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1 **Identification of the major immune differences in severe asthmatic children**
2 **according to their atopic dermatitis status**

3 **Running title: Children with severe asthma and atopic dermatitis**

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34 **Author contributions**

35 G.L., K.A.P., and M.L.M. designed research; M.G., and C.D., performed research; G.L., C.P., K.A.P., and
36 M.L.M. analyzed data; and G.L., K.A.P., and M.L.M. wrote the paper.

37 **ABSTRACT**

38 Severe asthma (SA) affects 2% to 5% of asthmatic children. Atopic dermatitis can affect up to 34% of
39 children with SA (cwSA). Atopic dermatitis and asthma share common genetic and immunological
40 features. However, not all children with SA suffer from AD, and it remains unclear whether the overall
41 immune profiles of these children are similar. In this study, seventeen cwSA (9.8 [7.1-13.2] years; seven
42 with and ten without AD) were enrolled. Bronchoalveolar lavage (BAL) and blood samples were
43 collected from these patients. Seventy-three cytokines/chemokines and distinct immune T cell
44 populations were evaluated in blood and BAL. We found that BAL and blood immune profiles of cwSA
45 with and without AD were globally similar. However, specific differences were observed, namely lower
46 frequency of Tc2, Th17 and IL-17-producing mucosal associated invariant T (MAIT-17) cells and higher
47 CD8/CD4 ratio and IL-22 concentrations in BAL and of CCL19 concentrations in plasma from cwSA with
48 AD. Further, in contrast with cwSA without AD, we found a positive correlation between a set of plasma
49 cytokines and almost all cytokines in BAL in cwSA with AD. In conclusion, this study shows the major
50 immune differences between cwSA with and without AD in BAL and blood suggesting that distinct
51 endotypes may be implicated in the inflammatory responses observed in these pediatric patients.

52

53 **Keywords:**

54 Asthma; atopic dermatitis; children; Bronchoalveolar lavage; T cells

55

56 **Abbreviations:**

57 AD, atopic dermatitis; BAL, Bronchoalveolar lavage; cwSA, children with SA; ILT, innate-like T (ILT) cells;
58 MAIT, mucosal-associated invariant T; SA; severe asthma

59

60

61 1. Introduction

62 Asthma is a heterogeneous immunological condition usually characterized by a chronic airway
63 inflammation, with different underlying disease processes. It is defined by the history of respiratory
64 symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in
65 intensity, together with variable expiratory airflow limitation (1-3). Asthma is a common chronic
66 respiratory disease affecting 1 to 18% of the general population depending on the country (4,5). The
67 prevalence of asthma in childhood is elevated and up to 7% of adolescents had severe asthma symptoms
68 (5).

69 It is currently accepted that the term asthma refers to several conditions with distinct mechanistic
70 pathways (endotypes) and variable clinical presentations (phenotypes) (6,7). This is why a better
71 understanding of the immune mechanisms involved in each endotype is crucial to the management of
72 asthma (8,9). Severe asthma (SA) affects 2% to 5% of children with asthma. Children with SA (cwSA)
73 suffer from frequent exacerbations despite being treated with inhaled corticosteroids and at least
74 another controller, good treatment adherence and technique and removal of environmental factors
75 such as tobacco (10,11). cwSA have a reduced quality of life. Association between asthma and other
76 atopic conditions such as atopic dermatitis (AD) was described in children (5,12). AD, also known as
77 eczema and atopic eczema, is one of the most common inflammatory disorders characterized by
78 intense itching and recurrent eczematous lesions (5,13). AD affects up to 20% of children and 10% of
79 adults in high-income countries (5,14,15). Previous studies described the progression of atopic
80 disorders from AD in infants to allergic rhinitis and asthma in children (16,17). In addition, it was
81 reported that up to 25% of children with current AD also have asthma (18).

82 Asthma and AD share common genetic and immunological features (19,20). These two pathologies
83 were originally considered to be mediated by an imbalance towards a T helper 2 (Th2) and exaggerated
84 IgE responses to allergens (8,12,13,20,21). In this context, biologics such as dupilumab (anti-IL-4Ra)
85 and omalizumab (anti-IgE) are effective treatments of moderate to severe asthma and AD (22-24).
86 However, not all cwSA have AD, and clinical responses to biologics may vary among cwSA, children
87 with AD or both (25). In addition, it remains unclear whether cwSA with current AD and cwSA without
88 AD have distinct local and systemic immune profiles. Here, we carried out an in-depth analysis of the
89 immune profile of cwSA according to the presence or absence of AD to determine the main immune
90 features they share and those that distinguish them. For this, we analyzed distinct T cell populations,
91 including innate-like T (ILT) cells as mucosal-associated invariant T (MAIT), invariant Natural Killer T
92 (iNKT) and $\gamma\delta$ T cells (26-29), and cytokine levels in both bronchoalveolar lavage (BAL) and blood from
93 cwSA with current AD and cwSA without AD.

94 **2. Materials and Methods**

95 **2.1. Subjects and sample collection**

96 CwSA included in this study were from the CLASSE (Cellules Lymphoïdes Innées dans l'Asthme Sévère
97 de l'Enfant) cohort (ClinicalTrials.gov Identifier: NCT03784781). They were recruited in the department
98 of Paediatric Pulmonology and Allergy of Necker Hospital (Paris, France). Institutional ethical approval
99 and written informed consent were obtained.

