

Circulating IL-4, IFN γ and IL-17 conventional and Innate-like T-cell producers in adult asthma

To the Editor,

Compelling evidence outlined the importance of conventional T-helper (Th)2 and Th17 CD4⁺ T cells and of innate-like T cells (ILT), namely invariant natural killer T (iNKT), $\gamma\delta$ T and mucosal-associated invariant T (MAIT) cells, as sources of inflammatory cytokines involved in asthma pathology.¹⁻⁵ Ideally, the analysis of the major inflammatory cells implicated in asthma severity should be performed in the airways. However, for several reasons, these studies were realized in a limited number of patients.^{6,7} Here, we systematically profiled in peripheral blood Th1, Th2 and Th17, respectively, identified as interferon- γ (IFN γ), interleukin (IL)-4- and IL-17A (hereafter referred as IL-17)-producing CD4⁺ T cells. Type 1 and Type 2 cytotoxic (Tc1 and Tc2) CD8⁺ T cells (IFN γ and IL-4 producers, respectively) and Foxp3⁺CD25⁺CD4⁺ regulatory T cells (Treg) were also analysed. Among ILT cells, we focused on IFN γ -, IL-4- and IL-17-producing iNKT, MAIT and V δ 2⁺ $\gamma\delta$ T cells. The frequency of these T cells was compared in peripheral blood of 50 healthy donors (HD), 28 mild-to-moderate (GINA steps 1-3) and 72 severe asthmatics (GINA step 4 = 33 and GINA step 5 = 39) who have been included over 4 years, in the multicentre Cohort of Bronchial Obstruction and Asthma (COBRA) (Table S1).⁸ The group merging all asthmatics was referred as "ALL."

The proportion of CD4⁺ (Figure S1A), but not CD8⁺ (Figure S1B), T cells was lower in ALL than in HD. However, this difference disappeared when asthmatic patients were separated into GINA steps 1-3, 4 and 5 sub-groups (Table 1). Within the CD4⁺ and CD8⁺ T-cell populations, Th1, Th2, Th17 and Tc1 were greater in ALL than in HD (Figure S1C-F), whereas Tc2 frequency was similar between these groups (Figure S1G). Th2 were significantly higher in GINA steps 1-3 and 5, but lower in GINA step 4 than in step 3 (Table 1). A similar trend was observed for Tc1 (Table 1), whereas Tc2 ($P = .08$) and Th1 ($P = .06$) tended to behave as Th2 and Tc1, respectively (Table 1).

Although there is a consensus showing an association between pro-Th2 immune responses and allergic asthma,³ we found that Th2 or Tc2 were neither over-represented in atopic asthmatics (Table S2), nor they correlated with circulating IgE or blood eosinophils (Table 2). In contrast, Th1 were negatively associated with blood eosinophils and with daily dose of ICS (Table 2). ICS daily dose was also negatively correlated with Th2 (Table 2). To better understand

the possible contribution of these cells in the context of ICS use and atopic markers, we analysed the ratios Th1/Th2, Th2/Th17 and Th1/Th17. These ratios were similar between HD and ALL (Figure S1H, I and J). However, Th2/Th17 and Th1/Th17 positively correlated with circulating IgE, but not with ICS use (Table 2) indicating that low frequency of Th17 is a hallmark of elevated IgE levels.

Mirroring the higher frequency of activated T cells, we found lower Treg in ALL than in HD (Figure S2A), whereas no significant differences were observed when the asthmatics were considered as separate groups, according to GINA steps (Table 1). Further, the ratios Th1/Treg, Th17/Treg and, to a lesser extent, Th2/Treg showed that these activated T cells were over-represented in ALL compared to HD (Figure S2B to D). This latter ratio also positively correlated with circulating IgE (Table 2). These findings indicate that elevated frequencies of activated T cells and a lower incidence of Treg discriminated asthmatic patients from HD.

Among $\gamma\delta$ T cells, the frequency of V δ 2 was higher in ALL than in HD (Figure S3A). Further, we observed an elevated proportion of IFN γ - and IL-17-producing V δ 2 in ALL (Figure S3B and C). No significant differences were observed when the sub-groups of asthmatics were analysed according to GINA steps (Table 1), nor when IFN γ - and IL-17-producing V δ 2 were examined in the context of clinical parameters (Table 2). These findings indicate that the role of V δ 2 on asthma pathology requires confirmation.

ALL and asthmatics patient sub-groups had similar proportions of iNKT than HD (Figure S3D and Table 1). However, these cells highly and negatively correlated with circulating IgE (Table 2) and they tended to be lower in atopic than in nonatopic asthmatics ($P = .12$) (Table S2). These data suggest iNKT are mainly associated with nonatopic asthma.

Examination of activation status of iNKT showed no differences in IL-4 and IFN γ production between HD and ALL (Figure S3E and F). However, IL-4-producing iNKT were higher in GINA steps 1-3 than HD and GINA step 5 (Table 1). Both IL-4 and IFN γ -producing iNKT correlated negatively with the daily dose of ICS (Table 2), suggesting a possible influence of this treatment on their frequency. It is noteworthy that IL-4-producing iNKT were positively correlated with the ACT score (Table 2) indicating that this population may have an unexpected protective role in these patients.

Abbreviations: ACT, Asthma control test; ALL, All asthmatics; FEV₁, Forced expiratory volume in one sec; FVE, Forced vital capacity; GINA, Global Initiative for Asthma; HD, Healthy donors; ICS, Inhaled corticosteroids; IFN γ , Interferon- γ ; IL, Interleukin; IL-17, Interleukin-17A; ILT, innate-like T cells; iNKT, invariant Natural Killer T cells; MAIT, Mucosal-associated invariant T cells; MAIT17, IL-17-producing Mucosal-associated invariant T cells; MOD, mild-to-moderate asthmatics; OCS, Oral corticosteroids; SEV, Severe asthmatics; Tc1, Type 1 cytotoxic CD8 + T cells; Tc2, Type 2 cytotoxic CD8 + T cells; TCR, T-cell receptor; Th, T helper.

TABLE 1 Frequency of immune cells in peripheral blood of controls and asthmatics, according to GINA stages

Cell type (in %)	Controls	Asthmatics			P ^a
		GINA steps 1-3	GINA step 4	GINA step 5	
Number of subjects	50	28	33	39	–
CD4+/CD3+	69.8 (65.2-76.5)	64.5 (61.1-71.4)	67.5 (61.8-73.9)	69.6 (61.2-75.1)	.42
CD8+/CD3+	24.3 (19.7-27.7)	24.6 (18.9-28.8)	23.5 (15.6-28.6)	23.7 (18.3-29.2)	.96
IL-4+/CD4+ (Th2)	2.1 (1.2-2.8)	3.6 (1.8-4.7)†	2.1 (1.0-2.9) ‡	3.2 (2.1-4.3)†§	.04
IL-4+/CD8+ (Tc2)	0.74 (0.28-1.49)	1.1 (0.8-1.8)	0.8 (0.5-1.2)	1.2 (0.6-2.1)	.08
IFN γ +/CD4+ (Th1)	11.7 (8.1-16.1)	21.6 (14.8-26.2)	16.8 (10.6-20.9)	14.9 (11.3-21.0)	.06
IFN γ +/CD8+ (Tc1)	22.8 (15.2-38.5)	54.3 (38.5-65.7)†	50.4 (33.9-60.9)†	40.1 (28.9-57.4)†	.05
IL-17+/CD4+ (Th17)	0.88 (0.49-1.23)	1.2 (0.7-1.9)	1.0 (0.6-1.3)	1.1 (0.7-1.7)	.47
Foxp3+/CD25+/CD4+ (Treg)	6.5 (5.2-7.8)	5.6 (4.7-6.3)	4.8 (4.4-6.1)	5.2 (3.9-6.5)	.69
Ratio Th1/Th2	6.3 (3.7-8.7)	8.2 (4.7-9.3)	6.9 (5.5-9.7)	5.6 (3.5-8.1)	.06
Ratio Th2/Th17	2.7 (1.3-4.2)	2.4 (1.7-3.3)	1.9 (1.3-2.9)	2.8 (1.6-3.8)	.22
Ratio Th1/Th17	12.9 (9.0-21.8)	17.0 (10.3-27.2)	16.0 (8.4-22.2)	13.3 (8.3-18.1)	.44
Ratio Th1/Treg	2.0 (1.5-3.1)	3.8 (2.6-5.1)	3.3 (2.3-3.9)	2.9 (1.8-4.3)	.34
Ratio Th2/ Treg	0.40 (0.25-0.62)	0.58 (0.41-0.75)	0.50 (0.17-0.78)	0.67 (0.39-0.92)	.69
Ratio Th17/Treg	0.12 (0.05-0.27)	0.24 (0.13-0.40)	0.21 (0.13-0.33)	0.23 (0.11-0.36)	.36
iNKT/CD3+	0.09 (0.06-0.16)	0.06 (0.03-0.12)	0.09 (0.03-0.33)	0.07 (0.03-0.19)	.64
IL-4+/iNKT	6.9 (2.2-16.5)	16.5 (7.7-28.4)†	5.2 (3.6-22.2)‡	9.8 (4.4-17.4)‡	.05
IFN γ +/iNKT	47.1 (36.7-61.1)	53.2 (44.5-69.1)	47.6 (32.3-61.5)	45.9 (23.4-64.2)	.14
MAIT/CD3+ ^b	3.2 (1.8-4.3)	0.7 (0.3-2.7)	1.6 (1.1-3.4)	1.1 (0.7-2.1)	.18
IFN γ +/MAIT ^b	45.9 (24.8-56.0)	56.9 (53.3-60.9)	38.5 (20.1-60.7)	48.0 (42.9-66.3)	.38
IL-17+/MAIT (MAIT17) ^c	0.93 (0.49-1.88)	3.4 (2.5-7.6)	1.0 (0.8-2.2)	1.6 (0.8-5.6)	.33
V δ 2+/CD3+ ^d	1.5 (0.7-2.4)	3.5 (2.4-4.0)	3.1 (1.7-5.3)	2.2 (0.9-3.3)	.27
IFN γ +/V δ 2+ ^e	49.6 (36.0-63.8)	65.4 (49.9-77.4)	69.1 (44.8-77.6)	82.2 (63.8-87.1)	.29
IL-17+/ V δ 2+ ^e	0.07 (0.04-0.14)	0.43 (0.31-0.55)	0.15 (0.10-0.41)	0.33 (0.07-1.04)	.72

