



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

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

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25 M.L.M, M.A., M.P. designed the research.

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40 **ABBREVIATIONS**

41	ACT	Asthma control test
42	ALL	All asthmatics
43	FEV ₁	Forced expiratory volume in one sec
44	FVE	Forced vital capacity
45	HD	Healthy donors
46	GINA	Global Initiative for Asthma
47	ICS	Inhaled corticosteroids
48	IFN γ	Interferon- γ
49	IL	Interleukin
50	IL-17	Interleukin-17A
51	ILT	innate-like T cells
52	iNKT	invariant Natural Killer T cells
53	MAIT	Mucosal-associated invariant T cells
54	MAIT17	IL-17-producing Mucosal-associated invariant T cells
55	MOD	mild-to-moderate asthmatics
56	OCS	Oral corticosteroids
57	SEV	Severe asthmatics
58	Tc1	Type 1 cytotoxic CD8 ⁺ T cells
59	Tc2	Type 2 cytotoxic CD8 ⁺ T cells
60	TCR	T cell receptor
61	Th	T helper

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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 realised 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 ($P=0.08$) and Th1 ($P=0.06$) 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 overrepresented 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 overrepresented 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 non-atopic asthmatics ($P=0.12$) (Table S2). These data suggest iNKT are mainly associated with non-atopic 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.

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 -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

130 pathology. Further, MAIT17 were negatively correlated with IgE (Table 2) and their frequency
131 was higher in non-atopic than in atopic patients (Table S2). In addition, MAIT17 were
132 positively correlated with ACT score (Table 2). Together, these data suggest that these cells
133 may play a predominant role in non-atopic asthmatic patients. These results are in agreement
134 with our recent observations suggesting a role of MAIT17 in paediatric asthma.⁷ However,
135 further studies are required to confirm, or infirm the precise implication of MAIT in asthma.

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

141 Although descriptive, this study paves the way for future asthma analysis in large patient
142 cohorts. Overall, we suggest that the Th1/Th2 ratio and the frequency of MAIT17 should be
143 taken into consideration for better identifying distinct asthma endotypes and for testing the
144 clinical benefit of specific immunotherapies.

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

Cell type (in %)	Controls	Asthmatics			<i>P</i> ^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)	0.42
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)	0.96
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) †§	0.04
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)	0.08
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)	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) †	0.05
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)	0.47
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)	0.69
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)	0.06
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)	0.22
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)	0.44
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)	0.34
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)	0.69
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)	0.36
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)	0.64
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) ‡	0.05
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)	0.14
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)	0.18
IFN γ +/MAIT ^{c(0.92)}	45.9 (24.8-56.0)	56.9 (53.3-60.9)	38.5 (20.1-60.7)	48.0 (42.9-66.3)	0.38
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)	0.33
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)	0.27
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)	0.29
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)	0.72

bbreviations: IL, interleukin; IFN γ , interferon- γ ; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T cell
ata are medians (25-75 interquartile range)

Kruskal-Wallis test

55 asthmatics were included in the analysis

27 asthmatics were included in the analysis

28 asthmatics were included in the analysis

24 asthmatics were included in the analysis

P<0.05, as compared to controls, ‡ *P*<0.05, as compared to GINA 3 asthmatics; § *P*<0.05, as compared to GINA 4 asthmatics (Mann-Whitney U-test)

bold denotes statistical significance

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	0.640	-0.034	0.736	0.119	0.239	-0.013	0.902	0.114	0.304
CD8+/CD3+	-0.048	0.834	0.050	0.621	-0.089	0.378	0.073	0.487	-0.192	0.082
IL-4+/CD4+ (Th2)	-0.030	0.775	-0.183	0.078	-0.209	0.043	-0.004	0.967	-0.121	0.289
IL-4+/CD8+ (Tc2)	-0.022	0.835	-0.165	0.112	-0.122	0.239	-0.012	0.912	-0.104	0.362
IFN γ +/CD4+ (Th1)	-0.121	0.250	-0.217	0.035	-0.285	0.005	-0.147	0.178	0.029	0.799
IFN γ +/CD8+ (Tc1)	-0.134	0.204	-0.158	0.131	-0.185	0.075	-0.115	0.293	-0.038	0.740
IL-17+/CD4+ (Th17)	-0.134	0.200	-0.145	0.163	-0.166	0.109	-0.061	0.578	-0.005	0.961
Foxp3+/CD25+/CD4+ (Treg)	-0.100	0.346	-0.001	0.926	-0.056	0.594	-0.140	0.202	-0.017	0.884
Ratio Th1/Th2	-0.030	0.747	-0.030	0.790	-0.030	0.801	-0.060	0.587	0.140	0.219
Ratio Th2/Th17	0.280	0.007	-0.007	0.946	-0.079	0.447	-0.039	0.718	-0.045	0.690
Ratio Th1/Th17	0.218	0.036	-0.008	0.940	-0.106	0.307	-0.081	0.453	0.030	0.791
Ratio Th1/Treg	0.168	0.128	-0.188	0.084	-0.123	0.261	-0.116	0.307	0.062	0.604
Ratio Th2/ Treg	0.285	0.009	-0.190	0.080	-0.138	0.206	-0.0003	0.998	-0.069	0.563
Ratio Th17/Treg	-0.064	0.569	-0.143	0.192	-0.059	0.595	-0.072	0.526	0.084	0.480
iNKT/CD3+	-0.427	< 0.001	0.086	0.395	0.113	0.261	-0.049	0.644	0.106	0.342
IL-4+/iNKT	0.163	0.123	-0.185	0.078	-0.325	0.001	-0.054	0.625	0.255	0.025
IFN γ +/iNKT	-0.059	0.580	0.039	0.714	-0.234	0.026	-0.162	0.144	-0.026	0.821
MAIT/CD3+ ^b	-0.062	0.661	-0.082	0.551	0.218	0.110	0.093	0.515	-0.173	0.249
IFN γ +/MAIT ^c	0.279	0.167	-0.336	0.080	0.105	0.594	-0.276	0.202	0.295	0.194
IL-17+/MAIT (MAIT17) ^c	-0.439	0.025	0.120	0.543	-0.303	0.117	-0.113	0.607	0.451	0.040
V δ 2+/CD3+ ^c	-0.075	0.713	0.028	0.887	-0.010	0.956	-0.011	0.953	-0.037	0.858
IFN γ +/V δ 2+ ^c	-0.036	0.865	-0.198	0.331	0.310	0.135	0.216	0.310	-0.030	0.896
IL-17+/ V δ 2+ ^c	-0.239	0.260	-0.07	0.719	-0.133	0.515	-0.074	0.732	0.252	0.258