100 SA was defined by the persistence of symptoms despite the use of high doses of inhaled corticosteroid
101 (ICS) and another controller medication (10), confirmation of good adherence and inhalation
102 technique and control of underlying modifiable factors and environmental factors. Indeed, patients
103 were not included if their adherence or technique was considered poor by medical doctors, based on
104 both their history and how parents and children used the inhaler. Number of severe exacerbations
105 (SE), defined as exacerbations requiring bursts of systemic corticosteroids (≥ 3 days), were recorded.
106 Asthma control was assessed using the Asthma Control Test (ACT) based on a response scale from 1 to
107 25, where a score higher than 21 over the last 4 weeks, indicated complete control, between 16 to 20
108 somewhat controlled, below 15 poorly controlled, and below 7.5 not controlled (score 1).
109 Demographic data, age at asthma onset, total serum IgE and medication were recorded for each
110 patient. Children underwent clinical investigations, including flexible endoscopy with bronchoalveolar
111 lavage (BAL) collection and blood sampling, as previously described (30).

112 Moreover, atopic dermatitis (AD) status was defined by clinician's assessment as recommended (13)
113 and cwSA were divided into two groups based on their current AD. No cwSA had symptoms or findings
114 suggestive of current infection at the time of the bronchoscopy.

115 **2.2. Flow cytometry**

116 Blood and BAL were obtained from each patient on the same day. Freshly isolated peripheral blood
117 mononuclear cells (PBMC) were obtained by using Ficoll-Paque density centrifugation (1.077 g/mL;
118 PAA Laboratories GmbH, Velizy Villacoublay, France). BAL was immediately filtered through MACS
119 SmartStrainers (100 μ m) (Miltenyi Biotec, Paris, France) to remove mucus. BAL was centrifuged to
120 separate cells from fluids. BAL and blood samples were obtained at the same time and immediately
121 analyzed for T cells. No cells were frozen/thawed.

122 PBMC and BAL cells were washed in fluorescence-activated cell sorting buffer (2% w/v BSA, 0.1%
123 NaN_3 sodium azide in PBS) and then stained with eFluor506–fixable viability dye (ThermoFisher
124 Scientific, Illkirch, France) for 10 minutes at 4°C. Then, cells were stained with the following
125 fluorochrome-conjugated antibodies for 30 min at 4°C: anti-CD3, anti-CD4, anti-CD8, anti-CD161, anti-

126 V δ 2 and anti-TCRV α 7.2. Doublets were excluded using pulse-width and pulse-area and side scatter
127 properties. MAIT and V δ 2 cells were identified as CD3⁺TCRV α 7.2⁺CD161⁺ and CD3⁺V δ 2⁺ cells,
128 respectively. For intracellular cytokine analysis allowing the identification of IL-4, IL-13, IL-17A, and
129 IFN γ -producing T cells, PBMC and BAL cells were cultured during 5h with 25 ng/ml phorbol 12-
130 myristate 13-acetate (PMA) and 1 μ M ionomycin, in the presence of 10 ng/ml Brefeldin A (all from
131 Sigma-Aldrich, Saint-Quentin Fallavier, France). Events were acquired on a FACS LSR Fortessa (BD
132 Biosciences) and analyzed using FlowJo software v10.8.1 (Becton, Dickinson and Company, Ashland,
133 USA). CD8⁺/CD4⁺ ratio was calculated as the percentage of CD8⁺ among gated CD3⁺ cells divided by
134 the percentage of CD4⁺ among gated CD3⁺ cells. Gate strategies are showed in **Figure 1** and the
135 antibodies and reagents used for flow cytometry analysis are listed in **Supplementary Table 1**.

136 **2.3. Cytokines in BAL and plasma**

137 Cytokines were analysed using xMAP[®] Luminex technology and the associated apparatus
138 (Bioplex[®]200, Biorad, Marnes-la-Coquette, France). Forty chemokines (Bio-Plex Pro[™] Human
139 chemokine assays, 40-plex; BioRad) and 37 inflammation markers (Bio-Plex Pro[™] Human Inflammation
140 Panel I, 37-plex; BioRad) were analysed on BAL diluted 1/2 and plasma diluted 1/4, following the
141 manufacturer's recommendations, as previously reported (20). Due to a small amount of redundancy
142 between the two multiplex assays used, 73 cytokines were assayed: APRIL/TNFSF13, BAFF/TNFSF13B,
143 sCD30/TNFRSF8, sCD163, Chitinase 3-like 1 (CHI3L1), CCL21 (6Ckine), CXCL13 (BCA-1), CCL27 (CTACK),
144 CXCL25 (ENA-78), CCL11 (Eotaxin), CCL24 (Eotaxin-2), CCL26 (Eotaxin-3), CX3CL1 (Fractalkine), CXCL6
145 (GCP-2), GM-CSF, CXCL1 (Gro- α), CXCL2 (Gro- β), CCL1 (I-309), gp130/sIL-6RB, sIL-6RA, IFN α 2, IFN β ,
146 IFN γ , IL-1 β , IL-2, IL-4, IL-6, IL-8 (CXCL8), IL-10, IL-11, IL-12p40, IL-12p70, IL-16, IL-19, IL-20, IL-22, IL-26,
147 IL-27 (p28), IL-28A (IFN- λ 2), IL-29 (IFN- λ 1), IL-32, IL-34, IL-35, CXCL10 (IP-10), CXCL11 (I-TAC), CCL2
148 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP4), CCL22 (MDC), MIF, CXCL9 (MIG), CCL3 (MIP-1 α),
149 CCL15 (MIP-1 δ), CCL20 (MIP-3 α), CCL19 (MIP-3 β), CCL23 (MPIF-1), CXCL16 (SCYB16), CXCL12 (SDF-
150 1 α + β), CCL17 (TARC), CCL25 (TECK), LIGHT/TNFSF14, MMP-1, MMP-2, MMP-3, Osteocalcin,
151 Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TLSP, TNF α and TWEAK/TNFSF12.