Note: Bold denotes statistical significance. Data are medians (25-75 interquartile range). †P < .05, as compared to controls, ‡P < .05, as compared to GINA 3 asthmatics; §P < .05, as compared to GINA 4 asthmatics (Mann-Whitney U test).

Abbreviations: IFN γ , interferon- γ ; IL, interleukin; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cells.

^aKruskal-Wallis test.

^b55 asthmatics were included in the analysis.

^c27 asthmatics were included in the analysis.

^d28 asthmatics were included in the analysis.

^e24 asthmatics were included in the analysis.

In agreement with previous published data,⁶ MAIT were significantly lower in ALL than in HD (Figure S3G), but not between HD and asthmatic patient sub-groups (Table 1). Whereas no differences were observed in the frequency of IFN γ +/MAIT (Figure S3H and Table 1), IL-17-producing MAIT (MAIT17) were higher in ALL than in HD (Figure S3I). Given the absence of correlation between MAIT17 and ICS use (Table 2) and the lack of difference in their frequency in OCS-treated and OCS-untreated asthmatics of the GINA 5 step (Table S3), it may be speculated that MAIT17 represent a steroid less sensitive cell component involved in asthma pathology. Further, MAIT17 were negatively correlated with IgE (Table 2) and their frequency was higher in nonatopic than in atopic patients (Table S2). In addition, MAIT17 were positively correlated with ACT score (Table 2). Together, these data suggest that

these cells may play a predominant role in nonatopic asthmatic patients. These results are in agreement with our recent observations suggesting a role of MAIT17 in paediatric asthma.⁷ However, further studies are required to confirm or infirm the precise implication of MAIT in asthma.

Overall, our findings indicate that distinct T-cell populations and their ratio should be considered to better understand their role in distinct asthma endotypes. It is noteworthy that the positive correlation between MAIT17 and ACT score, associated with our previous studies in asthmatic children,⁷ suggests that these cells may play distinct roles in both adult and paediatric asthma pathology.

Although descriptive, this study paves the way for future asthma analysis in large patient cohorts. Overall, we suggest that the Th1/Th2 ratio and the frequency of MAIT17 should be taken into

TABLE 2 Correlation analyses between the frequency of immune cells in peripheral blood and main clinical parameters in all asthmatics^a

Cell type (in %)	Circulating IgE (IU/mL)		Blood EOS (per mm ³)		Daily dose of ICS (μg)		Number of exacerbations		ACT score	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
CD4+/CD3+	0.048	.640	-0.034	.736	0.119	.239	-0.013	.902	0.114	.304
CD8+/CD3+	-0.048	.834	0.050	.621	-0.089	.378	0.073	.487	-0.192	.082
IL-4+/CD4+ (Th2)	-0.030	.775	-0.183	.078	-0.209	.043	-0.004	.967	-0.121	.289
IL-4+/CD8+ (Tc2)	-0.022	.835	-0.165	.112	-0.122	.239	-0.012	.912	-0.104	.362
IFN γ +/CD4+ (Th1)	-0.121	.250	-0.217	.035	-0.285	.005	-0.147	.178	0.029	.799
IFN γ +/CD8+ (Tc1)	-0.134	.204	-0.158	.131	-0.185	.075	-0.115	.293	-0.038	.740
IL-17+/CD4+ (Th17)	-0.134	.200	-0.145	.163	-0.166	.109	-0.061	.578	-0.005	.961
Foxp3+/CD25+/CD4+ (Treg)	-0.100	.346	-0.001	.926	-0.056	.594	-0.140	.202	-0.017	.884
Ratio Th1/Th2	-0.030	.747	-0.030	.790	-0.030	.801	-0.060	.587	0.140	.219
Ratio Th2/Th17	0.280	.007	-0.007	.946	-0.079	.447	-0.039	.718	-0.045	.690
Ratio Th1/Th17	0.218	.036	-0.008	.940	-0.106	.307	-0.081	.453	0.030	.791
Ratio Th1/Treg	0.168	.128	-0.188	.084	-0.123	.261	-0.116	.307	0.062	.604
Ratio Th2/ Treg	0.285	.009	-0.190	.080	-0.138	.206	-0.0003	.998	-0.069	.563
Ratio Th17/Treg	-0.064	.569	-0.143	.192	-0.059	.595	-0.072	.526	0.084	.480
iNKT/CD3+	-0.427	<.1	0.086	.395	0.113	.261	-0.049	.644	0.106	.342
IL-4+/iNKT	0.163	.123	-0.185	.078	-0.325	.001	-0.054	.625	0.255	.025
IFN γ +/iNKT	-0.059	.580	0.039	.714	-0.234	.026	-0.162	.144	-0.026	.821
MAIT/CD3+ ^b	-0.062	.661	-0.082	.551	0.218	.110	0.093	.515	-0.173	.249
IFN γ +/MAIT ^c	0.279	.167	-0.336	.080	0.105	.594	-0.276	.202	0.295	.194
IL-17+/MAIT (MAIT17) ^c	-0.439	.025	0.120	.543	-0.303	.117	-0.113	.607	0.451	.040
V δ 2+/CD3+ ^c	-0.075	.713	0.028	.887	-0.010	.956	-0.011	.953	-0.037	.858
IFN γ +/V δ 2+ ^c	-0.036	.865	-0.198	.331	0.310	.135	0.216	.310	-0.030	.896
IL-17+/V δ 2+ ^c	-0.239	.260	-0.07	.719	-0.133	.515	-0.074	.732	0.252	.258

Note: Bold denotes statistical significance.

Abbreviations: EOS, eosinophils; ICS, inhaled corticosteroids; IFN γ , interferon- γ ; IL, interleukin; iNKT, invariant natural killer T; IU, international units; MAIT, mucosal-associated invariant T cells.

^aSpearman's rank-order method (n = 100).

^b56 asthmatics were included in the analysis.

^c28 asthmatics were included in the analysis.

^d24 asthmatics were included in the analysis.

consideration for better identifying distinct asthma endotypes and for testing the clinical benefit of specific immunotherapies.

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CONFLICT OF INTEREST

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Maria Leite-de-Moraes^{1,2} 

Renata Belo^{1,2}

Céline Dietrich^{1,2}

David Soussan^{3,4,5,6}

Michel Aubier^{3,4,5,6}

Marina Pretolani^{3,4,5,6}

¹Laboratory of Immunoregulation and Immunopathology, Institut Necker-Enfants Malades (INEM), CNRS UMR8253 and Inserm UMR1151, Paris, France

²Université Paris Descartes Sorbonne Paris Cité, Université de Paris, Paris, France

³Inserm UMR1152, Physiopathologie et Epidémiologie des Maladies Respiratoires, Paris, France

⁴Faculté de Médecine, Université Paris Diderot, Université de Paris, Paris, France

⁵Laboratory of Excellence INFLAMEX, Université Sorbonne Paris-Cité, Paris, France

⁶Département Hospitalo, Universitaire FIRE, Paris, France

Correspondence

Maria Leite-de-Moraes, Institut Necker-Enfants Malades, CNRS UMR 8253 and Inserm UMR1151, Faculté de Médecine Necker, 156 rue de Vaugirard, 75015, Paris, France.

