post-vaccination (Table 1).