152 **2.4. Statistical analysis**

153 Data were expressed as the median (interquartile range). Differences between groups were assessed
154 with the Mann-Whitney or Fischer's exact test (two tailed) for categorical data. Correlation analyses
155 were made using Spearman rank correlation analysis (two tailed). $p < 0.05$ was considered to be
156 significant. The Benjamini-Hochberg FDR method was used to correct for multiple comparisons. Data
157 were analysed using GraphPad Prism version 10.1.0 (GraphPad Software, Inc, San Diego, Calif).

158

159 **3. Results**

160 **3.1. Clinical characteristics of patients**

161 Data from seventeen cwSA (9.8 [7.1-13.2] years) were analyzed. Seven children had concomitant AD
162 (cwSA with AD) and ten did not (cwSA without AD). Demographic and clinical characteristics are
163 summarized in **Table 1**. The number of severe exacerbations in the past year, the number of
164 exacerbators among cwSA, ACT score, lung function, IgE levels and the number of blood cells
165 (leukocytes, eosinophils, neutrophils and lymphocytes) did not differ between groups. Among the
166 clinical parameters, only the dose of ICS was higher in cwSA with AD ($p=0.03$).

167 **3.2. The frequency of IL-17-producing MAIT (MAIT-17) and CD4+ (Th17) cells was significantly lower**
168 **in BAL from cwSA with AD**

169 Here, we systematically profiled in BAL Th1, Th2 and Th17 cells identified as IFN γ -, IL-4- and IL-13- and
170 IL-17-producing CD4+ T cells, respectively. Type 1 and Type 2 cytotoxic (Tc1 and Tc2) CD8+ T cells (IFN γ
171 and IL-4 producers, respectively) were also analyzed. Among ILT cells, we focused on IFN γ - and IL-17-
172 producing MAIT and on IFN γ -producing V δ 2+ γ δ T cells. No significant differences were observed
173 between the frequency of the majority of these T cell populations in BAL (**Table 2**). Despite all these
174 similarities, the ratio CD8/CD4 was higher while the frequency of Tc2, Th17 and IL-17-producing MAIT
175 (MAIT-17) cells in BAL was lower in cwSA with AD than in those without AD (**Figure 2A – 2D**). Further,
176 we found a negative correlation between the frequency of Th2 and Th17 cells in BAL from cwSA with
177 AD ($p=0.048$, $r= -0.786$) but not without AD ($p=0.263$, $r= 0.394$).

178 The analysis of circulating Th1, Th2, Th17, Tc1, Tc2 and cytokine-producing MAIT and V δ 2+ γ δ T cells
179 showed no significant difference between these populations in the peripheral blood of cwSA with AD
180 compared to those without AD (**Table 3**).

181 **3.3. IL-22 and CCL19 were significantly enhanced in cwSA with AD**

182 Further, we analyzed distinct cytokines in BAL and plasma from cwSA with versus without AD. No major
183 differences were observed except for IL-22 and CCL19 levels that were respectively higher in BAL and
184 plasma from cwSA with AD compared to cwSA without AD (**Table 4**). A positive correlation was
185 observed between the levels of BAL and plasma CCL19 in cwSA with AD ($r=0.793$; $p=0.04$) but not in
186 CwSA without AD ($r=0.292$; $p=0.56$).

187 **3.4. Correlation of soluble cytokines between BAL and plasma**

188 Spearman correlations showed that most of the cytokines analyzed were positively correlated with
189 each other in BAL from cwSA without AD (**Figure 3A**) and with AD (**Figure 3B**). In plasma, a particular

190 core of cytokines was more strongly positively correlated with each other, mainly in samples from
191 cwSA with AD (**Figure 3B**). This core included IFN α 2, IFN β , IFN δ 1, IFN δ 2, IFN γ , IL-10, IL-11, IL-12p70, IL-
192 19, IL-2, IL-20, IL-22, IL-27p28, IL-32, IL-34, IL-35, IL-4, IL-6 (green square).

193 We also determined whether correlations could also exist between cytokines present in BAL and those
194 present in plasma. This was not the case for the majority of cytokines from cwSA without AD (**Figure**
195 **3A**). However, we found in cwSA with AD a clear positive correlation between a set of plasma cytokines
196 (IFN α 2, IFN β , IFN δ 1, IFN δ 2, IFN γ , IL-11, IL-12p40, IL-19, IL-2, IL-20, IL-22, IL-32, IL-34, IL-35, IL-4 and IL-
197 6) and almost all cytokines present in BAL (**Figure 3B**, orange rectangle).

198 **4. Discussion**

199 Our study provided a deep comparative analyze of the immune profile of cwSA with versus without
200 AD. A large set of variables, including cytokines, chemokines, immune cells, and immunoglobulins,
201 were analyzed both in periphery and in BAL. The access to these samples is crucial because the levels
202 of many variables in the airways and blood did not correlate with each other in cwSA (30-32) and here
203 we measured several variables in both BAL and blood. Among the immune parameters analyzed, six
204 were significantly different between the groups and among them five in BAL. In fact, cwSA with AD had
205 less Th17, MAIT-17 and Tc2 cells but enhanced CD8/CD4 T cell ratio and IL-22 in BAL and higher levels
206 of CCL19 in plasma compare to cwSA without AD. This confirms that invasive analyses performed in
207 BAL are necessary for a better understanding of the local actors and mechanisms responsible for SA
208 associated with other pathologies as AD.