Email: maria.leite-de-moraes@parisdescartes.fr or maria.leite-de-moraes@inserm.fr

ORCID

Maria Leite-de-Moraes  <https://orcid.org/0000-0002-2891-2269>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

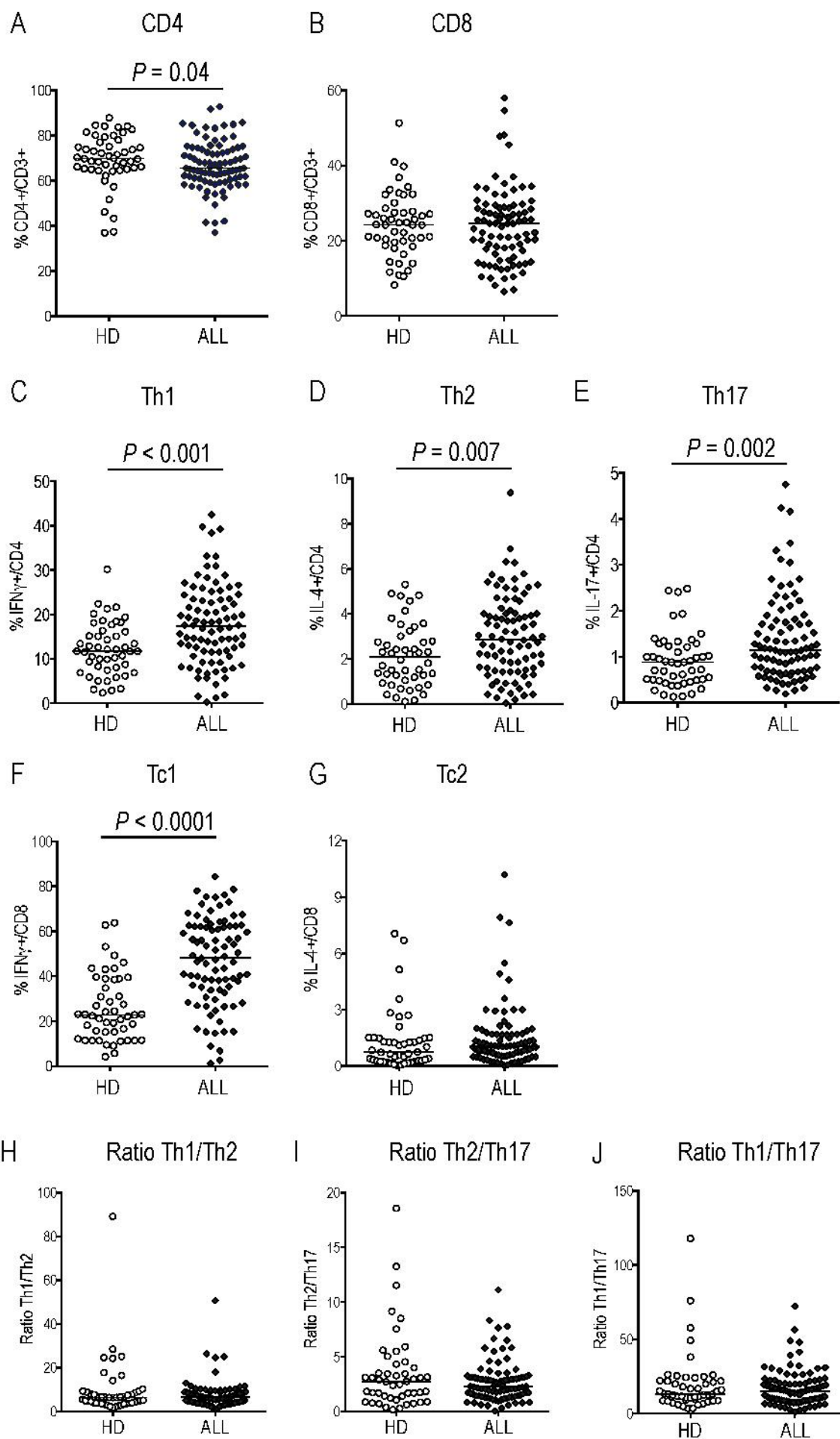


Figure S1

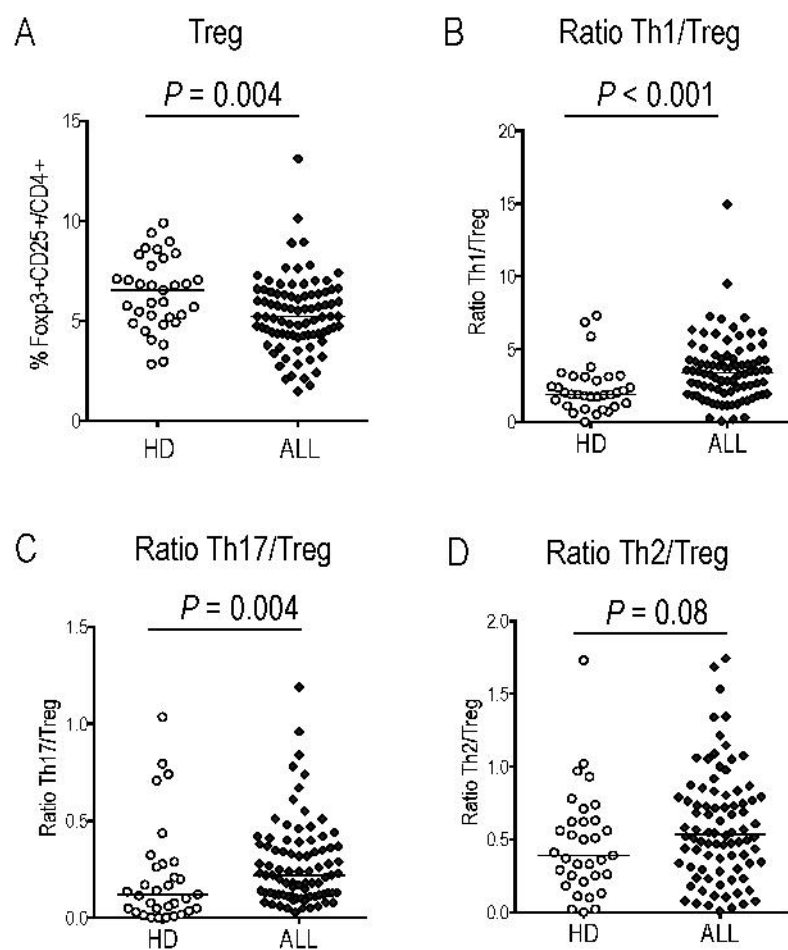


Figure S2

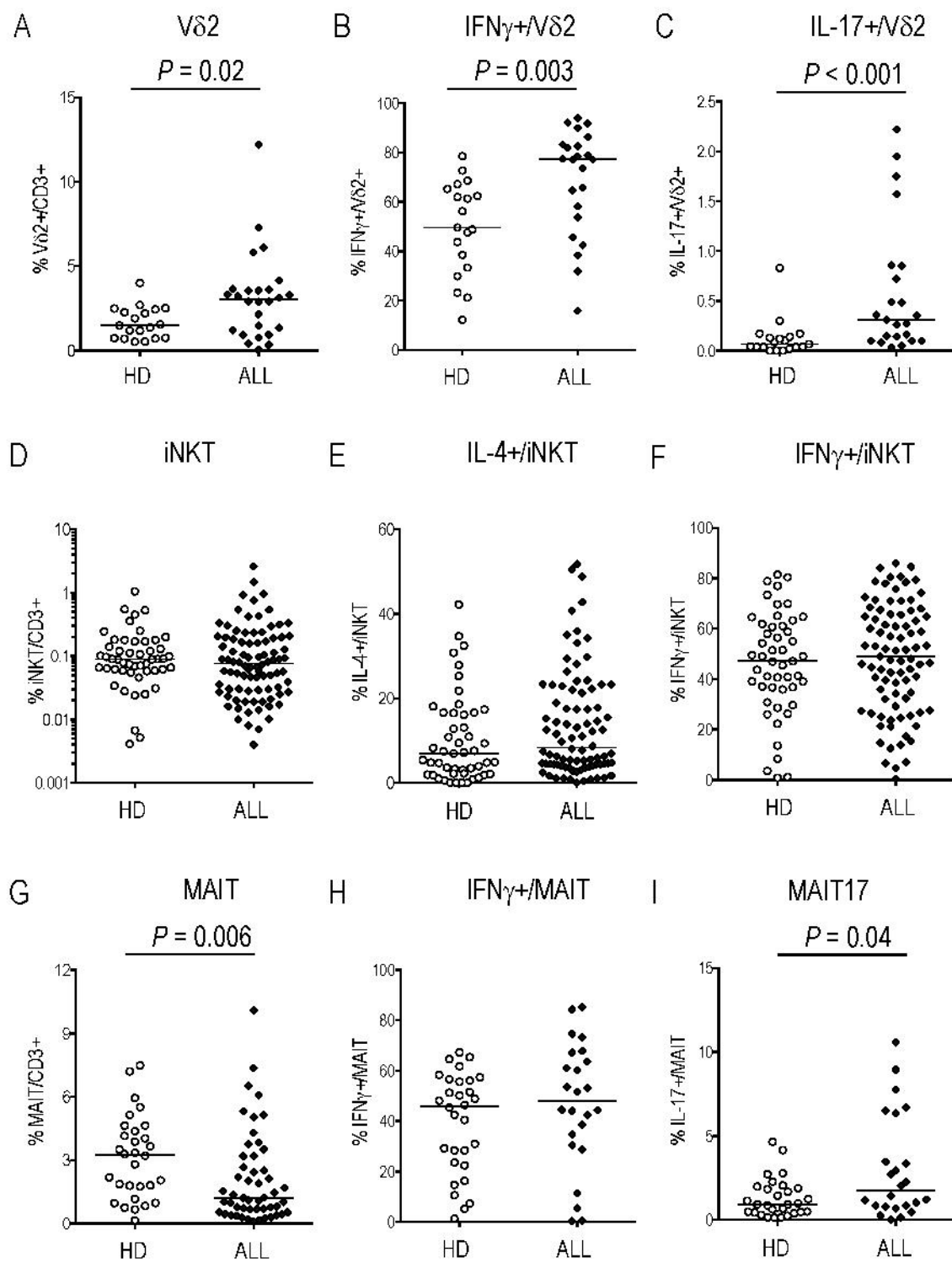


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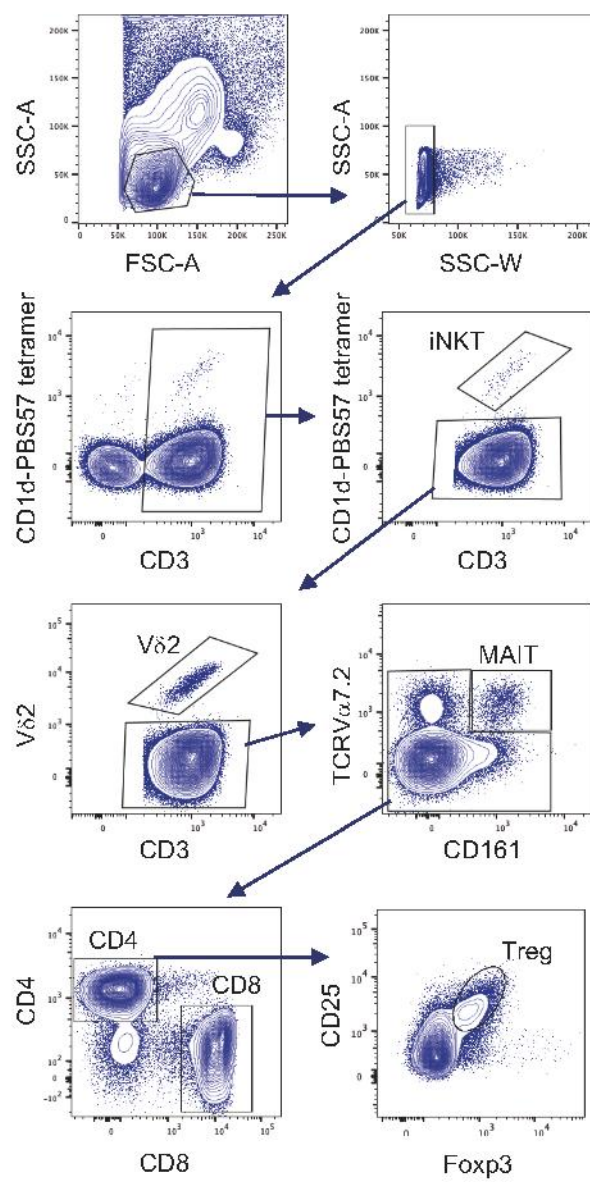


Figure S4

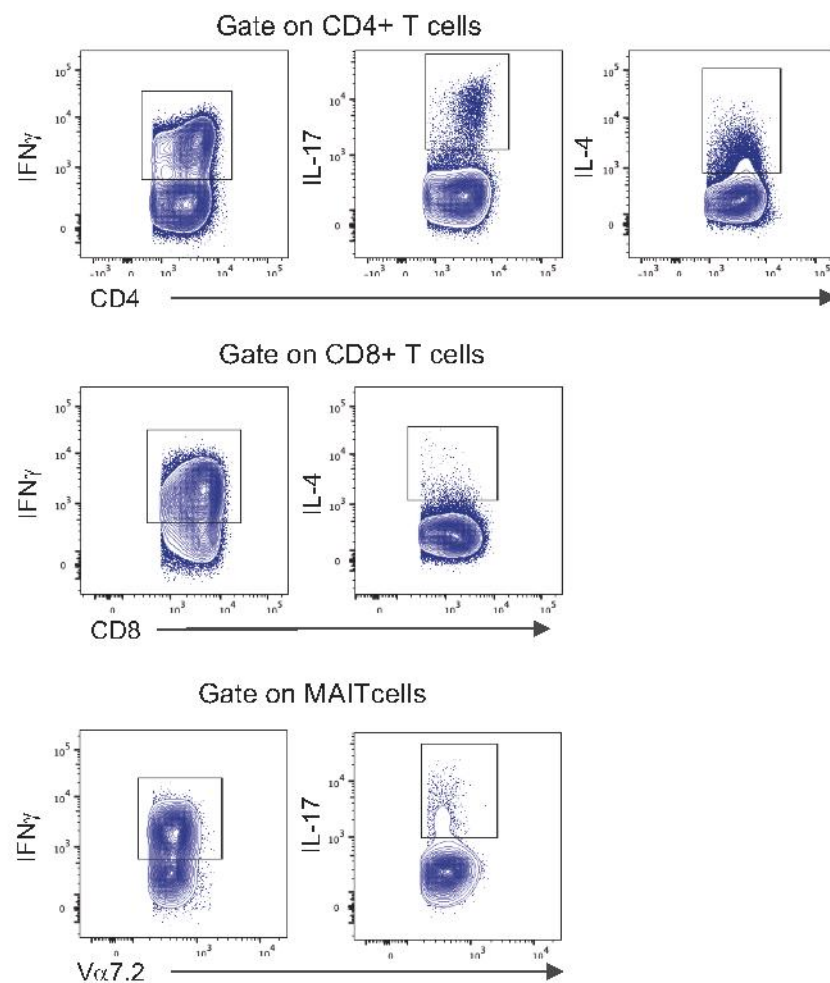


Figure S5

Table S1: Characteristics of mild-to-moderate and severe asthmatics providing blood samples for determining the frequency of immune cells

Parameter	Asthmatics, according to severity			P ^a
	MOD	SEV		
	GINA steps 1-3 ^b	GINA step 4	GINA step 5	
No. of patients (%)	28	33	39	-
Women – no. (%)	16 (57.1)	18 (54.6)	24 (61.5)	0.84
Age – years	50.5 ± 17.4	51.5 ± 16.5	48.5 ± 12.3	0.56
BMI – kg per m ²	27.5 ± 4.5	26.4 ± 4.0	26.0 ± 5.0	0.22
Smoking history – former/never (%) ^c	9/18 (33/67)	7/23 (23/77)	14/25 (36/64)	0.52
Age of asthma onset – years	23.2 ± 20.6	25.7 ± 19.3	21.7 ± 17.4	0.69