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

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

^b 56 asthmatics were included in the analysis

^c 28 asthmatics were included in the analysis

^d 24 asthmatics were included in the analysis

Bold denotes statistical significance

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

Parameter	ALL	Asthmatics, according to severity			P ^a
		MOD	SEV		
		GINA steps 1-3 ^b	GINA step 4	GINA step 5	
No. of patients (%)	100	28	33	39	-
Women – no. (%)	58 (58.0)	16 (57.1)	18 (54.6)	24 (61.5)	0.84
Age – years	50.1 ± 15.2	50.5 ± 17.4	51.5 ± 16.5	48.5 ± 12.3	0.56
BMI – kg per m ²	26.6 ± 4.5	27.5 ± 4.5	26.4 ± 4.0	26.0 ± 5.0	0.22
Smoking history – former/never (%) ^c	30/66 (31/69)	9/18 (33/67)	7/23 (23/77)	14/25 (36/64)	0.52
Age of asthma onset – years	23.4 ± 18.9	23.2 ± 20.6	25.7 ± 19.3	21.7 ± 17.4	0.69
Asthma duration – years	27.0 ± 15.8	27.6 ± 14.0	25.2 ± 16.0	28.2 ± 17.0	0.84
With history of atopy – no. (%)	67 (67.0)	23 (82.1)	18 (54.6)	26 (66.7)	0.08
Blood eosinophils counts – 10 ⁹ per Liter	290 (180-450)	285 (200-385)	290 (190-550)	308 (110-510)	0.63
With ≥ 300 blood eosinophils – no. (%)	46 (46.0)	11 (39.3)	15 (45.5)	20 (51.3)	0.63
Total serum IgE – International Units per milliliter	221 (88-600)	415 (76-812)	176 (82-309)	220 (92-679)	0.17
Respiratory function					
Pre-bronchodilator FEV ₁ – % of predicted	73.9 ± 20.9	88.8 ± 16.0	71.1 ± 19.5 †	65.5 ± 19.9 †	< 0.0001
Post-bronchodilator FEV ₁ – % of predicted	79.9 ± 21.2	96.9 ± 14.3	76.8 ± 19.1 †	70.3 ± 20.2 †	< 0.0001
Pre-bronchodilator FEV ₁ /FVC – % of predicted	62.8 ± 12.3	66.8 ± 10.5	60.8 ± 12.2	61.6 ± 13.1	0.12
Post-bronchodilator FEV ₁ /FVC – % of predicted	65.5 ± 12.4	70.5 ± 9.0	63.8 ± 12.9 †	63.5 ± 13.4 †	0.03
Treatments					
On SABA – number (%)	100 (100.0)	28 (100.0)	33 (100.0)	39 (100.0)	-
On LABA – number (%)	93 (93.0)	23 (82.1)	32 (97.0)	38 (97.4) †	0.03
Daily dose of ICS ^c	1809 ± 1006	696 ± 249	2139 ± 761 †	2328 ± 907 †	< 0.0001
On maintenance use of OCS – no. (%)	16 (16.0)	0	0	16 (41.0)	-
Daily dose of oral prednisone – mg	25.3 ± 12.3	0	0	25.3 ± 12.3	-
On anti-IgE	18 (18.0)	0	0	18 (46.2)	-

Table S1 (continued)

Asthma control					
Score on ACT	13.5 ± 6.0	18.4 ± 4.5	13.1 ± 6.1 †	10.3 ± 4.5 †‡	< 0.0001
Annual rate of severe exacerbations requiring OCS bursts	3.0 ± 0.4	0.4 ± 0.2	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 χ² 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	P ^a	≤ 20 mg/day	> 20 mg/day	P ^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

¹
Table S4. Correlation analyses between Th2, Th1, Th17, Tc1 and Tc2 in healthy donors (HD) and asthmatic patients (ALL)^a

Cell types	HD		ALL	
	r	P	r	P
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

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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