209 The analysis of T cell populations showed that the ratio CD8/CD4 was significantly higher in BAL from
210 cwSA with AD than without AD. It is possible that the balance between these cells reflects the
211 involvement of distinct immune mechanisms. CD8+ T cells, mainly Tc1 cells, are critical to anti-viral
212 defense (33), but cwSA presented no viral infection at the time of the bronchoscopy. Nevertheless,
213 CD8+ Tc2 cells, another subset of CD8+ T cells, produce type 2 cytokines (34). The implication of type
214 2 cytokines on atopic diseases including asthma and AD was confirmed by the efficacy and safety of
215 dupilumab (anti-IL-4R α) treatment (22,35,36). Th2 cells are commonly considered as a major source
216 of IL-4 in asthma. However, Tc2 cells may also play an important role in asthma pathogenesis (34,37).
217 Indeed, Tc2 cells have been detected in blood and lung samples from adult asthmatic patients, their
218 numbers often correlating with long-term decline in lung function, poor symptom control or death
219 (34,38,39). Tc2 cells were also associated with severe AD (40). Here, we found that Tc2 cells were
220 reduced in BAL from cwSA with AD. It was suggested that Tc2 cells could be more steroid-resistant
221 type 2 cytokine producers than Th2 cells (34). However, this does not seem to explain our results, since
222 cwSA with AD received higher doses of ICS than cwSA without AD. Future studies focused at the in-
223 depth molecular and functional characterization of Tc2 cells and their role in pediatric SA will likely
224 provide valuable new insight into the pathophysiology of uncontrolled disease.

225 In addition to the implication of Th2-type cytokines in SA and AD, the involvement of Th17 cells in the
226 pathogenesis of these pathologies have also been reported (41-43). The percentage of circulating Th17
227 cells in AD patients was higher and associated with disease severity, but it has also been suggested
228 that the presence of IL-17 in the skin may be primarily a defense mechanism against infection (44,45).
229 Concerning pediatric SA, it was reported that the frequency of Th17 cells in the bronchial mucosa and
230 BAL did not differ between asthmatics and controls and we did not find significant association between

231 the frequency of Th17 cells and the number of severe exacerbations in cwSA (30-32). Here, we found
232 that cwSA with AD presented lower frequency of Th17 cells in BAL and that the frequency of Th17 and
233 Th2 negatively correlated in BAL from cwSA with AD. It is possible that in cwSA with AD, the
234 differentiation of CD4+ Th2 cells was favored at the expense of CD4 Th17 cells.

235 MAIT cells are currently described as being implicated on the mucosal defense against infections
236 (46,47) but they can also contribute to SA pathology since they are present in BAL from cwSA (30). Our
237 previous report (30) identified two groups of cwSA according to MAIT-17 frequency in BAL: a group
238 with high (MAIT-17^{high}) and a group with low (MAIT-17^{low}) frequency of MAIT-17 cells. Children in the
239 MAIT-17^{high} group was more prone to severe exacerbations (30). Here, the frequency of MAIT-17 was
240 lower in BAL from cwSA with DA, but no significant difference in the number of severe exacerbations
241 was observed between the two groups of patients. It was reported in adults with SA that oral
242 corticosteroid (OCS) could decrease the frequency of circulating MAIT cells, but this study did not
243 analyze MAIT-17 cells (48). Here, we found that cwSA with AD used higher doses of ICS (not of OCS)
244 than cwSA without AD. We therefore cannot exclude that ICS may contribute to the reduced frequency
245 of MAIT-17 cells observed in BAL from cwSA with DA.

246 IL-22 belongs to the IL-10 cytokine family and is produced by T cells as Th17, type 3 innate lymphoid
247 cells as well as neutrophils (49). Elevated BAL and serum IL-22 levels in adult patients with SA have
248 been reported (50,51). It was suggested that IL-22 could play a role in certain endotypes of asthma as
249 neutrophilic asthma (52). Further, IL-22 was reported to be implicated in AD and the anti-IL-22
250 monoclonal antibody (fezakinumab) improved AD clinical scores (53,54). The levels of IL-22 were
251 higher in BAL from cwSA with DA but these patients presented no significant increase in the frequency
252 of neutrophils in BAL when compared to cwSA without DA. Experimental models reported that IL-22
253 promoted allergic airway inflammation and hyperreactivity in intranasal antigen challenge of mice
254 sensitized epicutaneously (55). These results associated with our observations suggest that cutaneous
255 sensitization, as can occur in cwSA with AD, may elicit IL-22 production in BAL then contributing to lung
256 inflammation.

257 CCL19, which primarily regulates cell trafficking, plays an important role in the balance between
258 immunity and tolerance (56,57). The higher levels of CCL19 in the plasma of cwSAs with AD could result
259 from the production of this chemokine by fibroblasts in the area infiltrated by leukocytes in AD lesions,
260 since it has been described that CCL19 gene expression was strongly upregulated in the lesions of adult
261 AD patients (58). A positive correlation was found between CCL19 levels in BAL and plasma of cwSA
262 with AD, suggesting that this chemokine may also be involved in airway inflammation in these patients.