Asthma duration – years	27.6 ± 14.0	25.2 ± 16.0	28.2 ± 17.0	0.84
With history of atopy – no. (%)	23 (82.1)	18 (54.6)	26 (66.7)	0.08
Blood eosinophils counts – 10 ⁹ per Liter	285 (200-385)	290 (190-550)	308 (110-510)	0.63
With ≥ 300 blood eosinophils – no. (%)	11 (39.3)	15 (45.5)	20 (51.3)	0.63
Total serum IgE – International Units per milliliter	415 (76-812)	176 (82-309)	220 (92-679)	0.17
Respiratory function				
Pre-bronchodilator FEV ₁ – % of predicted	88.8 ± 16.0	71.1 ± 19.5 †	65.5 ± 19.9 †	< 0.0001
Post-bronchodilator FEV ₁ – % of predicted	96.9 ± 14.3	76.8 ± 19.1 †	70.3 ± 20.2 †	< 0.0001
Pre-bronchodilator FEV ₁ /FVC – % of predicted	66.8 ± 10.5	60.8 ± 12.2	61.6 ± 13.1	0.12
Post-bronchodilator FEV ₁ /FVC – % of predicted	70.5 ± 9.0	63.8 ± 12.9 †	63.5 ± 13.4 †	0.03
Treatments				
On SABA – number (%)	28 (100.0)	33 (100.0)	39 (100.0)	-
On LABA – number (%)	23 (82.1)	32 (97.0)	38 (97.4) †	0.03
Daily dose of ICS ^c	696 ± 249	2139 ± 761 †	2328 ± 907 †	< 0.0001
On maintenance use of OCS – no. (%)	0	0	16 (41.0)	-
Daily dose of oral prednisone – mg	0	0	25.3 ± 12.3	-
On anti-IgE	0	0	18 (46.2)	-