263 A compelling finding of the present study is the observed positive correlation between a panel of
264 cytokines present in plasma, namely IFN α 2, IFN β , IFN δ 1, IFN δ 2, IFN γ , IL-11, IL-12p40, IL-19, IL-2, IL-20,
265 IL-22, IL-32, IL-34, IL-35, IL-4 and IL-6, with the majority of cytokines present in BAL from cwSA with
266 AD. This phenomenon was specific to this group, as it was not observed in cwSA without AD. Prior to
267 the present study, the correlation between BAL and plasma cytokines in cwSA with AD have not been
268 studied in detail. In our previous study, we reported that almost no correlation between BAL and blood
269 concentrations of any soluble constituents was observed in cwSA (31,32). Our results may indicate
270 that, in cwSA with AD, increased levels of this group of cytokines in their plasma may reflect increased
271 levels of these cytokines in their BAL. These observations could provide valuable information on airway
272 inflammation in cwSA with AD. The present results need to be confirmed in another cohort with more
273 patients but, if confirmed, it could contribute to better understand the global immune profile from
274 cwSA with compared to without AD.

275 Our study has limitations. We are aware that our sample size was small and future studies performed
276 on independent cohorts are required to confirm our results. However, we would like to draw attention
277 to the fact that it is difficult to obtain BAL samples from cwSA and that this may explain the limited
278 number of studies on T cells present in this type of invasive sample. The severity of AD was not assessed
279 and no healthy controls were included due to ethical reasons, so we were not able to distinguish
280 pathological variation from normal expression.

281 In summary, this study compared for the first time the immune profile in BAL and blood of cwSA with
282 or without AD. Our results showed that immune profiles of these groups were globally similar but
283 specific differences were observed, namely lower frequency of Tc2, Th17 and MAIT-17 cells and higher
284 CD8/CD4 ratio and IL-22 concentrations in BAL and enhanced CCL19 concentrations in plasma from
285 cwSA with AD.

286 Our results support the idea that targeted analyses in cwSA with versus without AD will potentially
287 allow us to dissect the relative contribution of different endotypes to the inflammatory responses
288 observed in these patients.

289

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501 **Legends to figures.**

502 **Figure 1.** Representative flow cytometric gating strategies used to identify distinct BAL and circulating
503 T cell populations. (A) Flow cytometric gating strategy to identify CD3⁺ T cells among gated CD45⁺
504 cells. Debris, dead cells and doublets were gated out. (B) Flow cytometric gating strategy to identify
505 invariant Natural Killer T (iNKT) (CD1d-PBS-57 tetramers+CD3⁺), V δ 2⁺ $\gamma\delta$ T cells, mucosal-associated
506 invariant T (MAIT) (TCRV α 7.2+CD161+iNKT-V δ 2⁻), CD4⁺ and CD8⁺ cells within CD3⁺ T cells (MAIT-iNKT-
507 V δ 2⁻). (C) Gate strategy to identify IL-4⁻, IL-13⁻, IL-17A⁻ and IFN γ -producing cells among gated CD4⁺,
508 CD8⁺, MAIT, V δ 2⁺ as represented in B. Data represent PBMC cells.

509

510 **Figure 2.** Ratio and frequency of distinct T cell populations present in BAL from cwSA without (green
511 circles) and with (blue squares) AD. **A.** Ratio of CD8⁺/CD4⁺ among gated CD3⁺ T cells. **B-D.** Percentages
512 of Tc2 (IL-4⁺ among gated CD8⁺ T cells), Th17 (IL-17A⁺ among gated CD4⁺ T cells) and MAIT-17 (IL-17⁺
513 among gated CD3⁺TCRV α 7.2⁺CD161⁺ MAIT cells) in BAL from cwSA with or without AD. p values are
514 indicated. p<0.05 was considered as significant. Mann-Whitney test.

515

516 **Figure 3.** Heatmap of Spearman correlations for cytokines analyzed in BAL and plasma from cwSA
517 without (**A**) and with (**B**) AD. Data for 66 cytokines, representing the most significant correlations, are
518 shown. Positive correlations are shown in red, the absence of a correlation in white, and negative
519 correlations in blue. The intensity of the color indicates the intensity of the correlation. Blue squares
520 indicate correlations between soluble components from BAL and BAL and plasma and plasma, while
521 yellow and green squares indicate respectively the major correlations between soluble components
522 from BAL and plasma or plasma and plasma.

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524

525 **Table I.** Demographic and clinical characteristics of children with SA belonging to the group with AD
 526 or the group without AD.

Characteristics	cwSA without AD	cwSA with AD	p value
Study participants, n	10	7	
Age (y)	9.7 (7.7 - 11.4)	12 (7-14.6)	0.59
Sex: Female/male	4/6	2/5	>0.99
BMI (kg/m ²)	16 (15 - 22)	21 (16 - 28)	0.25
Post-BD FEV1 (%)	113 (98 - 118)	109 (99 - 116)	0.94
Post-BD FEV1/FVC (%)	87 (79 - 92)	91 (81.7 - 95.9)	0.41
SE in the past year, n	5 (1 - 10)	3 (2 - 7)	0.87
Exacerbators, n	7	4	0.64
ACT score	18 (13 - 21)	14 (8 - 22)	0.42
Allergic rhinitis	7/10	6/7	0.60
ICS (mg/d equivalent fluticasone)	500 (500 - 500)	750 (500 - 1000)	0.03
Circulating total IgE levels (ku/ L)	801 (229 - 1016)	1054 (150 - 1432)	0.51
Blood leukocytes (x 10 ⁻³ /mL)	6.8 (5 - 7.8)	6.6 (4.6 - 7.8)	>0.99
Blood eosinophils x 10 ⁻³ /mL)	0.6 (0.25 - 1.15)	0.4 (0.3 - 1)	0.87
Blood neutrophils x 10 ⁻³ /mL)	2.7 (1.95 - 3.95)	2.9 (1.9 - 3.8)	>0.99
Blood lymphocytes x 10 ⁻³ /mL)	2.6 (1.80 - 2.95)	2.2 (1.6 - 2.8)	0.63