Table S1 (continued)

Asthma control				
Score on ACT	13.5 ± 6.0	13.1 ± 6.1 †	10.3 ± 4.5 †‡	< 0.0001
Annual rate of severe exacerbations requiring OCS bursts	3.0 ± 0.4	2.5 ± 0.6 †	5.3 ± 0.8 †‡	< 0.0001

Abbreviations: BMI, body mass index; FEV₁, Forced Expiratory Volume in One second; FVC, Forced Vital Capacity; SABA, short-acting β₂-agonists; LABA, long-acting β₂-agonists; ICS, inhaled corticosteroids; OCS, oral corticosteroids; ACT, asthma control test

Data are n (%), or means ± SD, or medians (interquartile range). Results on annual rates of severe exacerbations are expressed as means ± SE

^a ANOVA, or Kruskal-Wallis test or χ^2 test

^b Data are available for 2 GINA 1, 17 GINA 2 and 9 GINA 3 asthmatics

^c In µg of beclomethasone equivalents

† $P < 0.05$, as compared to GINA 1-3 asthmatics, ‡ $P < 0.05$, as compared to GINA 4 asthmatics (Student's t test for unpaired value, Mann-Whitney U-test or Fisher test 2-tailed)

Bold denotes statistical significance

Table S2: Frequency of immune cells in peripheral blood of asthmatics according to atopy ^a

Cell type (in %)	Non atopic	Atopic	<i>P</i> ^a
Number of patients	32	67	-
CD4+/CD3+	64.6 (60.9-73.8)	67.4 (61.7-74.6)	0.55
CD8+/CD3+	23.5 (14.7-28.6)	24.6 (18.8-29.2)	0.39
IL-4+/CD4+ (Th2)	2.8 (1.0-3.9)	2.8 (1.8-4.0)	0.58
IL-4+/CD8+ (Tc2)	1.0 (0.6-2.0)	1.0 (0.6-1.7)	0.79
IFN γ +/CD4+ (Th1)	17.9 (11.3-24.0)	16.8 (12.1-22.4)	0.79
IFN γ +/CD8+ (Tc1)	53.5 (39.6-61.3)	40.9 (28.7-62.2)	0.21
IL-17+/CD4+ (Th17)	1.3 (0.7-1.9)	1.0 (0.7-1.7)	0.52
Foxp3+/CD25+/CD4+ (Treg)	5.6 (4.4-6.4)	5.2 (4.3-6.2)	0.38
Ratio Th1/Th2	7.6 (4.5-9.2)	6.4 (4.2-8.3)	0.38
Ratio Th2/Th17	1.9 (1.1-2.9)	2.7 (1.7-3.1)	0.09
Ratio Th1/Th17	12.6 (9.2-20.7)	15.9 (8.7-21.3)	0.58
Ratio Th1/Treg	3.3 (1.8-4.2)	3.6 (2.4-4.3)	0.73
Ratio Th2/ Treg	0.52 (0.23-0.76)	0.65 (0.34-0.84)	0.30
Ratio Th17/Treg	0.25 (0.12-0.35)	0.21 (0.12-0.38)	0.96
iNKT/CD3+	0.11 (0.06-0.20)	0.06 (0.03-0.18)	0.18
IL-4+/iNKT	6.1 (3.6-14.6)	12.5 (4.4-23.1)	0.12
IFN γ +/iNKT	45.6 (32.7-64.5)	51.7 (32.4-65.5)	0.74
MAIT/CD3+ ^b	1.4 (0.7-4.0)	1.0 (0.5-2.0)	0.11
IFN γ +/MAIT ^c	43.4 (32.5-56.3)	53.2 (36.2-67.9)	0.31
IL-17+/MAIT (MAIT17) ^c	3.2 (1.6-6.5)	1.0 (0.6-2.7)	0.05
V δ 2+/CD3+ ^d	2.9 (1.1-3.9)	3.1 (1.2-3.6)	0.89
IFN γ +/V δ 2+ ^e	76.0 (59.1-87.1)	77.2 (51.7-79.7)	0.67
IL-17+/ V δ 2+ ^e	0.29 (0.15-0.75)	0.25 (0.10-0.58)	0.89

Abbreviations: IU, international units; IL, interleukin; IFN γ , interferon- γ ; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cells

Data are medians (25-75 interquartile range)

^a Mann-Whitney U-test

^b 55 asthmatics were included in the analysis

^c 27 asthmatics were included in the analysis

^d 28 asthmatics were included in the analysis

^e 24 asthmatics were included in the analysis

Bold denotes statistical significance

Table S3: Frequency of immune cells in peripheral blood of severe asthmatics of GINA 5 step, according to OCS use

Cell type (in %)	Without OCS	On regular OCS				
		All	<i>P</i> ^a	≤ 20 mg/day	> 20 mg/day	<i>P</i> ^b
Number of patients	23	16	-	9	7	-
CD4+/CD3+	71.7 (64.7-75.1)	64.7 (59.7-74.9)	0.53	67.8 (63.7-84.7)	53.0 (44.0-56.0)	0.06
CD8+/CD3+	22.2 (18.3-26.7)	27.3 (17.9-29.7)	0.31	29.5 (13.8-29.7)	26.9 (24.6-28.3)	0.99
IL-4+/CD4+ (Th2)	3.2 (2.2-4.0)	3.4 (2.2-4.8)	0.73	2.9 (1.6-4.1)	4.3 (3.2-4.8)	0.61
IL-4+/CD8+ (Tc2)	1.2 (0.6-2.1)	1.3 (0.5-1.9)	0.84	1.0 (0.4-4.2)	1.7 (1.0-1.7)	0.99
IFN γ +/CD4+ (Th1)	14.4 (10.6-19.7)	16.9 (11.7-23.5)	0.51	14.5 (10.7-22.9)	19.3 (14.4-23.6)	0.69
IFN γ +/CD8+ (Tc1)	38.4 (26.5-53.5)	40.9 (33.9-65.8)	0.25	39.8 (34.5-57.6)	55.9 (33.3-72.2)	0.69
IL-17+/CD4+ (Th17)	1.2 (0.8-1.8)	1.0 (0.7-1.6)	0.51	1.0 (0.9-1.6)	0.9 (0.6-1.6)	0.61
Foxp3+/CD25+/CD4+ (Treg)	5.4 (4.0-6.1)	4.4 (4.0-6.7)	0.75	4.4 (3.5-5.2)	5.8 (4.3-4.3-8.5)	0.39
Ratio Th1/Th2	5.8 (3.4-8.1)	5.5 (3.6-7.4)	0.75	5.9 (4.7-8.1)	3.8 (3.6-5.3)	0.53
Ratio Th2/Th17	2.8 (1.6-3.1)	2.8 (1.8-3.4)	0.68	2.1 (1.9-2.9)	3.0 (2.1-5.8)	0.39
Ratio Th1/Th17	10.7 (7.4-16.6)	15.2 (11.8-18.2)	0.36	15.7 (10.8-17.1)	13.5 (11.9-24.3)	0.69
Ratio Th1/Treg	2.7 (1.6-4.2)	3.5 (1.6-4.5)	0.52	3.5 (2.0-4.5)	3.6 (1.3-5.2)	0.71
Ratio Th2/ Treg	0.67 (0.41-0.77)	0.63 (0.35-0.99)	0.73	0.43 (0.38-0.92)	0.92 (0.36-1.02)	0.90
Ratio Th17/Treg	0.24 (0.13-0.42)	0.17 (0.10-0.35)	0.44	0.26 (0.17-0.35)	0.12 (0.09-0.24)	0.31
iNKT/CD3+	0.08 (0.03-0.17)	0.07 (0.03-0.20)	0.99	0.05 (0.03-0.12)	0.07 (0.04-0.25)	0.60
IL-4+/iNKT	13.1 (4.6-17.8)	5.7 (2.9-11.5)	0.11	7.8 (4.3-12.2)	4.8 (2.9-9.7)	0.57
IFN γ +/iNKT	43.9 (21.4-63.6)	46.7 (30.1-63.9)	0.72	52.8 (37.3-72.3)	42.3 (30.1-57.2)	0.41
MAIT/CD3+	0.8 (0.4-1.8)	1.3 (0.7-2.5)	0.29	1.1 (0.8-2.4)	1.5 (0.7-2.4)	0.99
IFN γ +/MAIT (MAIT17) ^c	44.4 (42.5-73.6)	51.6 (44.4-63.6)	0.99	54.0 (49.2-58.8)	51.6 (43.2-59.4)	0.99
IL-17+/MAIT ^c	6.5 (1.2-6.7)	1.2 (0.7-2.0)	0.30	1.0 (0.8-1.1)	2.0 (1.2-2.4)	0.78
V δ 2+/CD3+ ^d	3.1 (1.7-3.3)	1.5 (1.0-1.0-3.2)	0.99	1.5 (1.2-2.9)	2.1 (0.9-4.2)	0.91
IFN γ +/V δ 2+ ^d	86.2 (81.9-88.0)	81.9 (58.2-83.2)	0.46	65.7 (31.0-82.5)	86.8 (76.0-01.7)	0.28
IL-17+/ V δ 2+d	0.05 (0.03-0.16)	0.48 (0.31-1.57)	0.08	0.48 (0.35-0.86)	0.94 (0.25-1.67)	0.91