527 Exacerbators: > 2 SE in the past year

528 *Abbreviations:* AD, atopic dermatitis; BMI, body mass index; BD, bronchodilator; cwSA, children with
 529 severe asthma; FVC, force vital capacity; ICS, inhaled corticosteroid; SA, severe asthma; SE, severe
 530 exacerbations. Data are represented as the median (interquartile range). Mann-Whitney test (2-
 531 tailed) or Fischer exact test (2-tailed) were used. Boldface denotes statistical significance (p<0.05).

532

533 **Table 2.** Frequency of immune cells in bronchoalveolar lavage of children with severe asthma (cwSA)
 534 with or without atopic dermatitis (AD).

Cell type (%)	cwSA without AD	cwSA with AD	p value
CD3+/CD45+	81.9 (77.8-90.3)	81.6 (66.2-91.4)	0.89
CD4+/CD3+	33.1 (31.5-39.1)	30.7 (19.9-35)	0.19
IFN γ +/CD4+ (Th1)	33.5 (18.4-75.1)	46.0 (15.2-60.7)	0.36
IL-4+/CD4+ (Th2)	3.7 (1.9-4.8)	2.9 (0.9-10.5)	0.60
IL-13+/CD4+	3.3 (1.4-5.8)	4.7 (1.5-19.6)	0.54
CD8+/CD3+	54.7 (45.1-59.7)	61.3 (54.8-72)	0.23
IFN γ +/CD8+ (Tc1)	56.2 (23.7-92)	44.4 (32.8-90.8)	0.54
IL-13+/ CD8+	0.3 (0.1-1.1)	0.1 (0.1-1.1)	0.87
IL-17+/ CD8+	1.9 (0.6-7.3)	1.1 (0.6-4.1)	0.54
iNKT/CD3+	0.06 (0.02-0.1)	0.01 (0-0.10)	0.30
MAIT/CD3+	1.1 (0.8-2.1)	1.3 (0.9-2.3)	0.74
IFN γ +/MAIT	50.1 (30.1-61.9)	57.9 (45-74.6)	0.23
IL-4+/ MAIT	1.6 (0.4-3.3)	0.6 (0-15)	0.79
IL-13+/ MAIT	2.4 (0-5.6)	5.0 (0.9-12.9)	0.43
V δ 2+/CD3+	1.4 (0.3-9.1)	1.3 (0.5-2.2)	0.72
IFN γ +/V δ 2+	54.0 (43.3-72.5)	66.7 (23.9-74.6)	>0.99
IL-4+/ V δ 2+	0.4 (0-1.9)	0 (0-0.6)	0.12
IL-13+/ V δ 2+	0.2 (0-0.9)	0.41 (0-7.7)	0.39

535

536 *Abbreviations:* MAIT, mucosal associated invariant T; Th, T helper; Tc, T cytotoxic.

537 In the column "cell type (%)", the symbol "/" means "among gated".

538 Data are represented as the median (interquartile range). Mann-Whitney test (2-tailed) was used.

539

540

541 **Table 3.** Frequency of immune cells in peripheral blood of children with severe asthma (cwSA) with or
 542 without atopic dermatitis (AD).

Cell type (%)	cwSA without AD	cwSA with AD	p value
CD3+/CD45+	70.9 (65.6-77.2)	67 (65.2-69.4)	0.23
CD4+/CD3+	63.8 (57.8-68.6)	64.7 (61.2-69.9)	0.98
IFN γ +/CD4+ (Th1)	6.2 (4.2-13.1)	7.5 (5.9-10)	0.69
IL-4+/CD4+ (Th2)	2.1 (1.5-3.1)	2 (1.7-2.5)	0.94
IL-13+/CD4+	2.4 (1.6-3.1)	2.5 (2.3-2.9)	0.81
IL-17+/CD4+ (Th17)	0.6 (0.5-1.2)	0.9 (0.5-1.4)	0.58
CD8+/CD3+	30.8 (24.7-35.3)	29 (24.7-34.2)	0.94
IFN γ +/CD8+ (Tc1)	21.1 (10.6-33.1)	24.3 (11.9-30.7)	>0.99
IL-4+/CD8+ (Tc2)	0.4 (0.2-0.6)	0.3 (0.2-1.3)	0.65
IL-13+/CD8+	0.4 (0.3-0.9)	0.3 (0.3-0.6)	0.58
IL-17+/CD8+	0.1 (0-0.1)	0.1 (0-0.2)	0.65
Ratio CD8+/CD4+	0.5 (0.4-0.6)	0.4 (0.3-0.6)	0.96
iNKT/CD3+	0.09 (0-0.1)	0.05 (0-0.1)	0.11
MAIT/CD3+	1.2 (0.6-2.8)	2.1 (1.2-4.2)	0.36
IFN γ +/MAIT	49.6 (40.1-63.6)	50.6 (18.4-65.3)	>0.99
IL-4+/MAIT	0.5 (0.2-0.8)	0.4 (0.2-0.8)	0.67
IL-13+/MAIT	0.8 (0.2-1.5)	0.7 (0.3-1.2)	0.89
IL-17+/MAIT	2.5 (1.7-3.2)	1.2 (0.7-2.4)	0.13
V δ 2+/CD3+	5.8 (3.2-7.7)	2.9 (2.7-7.9)	0.36
IFN γ +/V δ 2+	65 (48.3-85.6)	83 (66.4-84.7)	0.23
IL-4+/V δ 2+	0.6 (0-1.7)	0.3 (0-0.4)	0.40
IL-13+/V δ 2+	0.2 (0-1.2)	0.2 (0.1-0.4)	0.76

543

544 *Abbreviations: MAIT, mucosal associated invariant T; Th, T helper; Tc, T cytotoxic.*

545 In the column "cell type (%)", the symbol "/" means "among gated".

546 Data are represented as the median (interquartile range). Mann-Whitney test (2-tailed) was used.

547

548 **Table 4.** Cytokine levels in BAL and plasma from children with severe asthma (cwSA) with or without atopic
 549 dermatitis (AD).

	BAL			Plasma		
	cwSA without AD	cwSA withAD	<i>p</i>	cwSA without AD	cwSA withAD	<i>p</i>
APRIL*	81.7 (26.1-114.8)	107.8 (42.2-310.3)	0.315	101.4 (75.6-132.3)	106.1 (96.4-122.4)	0.618
BAFF*	0.87 (0.79-2.21)	1.48 (0.97-2.41)	0.459	6.46 (5.02-16.57)	6.55 (5.99-7.32)	0.813
CCL1	39 (36-66)	42 (36-48)	0.812	70 (57-87)	59 (50-78)	0.490
CCL11	13 (12-25)	19 (16-20)	0.459	78 (61-112)	74 (54-104)	>0.999
CCL13	88 (43-158)	114 (78-139)	0.601	200 (183-234)	213 (142-305)	0.740
CCL15	307 (202-866)	643 (460-831)	0.133	3079 (2493-5421)	2931 (2280-4080)	0.669
CCL17	16 (14-19)	19 (18-23)	0.126	83 (72-182)	197 (65-219)	0.459
CCL19	21 (18-74)	45 (26-47)	0.377	138 (115-164)	175 (174-188)	0.031
CCL2	12 (8-19)	23 (12-26)	0.161	93 (66-535)	86 (81-129)	0.887
CCL20	17 (8-318)	21 (15-81)	0.887	18 (13-61)	16 (12-21)	0.832
CCL21*	0.35 (0.27-1.01)	0.64 (0.46-0.74)	0.601	7.59 (5.64-10.07)	11.12 (8.66-12.22)	0.070
CCL22	38 (28-80)	47 (40-71)	0.650	455 (387-585)	595 (564-621)	0.133
CCL23	32 (13-42)	36 (28-57)	0.230	182 (35-231)	114 (95-335)	0.813
CCL24	290 (70-660)	172 (126-344)	0.740	215 (117-493)	205 (129-254)	0.813
CCL25	246 (136-442)	217 (186-273)	0.690	325 (274-392)	256 (219-420)	0.270
CCL26	17 (10-36)	14 (11-17)	0.685	114 (87-136)	152 (87-199)	0.230
CCL27	11 (8-26)	17 (16-20)	0.268	401 (321-558)	472 (372-570)	0.315
CCL3	6 (4-14)	7 (6-11)	0.740	7 (6-118)	7 (6-12)	0.514
CCL7	47(33-97)	52 (40-66)	0.905	87 (70-131)	80 (66-87)	0.581
CCL8	1 (1-3)	1 (1-2)	0.867	17 (11-28)	20 (17-30)	0.364
CHI3L1*	3.97 (2.66-7.40)	6.62 (4.32-13.3)	0.315	9.98 (6.42-13.6)	13.3 (8.06-19.3)	0.364
CX3CL1	129 (69-664)	157 (117-224)	0.601	277 (210-424)	277 (218-326)	0.867
CXC13	4 (3-40)	4 (2-8)	0.431	165 (155-276)	160 (155-221)	0.813
CXCL1	3437 (1840-5101)	6592 (2472-20079)	0.669	49 (38-61)	42 (30-99)	0.887
CXCL10	128 (63-466)	143 (136-354)	0.887	68 (51-98)	87 (46-112)	>0.999
CXCL11	6 (2-23)	7 (3-20)	0.793	463 (357-521)	542 (463-609)	0.193
CXCL12	56 (46-97)	68 (51-76)	0.757	17 (13-25)	17 (13-24)	0.962
CXCL16	1254 (802-1385)	1376 (642-1582)	0.417	958 (718-1109)	1061 (854-1233)	0.315
CXCL2	19 (16-39)	27 (16-42)	0.457	49 (35-62)	43 (33-54)	0.826
CXCL5	418 (229-879)	697 (463-924)	0.193	168 (124-844)	97 (0-386)	0.254
CXCL6	673 (293-4442)	1298 (461-1875)	0.364	53 (31-112)	54 (49-98)	0.721
CXCL9	423 (102-1624)	575 (230-1165)	0.813	223 (159-272)	227 (220-475)	0.535
GMCSF	29 (24-49)	33 (26-40)	>0.999	29 (26-39)	28 (19-31)	0.458
IFNα2	2 (1-2)	2 (1-2)	0.868	10 (8-12)	7 (5-10)	0.161
IFNβ	28 (21-40)	39 (17-42)	0.584	130 (102-169)	123 (115-147)	0.813
IFNδ1	9 (0-37)	22 (9-35)	0.473	228 (199-401)	200 (183-288)	0.402
IFNδ2	8 (5-13)	14 (4-19)	0.459	111 (97-152)	94 (67-116)	0.260
IFNγ	11 (10-22)	13 (9-16)	0.867	62 (55-74)	60 (41-63)	0.417
IL-10	19 (17-30)	25 (23-29)	0.417	46 (39-66)	33 (30-43)	0.070
IL-11	1 (0-2)	1 (1-2)	0.549	24 (19-39)	20 (19-30)	0.489
IL-12p40	58 (45-89)	61 (40-81)	>0.999	525 (461-780)	481 (420-517)	0.520
IL-12p70	5 (0-5)	5 (1-5)	0.117	5 (0-15)	5 (0-5)	0.531
IL-16	437 (291-844)	545 (315-698)	0.887	291 (246-904)	410 (214-547)	0.962
IL-17	4 (3-5)	6 (5-7)	0.059	5 (0-12)	3 (2-135)	0.990
IL-19	69 (40-403)	101 (65-177)	0.417	211 (185-245)	175 (146-226)	0.298
IL-1β	67 (46-134)	101 (78-109)	0.536	20 (16-53)	17 (14-21)	0.403
IL-2	4 (3-6)	5 (4-5)	0.489	26 (23-32)	21 (20-35)	0.536
IL-20	14 (12-16)	14 (14-16)	0.722	92 (85-113)	87 (62-126)	0.475
IL-22	11 (9-12)	14 (12-21)	0.013	109 (85-133)	96 (73-154)	0.618