Abbreviations: OCS, oral corticosteroids; IL, interleukin; IFN γ , interferon- γ ; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cells
Data are medians (25-75 interquartile range)

^a Mann-Whitney U-test between severe OCS-untreated and –treated asthmatics

^b Mann-Whitney U-test between severe asthmatics treated with ≤ 20 mg/day and ≥ 20 mg/day OCS

^c 10 asthmatics were included in the analysis

^d 12 asthmatics were included in the analysis

Table S4. Correlation analyses between Th2, Th1, Th17, Tc1 and Tc2 in healthy donors (HD) and asthmatic patients (ALL)^a

Cell types	HD		ALL	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Th2 <i>versus</i>				
Th1	0.476	< 0.001	0.534	< 0.001
Th2 <i>versus</i>				
Th17	0.213	0.146	0.474	< 0.001
Th2 <i>versus</i>				
Tc1	0.312	0.030	0.348	< 0.001
Th2 <i>versus</i>				
Tc2	0.498	< 0.001	0.460	< 0.001
Th1 <i>versus</i>				
Th17	0.393	0.005	0.419	< 0.001
Th1 <i>versus</i>				
Tc1	0.730	< 0.001	0.671	< 0.001
Th1 <i>versus</i>				
Tc2	0.208	0.156	0.320	< 0.001
Th17 <i>versus</i>				
Tc1	0.282	0.049	0.190	0.071
Th17 <i>versus</i>				
Tc2	0.099	0.503	0.238	0.020
Tc1 <i>versus</i> Tc2	0.303	0.036	0.239	0.022
Th1 <i>versus</i>				
Treg	-0.253	0.161	0.145	0.182
Th2 <i>versus</i>				
Treg	-0.263	0.146	0.125	0.251
Th17 <i>versus</i>				
Treg	-0.067	0.716	0.022	0.836
Tc1 <i>versus</i>				
Treg	0.079	0.667	0.095	0.385
Tc2 <i>versus</i>				
Treg	0.058	0.750	0.119	0.275

^a Spearman's rank-order method (n = 100)

Bold denotes statistical significance

Table S5: Frequency of immune cells in peripheral blood of severe asthmatics, according to treatment with omalizumab ^a

Cell type (in %)	Without omalizumab	With omalizumab	<i>P</i> ^b
Number of patients	21	18	-
CD4+/CD3+	71.7 (60.6-79.7)	69.6 (63.0-72.7)	0.40
CD8+/CD3+	21.7 (14.7-29.7)	25.5 (20.9-28.4)	0.61
IL-4+/CD4+ (Th2)	3.7 (1.9-4.9)	3.2 (2.2-3.7)	0.53
IL-4+/CD8+ (Tc2)	1.3 (0.7-2.1)	1.1 (0.6-2.0)	0.66
IFN γ +/CD4+ (Th1)	14.6 (8.7-23.5)	15.2 (11.6-19.9)	0.84
IFN γ +/CD8+ (Tc1)	43.5 (28.1-58.9)	40.0 (30.7-53.5)	0.97
IL-17+/CD4+ (Th17)	1.2 (0.7-1.7)	1.0 (0.7-1.8)	0.98
Foxp3+/CD25+/CD4+ (Treg)	5.2 (4.0-6.3)	5.2 (4.0-6.5)	0.87
Ratio Th1/Th2	5.5 (3.4-8.1)	5.8 (3.9-7.8)	0.45
Ratio Th2/Th17	2.9 (1.8-3.0)	2.2 (1.6-3.2)	0.90
Ratio Th1/Th17	12.6 (7.4-16.7)	13.5 (8.9-18.5)	0.83
Ratio Th1/Treg	2.5 (1.5-4.4)	3.2 (2.3-4.2)	0.68
Ratio Th2/ Treg	0.69 (0.39-1.00)	0.51 (0.38-0.83)	0.82
Ratio Th17/Treg	0.22 (0.11-0.34)	0.27 (0.12-0.39)	0.61
iNKT/CD3+	0.09 (0.03-0.20)	0.06 (0.03-0.17)	0.60
IL-4+/iNKT	5.7 (4.3-13.6)	13.1 (4.5-17.8)	0.40
IFN γ +/iNKT	43.3 (26.6-63.7)	51.0 (21.4-63.6)	0.85
MAIT/CD3+ ^c	1.1 (0.7-2.5)	1.0 (0.7-1.7)	0.56
IFN γ +/MAIT ^d	43.4 (40.5-50.1)	57.6 (46.2-71.1)	0.46
IL-17+/MAIT (MAIT17) ^d	1.6 (1.0-3.2)	2.0 (0.8-5.7)	0.75
V δ 2+/CD3+ ^e	1.3 (0.7-3.3)	3.0 (2.4-3.2)	0.68
IFN γ +/V δ 2+ ^e	74.1 (51.6-87.6)	82.6 (80.8-84.9)	0.68
IL-17+/ V δ 2+ ^e	0.33 (0.06-0.80)	0.57 (0.22-1.04)	0.94

Abbreviations: OCS, oral corticosteroids; IL, interleukin; IFN γ , interferon- γ ; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cells

Data are medians (25-75 interquartile range)

^a Only severe asthmatics of GINA 5 step were included in the analysis (n=39)

^b Mann-Whitney U-test

^c 23 asthmatics were included in the analysis

^d 10 asthmatics were included in the analysis

^e 12 asthmatics were included in the analysis

Table S6: Frequency of immune cells in peripheral blood according to smoking history in all asthmatics ^a

Cell type (in %)	Never smokers	Former smokers	<i>P</i> ^b
Number of patients	67	31	-
CD4+/CD3+	67.4 (61.1-74.6)	67.6 (63.0-73.9)	0.69
CD8+/CD3+	22.8 (18.0-29.5)	25.6 (15.8-27.5)	0.76
IL-4+/CD4+ (Th2)	2.9 (1.7-4.5)	2.3 (1.4-3.6)	0.18
IL-4+/CD8+ (Tc2)	1.1 (0.6-1.8)	0.9 (0.5-1.7)	0.39
IFN γ +/CD4+ (Th1)	17.6 (11.6-22.6)	15.2 (11.6-25.4)	0.93
IFN γ +/CD8+ (Tc1)	47.7 (33.0-61.8)	39.5 (33.7-60.9)	0.62
IL-17+/CD4+ (Th17)	1.3 (0.7-2.0)	0.9 (0.7-1.2)	0.04
Foxp3+/CD25+/CD4+ (Treg)	5.3 (4.3-6.3)	4.8 (4.3-6.1)	0.67
Ratio Th1/Th2	6.1 (4.0-8.2)	7.6 (5.7-9.5)	0.13
Ratio Th2/Th17	2.1 (1.4-3.0)	2.9 (1.6-3.2)	0.41
Ratio Th1/Th17	12.7 (8.6-19.3)	16.9 (10.7-23.3)	0.12
Ratio Th1/Treg	3.5 (2.3-4.2)	2.9 (1.9-4.8)	0.83
Ratio Th2/ Treg	0.57 (0.37-0.83)	0.47 (0.25-0.77)	0.40
Ratio Th17/Treg	0.25 (0.12-0.40)	0.17 (0.12-0.31)	0.18
iNKT/CD3+	0.08 (0.03-0.20)	0.06 (0.02-0.16)	0.38
IL-4+/iNKT	11.9 (4.6-23.2)	6.9 (3.4-14.8)	0.10
IFN γ +/iNKT	51.0 (35.9-65.5)	45.9 (27.8-63.4)	0.66
MAIT/CD3+ ^c	1.1 (0.5-2.3)	1.6 (0.7-3.2)	0.57
IFN γ +/MAIT ^d	48.2 (36.2-63.6)	62.6 (46.2-81.6)	0.24
IL-17+/MAIT (MAIT17) ^d	2.3 (0.8-6.4)	1.1 (0.7-2.3)	0.34
V δ 2+/CD3+ ^d	3.2 (1.4-3.6)	3.0 (1.1-4.1)	0.92
IFN γ +/V δ 2+ ^e	69.6 (51.7-79.8)	81.9 (77.4-91.0)	0.14
IL-17+/ V δ 2+ ^e	0.29 (0.13-0.55)	0.27 (0.10-0.96)	0.98

Abbreviations: IL, interleukin; IFN γ , interferon- γ ; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cells

Data are medians (25-75 interquartile range)

^a Data are available for 98 out of the 100 asthmatics included in the study

^b Mann-Whitney U-test

^c 55 asthmatics were included in the analysis

^d 27 asthmatics were included in the analysis

^e 23 asthmatics were included in the analysis

Bold denotes statistical significance

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3 **Circulating IL-4, IFN γ and IL-17 conventional and**
4 **Innate-like T cell producers in adult asthma**
5
6

7 Maria Leite-de-Moraes, PhD, Renata Belo, PharmD, PhD, Céline Dietrich, MSc,
8 David Soussan, MSc, Michel Aubier, MD and Marina Pretolani, PharmD, PhD
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16 **On-line repository**
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METHODS

Subjects

Between 2011 and 2014, 100 asthmatic patients belonging to the longitudinal multi-centre french COhort Bronchial Obstruction and Asthma (COBRA) were recruited at the Pneumology A Department of Bichat Hospital in Paris.^{S1} This cohort is aimed at identifying relevant outcomes and biomarkers in patients with asthma based on thorough initial investigation. We used the recommendations of the Global Initiative for Asthma (GINA) to classify the 100 asthmatics into mild-to-moderate persistent (n=28) and severe persistent (n=72). Among the 28 mild-to-moderated asthmatics, 2, 17 and 9 patients belonged to GINA 1, GINA 2 and GINA 3 steps, respectively and the 72 severe asthmatics were of GINA 4 (n=33) and GINA 5 (n=39) steps (Table S1). The group merging all asthmatics was referred as 'ALL'. We considered the frequency and the intensity of daytime and night-time asthma symptoms, the need and dose of medications and values of forced-expiratory volume in one sec (FEV₁) and forced vital capacity (FVC), before and after the inhalation of 400 µg of salbutamol (Table S1).^{S2} Severe asthmatics were recruited if they had a known diagnosis of severe asthma for at least 1 year and they underwent all clinical and paraclinical examinations, as described below. Severity at inclusion was confirmed according to inadequate symptom control and/or 2 or more exacerbations requiring oral corticosteroid (OCS) bursts in the 12 previous months before inclusion, despite at least 1000 µg of fluticasone equivalents, combined with long-lasting β₂-agonists. Patients declaring non-adherence to their asthma treatments were not considered for the study.

Demographic data, age at asthma onset, clinical examination, atopic manifestation and total serum IgE, smoking history, number of the exacerbations (defined as a worsening of asthma symptoms requiring OCS bursts) and medication were recorded. Asthma control was assessed using the score of Asthma Control Test (ACT) that is based on a response scale from 1 to 25, where a score higher than 21 over the last 4 weeks, indicated complete control, between 16 to 20 somewhat controlled, below 15 poorly controlled, and below 7.5 not controlled.^{S3,S4}

Fifty control healthy donors (HD) obtained from the EFS (*Etablissement Français du Sang*) were included for comparisons.

Cell type analyses

T-helper (Th)1, Th2, Th17, regulatory T-cells (Treg) (Foxp3⁺/CD25⁺/CD4⁺ type), type 1 and type 2 cytotoxic CD8⁺ T cells (Tc1, Tc2) and invariant Natural Killer T (iNKT) cells were

analysed in the 50 HC and in the 100 asthmatics, whereas Mucosal-associated invariant T (MAIT) and V δ 2 $\gamma\delta$ T (the major peripheral blood $\gamma\delta$ T subset) cells, were examined during the last two years of the study in 56 and in 24-28 asthmatics, respectively.

We also determined the ratios Th1/Th2, Th2/Th17, Th1/Treg, Th2/Treg, Th17/Treg and Tc1/Treg between HD and mild-to-moderate and severe asthmatics, that were considered as a whole group and defined as 'ALL' (Figure S2 and S3). Indeed, an imbalance in the frequency of these cells has been shown to participate in the pathogenesis of a variety of diseases, including asthma.^{S5-S8}

Flow cytometry analyses

Freshly isolated peripheral blood mononuclear cells (PBMC) were obtained by using Ficoll-Paque density centrifugation (1.077 g/mL; PAA Laboratories GmbH, Velizy Villacoublay, France). Cells were then resuspended and washed in fluorescence activated cell sorting (FACS) buffer (2% (w/v) Bovine Serum Albumin, 0.1% NaN₃ sodium azide in Phosphate Buffered Saline) then stained with fluorochrome-conjugated antibodies for 30 min at 4°C. Doublets were excluded using pulse-width and pulse-area and side scatter properties. MAIT, iNKT, V δ 2 and Treg cells were identified as CD3⁺TCRV α 7.2⁺CD161⁺, CD3⁺CD1d-PBS57 tetramers⁺, CD3⁺V δ 2⁺ and CD3⁺CD4⁺CD25⁺Foxp3⁺ cells, respectively (gate strategy in Figure S4). For intracellular cytokine analysis allowing the identification of IL-4, IL-17 and IFN γ -producing T cells (gate strategy in Figure S4), freshly isolated PBMC were cultured during 6h with 25 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μ M ionomycin, in the presence of 10 ng/ml Brefeldin A (all from Sigma-Aldrich, Saint-Quentin Fallavier, France). Cytokine-producing iNKT, MAIT, V δ 2⁺, CD4⁺ and CD8⁺ T cell subsets were identified by intracellular staining for 45 min with the following monoclonal antibodies: anti-IFN γ -PE-Cy7 (clone 4S.B3, eBioscience), anti-IL-17-PerCP-Cy5.5 (clone eBio64DEC17, eBioscience), as described (gate strategy in Figure S5).^{S9,S10} Cells were identified by flow cytometry using an eight-colour FACS CantoTM (BD Biosciences) and analysed with FlowJo 10.0.8r1 software (FlowJo).

Statistical analysis

Qualitative variables are reported as means $\pm$ SD and percentages and they were compared using Student's t test, or Fisher's exact test 2-tailed. Normally distributed quantitative variables were compared χ^2 test or one-way analysis of variance (ANOVA). Non-normal

variables were reported as median and 25-75 interquartile range (IQR) and compared using Kruskal-Wallis and Mann-Whitney U-tests.

Potential associations between biological and clinical variables have been assessed by using the nonparametric Spearman rank correlation coefficient (r). P values of less than 0.05 were considered significant. Data were analysed statistically with Prism 6.0 (GraphPad Software).

RESULTS

Clinical characteristics of asthmatics

Mild-to-moderate asthmatics were mostly atopic (82.1%), the majority were never smokers (55%), they had normal lung function, daytime and nighttime symptoms, and they were all treated with short-acting β 2-agonists alone, or in association with long-acting β 2-agonists (84.6%) and/or inhaled corticosteroids (ICS) (between 500-1,000 μ g/day of inhaled equivalents of beclomethasone) (Table S1). These patients showed partial or complete asthma controls with a mean ACT score of 18.4 and ≤ 1 exacerbations during the previous 12 months (mean = 0.4) (Table S1).

Severe asthmatics were also mainly never smokers (77% and 64% in GINA 4 and 5 steps, respectively) and their incidence of atopy tended to be lower than that of patients with mild-to-moderate disease (54.6 % and 67.7 % in GINA steps 4 and 5, respectively, as compared to 82.1 % in GINA 1-3 steps, overall $P=0.08$) (Table S1). These severe asthmatics also showed airflow obstruction, with mean values of pre- and post-bronchodilator $FEV_1 < 80\%$ predicted and of pre- and post-bronchodilator $FEV_1/FVC < 70\%$ and they were poorly controlled, with a mean ACT value ≤ 15 (mean values = 13.1 and 11.6 in GINA steps 4 and 5, respectively, $P=0.04$) and ≥ 3 severe exacerbations during the previous year (mean values = 2.5 and 5.3 in GINA steps 4 and 5, respectively, $P= 0.002$) (Table S1).