IL-26*	0.52 (0.35-0.69)	0.49 (0.35-0.79)	0.865	7.54 (6.63-7.92)	7.08 (5.94-7.50)	0.364
IL-27p28	72 (52-84)	82 (67-111)	0.183	335 (289-384)	316 (242-371)	0.758
IL-32	291 (268-352)	326 (288-432)	0.364	659 (590-912)	616 (421-799)	0.459
IL-34	384 (353-514)	407 (384-529)	0.349	729 (570-960)	562 (480-937)	0.521
IL-35	133 (97-188)	138 (108-214)	0.489	963 (828-1117)	698 (639-903)	0.059
IL-4	34 (20-48)	20 (15-30)	0.228	40 (29-56)	26 (23-44)	0.165
IL-6	3 (2-7)	6 (3-6)	0.304	4 (3-62)	4 (3-6)	0.128
IL-8	86 (52-444)	159 (91-367)	0.417	182 (146-1449)	127 (117-221)	0.984
MIF*	34.6 (26.9-49.3)	36.7 (18.2-47.7)	0.962	1.45 (0.81-4.38)	2.22 (1.22-3.90)	0.549
MMP1	119 (94-143)	95 (82-127)	0.349	529 (494-723)	475 (419-689)	0.364
MMP2*	0.78 (0.51-0.99)	0.60 (0.15-0.94)	0.304	105.9 (89.7-139.3)	90.6 (62.6-136.8)	0.459
MMP3*	0.32 (0.02-0.78)	0.63 (0.02-0.86)	0.518	7.93 (6.28-9.80)	7.41 (6.77-9.16)	0.315
Osteocalcin*	0.05 (0.03-0.07)	0.02 (0.09-0.05)	0.103	28.4 (13.4-33.1)	23.5 (16.9-27.1)	0.887
Osteopontin*	0.56 (0.37-0.86)	0.57 (0.46-0.61)	0.718	132.5 (73.5-194.4)	93.0 (60.9-139.3)	0.813
Pentraxin3*	0.43 (0.21-1.16)	0.78 (0.35-1.47)	0.377	45.1 (36.1-54.1)	38.5 (21.1-57.7)	0.417
sCD163*	43.8 (26.6-80.8)	75.7 (28-84.5)	0.620	180.5 (119.2-219.7)	175.6 (121-195)	0.364
sCD30	39 (27-44)	38 (27-47)	0.982	3497 (2502-4651)	3266 (1929-5143)	0.601
sIL-16RB*	5.66 (2.53-9.67)	7.20 (3.61-12.1)	0.475	118.3 (83.4-141.1)	99.1 (85.8-127.7)	0.601
sIL-6RA*	0.38 (0.23-0.65)	0.39 (0.20-0.91)	0.740	28.4 (23.0-42.9)	31.7 (23.4-53.8)	0.887
sTNFR1	251 (131-407)	245 (224-379)	0.813	1470 (1049-1895)	1335 (1008-1602)	0.887
sTNFR2	112 (100-156)	136 (100-164)	0.553	957 (914-1080)	945 (807-965)	0.962
TNFα	13 (10-35)	19 (13-21)	0.490	26 (23-39)	25 (14-36)	0.364
TNFSF14	22 (20-24)	25 (21-26)	0.258	78 (66-114)	95 (60-133)	0.651
TSLP	9 (6-13)	10 (6-12)	0.981	78 (66-96)	57 (55-87)	0.757
TWEAK	313 (313-170)	402 (258-680)	0.377	977 (784-1110)	620 (571-899)	0.193

550 All cytokine values are expressed in pg/ml, except those marked with *, which are expressed in ng/ml. Data are represented
551 by the median (interquartile range). The Mann-Whitney test (two-tailed) was used. p-values are indicated. p<0.05 was
552 considered significant and indicated in bold.

553