Correlation analyses between the distinct T cell populations

In a first series of analyses, the frequency of immune cells was compared between HD and ALL (Figures S1 to S3). In addition, correlation analyses between each cell type in HD and all asthmatics were performed (Table S4). We found highly positive associations between Th2 and Th1, Th2 and Th17, Th2 and Tc1 and Th2 and Tc2 in ALL (Table S4). This also applied to Th1 and Th17, Th1 and Tc1, Th1 and Tc2, Th17 and Tc2 and Tc1 and Tc2, but not to Th17 and Tc1 (Table S4). Results obtained in HD were globally similar to those of ALL, but with some discrepancies, since no significant associations were observed between Th2 and Th17, Th1 and Tc2 and Th17 and Tc2 (Table S4). No significant correlation was observed between these T cell populations and Treg, irrespective of the group analysed, eg. HD or ALL (Table

114 S4). Although these results indicated that Th1, Th2, Th17, Tc1 and Tc2 were globally present
115 in both HD and in ALL, the correlation between these T cell populations were stronger in the
116 latter than in the former group (in majority, $P < 0.001$) (Table S4). These findings support
117 previous studies showing a positive correlation between distinct immune cells in the
118 peripheral blood of asthmatic patients.^{S8,S11,S12} In addition, our results underline the
119 simultaneous presence of Th1, Th2, Th17, Tc1 and Tc2 profiles in asthmatics, irrespective of
120 their severity, and suggest that Treg do not influence the presence of these activated T cells.

121 **Effect of asthma treatments on T-cell populations**

122 We compared mild-to-moderate (GINA steps 1 to 3) and severe asthmatics (GINA steps 4 and
123 5) (Table S1), since the difference in the daily dose of ICS taken by these patient groups may
124 interfere with the incidence of circulating immune cells (Table S1). Indeed, the dose of ICS in
125 the first group (GINA 1,2 and 3) was below 1000 µg, making unlikely their systemic passage,
126 whereas systemic side effects have been reported for doses of ICS averaging 2000 µg (GINA
127 4 and 5) (Table S1).^{S13}

128 Severe asthmatics of the GINA 5 step were further divided into 2 groups, according to their
129 treatment with OCS (n=16 out of 39, i.e., 41%, Table S3) and/or omalizumab (n=18 out of 39,
130 i.e., 46.2%, Table S5) since these compounds may also modify the distribution of circulating
131 immune cells.^{S14-S16} Finally, in a further sub-analysis, severe GINA 5 asthmatics treated with
132 daily doses of ≤ 20 mg (n=9) or >20 mg (n=7) oral prednisone were compared (Table S3).

133 No differences in the frequency of immune cells were found in OCS-treated as compared to
134 -untreated severe asthmatics, although a trend towards a decrease in the proportion of IL-
135 4+/iNKT+ cells was observed ($P=0.11$, Table S3). When OCS-treated severe asthmatics were
136 separated into 2 groups according to the daily dose taken, the proportion of CD4+/CD3+ cells
137 was lower in the group treated with > 20 mg than those with ≤ 20 mg oral prednisone and this
138 difference was near to reach statistical significance ($P=0.06$, Table S3). These results confirm
139 early findings showing reduction of Th2-type cells by OCS in patients with asthma.^{S14}

140 Treatment with omalizumab of severe asthmatics of the GINA 5 step failed to alter
141 significantly the distribution of immune cells (Table S5). These findings contrast in part with
142 previous observations showing lower density of CD4+ and CD8+ T cells in the airways of
143 omalizumab-treated severe asthmatics,^{S17} although another study reported no effect on these
144 cells in nasal biopsy specimens of patients with seasonal allergic rhinitis.^{S18} To our best
145 knowledge, the modulatory effect of omalizumab on different T-cell sub-populations, or on
146 iNKT, $\gamma\delta$ -Tcells and MAIT cells in asthma, remains elusive. In addition, since our analyses, as

well as those considering treatment with OCS, have been conducted exclusively in severe patients of the GINA 5 step which number was relatively low, some differences in the proportion of immune cells in response to these treatments may have failed to achieve statistical significance.

Impact of atopy on T-cell populations

We also determined whether the atopic status would alter the incidence of the immune cells analysed in the whole group of asthmatics (Table S2).^{S19,S20} Atopy was established by positive skin prick tests to common allergens and serum levels of total IgE, with median values (25-75 interquartile range) of 102 (46-220) and 324 (150-817) international Units/mL in non-atopic (n=32) and atopic (n=67) asthmatics, respectively ($P<0.0001$). Using this classification, we found a significant lower incidence of MAIT17 cells ($P=0.05$) and a trend towards an increase in the proportion of IL-4+/iNKT cells ($P=0.12$) in atopic, as compared to atopic asthmatics (Table S2). These data support, in part, previous findings showing lower Th17 polarisation in non-atopic asthma,^{S21,S22} although other studies demonstrated that IL-17 may induce the production of Th2-type cytokines as well.^{S23}

Impact of smoking on T-cell distribution

Finally, to ascertain whether smoking history modified the distribution of immune cells,^{S24,S25} we compared never and former smoker asthmatics of all patient groups. We found lower proportions of IL-17+/CD4+ cells in former than in never smokers ($P=0.04$) (Table S6). This result was unexpected since tobacco smoke was shown to enhance IL-17 expression, at least in the airways.^{S26} However, in our patient cohort, active smokers were not included and the analyses were performed in the circulating and not the airway compartment.

FIGURE LEGENDS

Figure S1. Percentages of CD4⁺ or CD8⁺ among gated CD3⁺ (A, B) T cells. Frequency of IFN γ ⁺ (Th1) (C), IL-4⁺ (Th2) (D), IL-17⁺ (Th17) (E) among gated CD3⁺CD4⁺ or IFN γ ⁺ (Tc1) (F), IL-4⁺ (Tc2) (G) among gated live CD3⁺CD8⁺ cells. Ratio Th1/Th2 (H), Th2/Th2 (I) and Th1/Th17 (J) are represented. Analyses were performed in peripheral blood from healthy donors (HD) and mild-to-moderate plus severe asthmatics (ALL). Horizontal bars represent median values. *P* values are obtained by Mann-Whitney test.

Figure S2. Frequency of CD25⁺Foxp3⁺ (Treg) (A) among gated CD3⁺CD4⁺ T cells. Ratio Th1/Treg ratio (B), Th17/Treg (C), Tc1/ Treg (D) and Th2 /Treg (E) were calculated in healthy donors (HD) and in mild-to-moderate and severe asthmatics, who were considered as a whole group (ALL). Horizontal bars represent median values. *P* values were obtained by Mann-Whitney test.

Figure S3. Frequency of innate-like T cells. Percentages of V δ 2⁺ $\gamma\delta$ T cells among gated CD3⁺ (A) T cells. Frequency of IFN γ ⁺ (B), IL-17⁺ (C), among gated V δ 2⁺ $\gamma\delta$ T cells. Proportions of iNKT (CD1d-PBS57 tetramer⁺) among gated CD3⁺ (D) T cells. Frequency of IL-4⁺ (E), IFN γ ⁺ (F) among gated iNKT cells. Percentages of MAIT (TCRV α 7.2⁺CD161⁺) among gated CD3⁺ (G) T cells. Frequency of IFN γ ⁺ (H), IL-17⁺ (I), among gated MAIT cells. Cells were identified by FACS analysis in the peripheral blood of healthy controls (HD) and of mild-to-moderate and severe asthmatics, who were considered as a whole group (ALL). Horizontal bars show median values. *P* values were calculated using Mann-Whitney test.

Figure S4. Flow cytometry gating strategy used to identify iNKT (CD3⁺ CD1d-PBS57 tetramer⁺), V δ 2 $\gamma\delta$ T cells (CD3⁺ CD1d-PBS57 tetramer⁻ V δ 2⁺), MAIT (CD3⁺ CD1d-PBS57 tetramer⁻ V δ 2⁻ CD161⁺ TCRV α 7.2⁺), CD4⁺ (CD3⁺ CD1d-PBS57 tetramer⁻ V δ 2⁻ CD161⁻ TCRV α 7.2⁻ CD8⁻ CD4⁺), CD8⁺ (CD3⁺ CD1d-PBS57 tetramer⁻ V δ 2⁻ CD161⁻ TCRV α 7.2⁻ CD8⁺ CD4⁻) and Treg cells (CD3⁺ CD1d-PBS57 tetramer⁻ V δ 2⁻ CD161⁻ TCRV α 7.2⁻ CD8⁻ CD4⁺ CD25⁺ Foxp3⁺) from peripheral blood.

Figure S5. Flow cytometric gating strategy used to identify IFN γ ⁺, IL-17⁺ and IL-4⁺ among gated CD4⁺, CD8⁺ and MAIT cells, as identified in Figure S4.

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