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1 **IL-4 and IL-17 are required for house dust mite-driven airway hyperresponsiveness in**
2 **autoimmune diabetes-prone non obese diabetic mice**

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14
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20 M.L.M., L.C. designed the research.

21 A-P.F., F.M., C.D., C.P. performed the research.

22 A-P.F., M.L.M. analyzed the data.

23 A-P.F., M.L.M., L.C. wrote the manuscript.

24 **KEY WORDS:** asthma, NOD mice, IL-17, exacerbations, iNKT cells

25

26 **Abbreviations**

27 AHR: airway hyperreactivity

28 BALF: bronchoalveolar lavage fluid

29 HDM: house dust mite

30 ILT: innate-like T cells

31 iNKT: invariant Natural Killer T cells

32 NOD: Non Obese Diabetic

33

34

35 **Abstract**

36 Allergic asthma is characterized by airway inflammation with a Th2-type cytokine profile, hyper-IgE
37 production, mucus hypersecretion and airway hyperreactivity (AHR). It is increasingly recognized that
38 asthma is a heterogeneous disease implicating complex immune mechanisms resulting in distinct
39 endotypes observed in patients. In this study, we showed that Non Obese Diabetic (NOD) mice, which
40 spontaneously develop autoimmune diabetes, undergo more severe allergic asthma airway
41 inflammation and AHR than pro-Th2 BALB/c mice upon house dust mite (HDM) sensitization and
42 challenge. The use of IL-4-deficient NOD mice and the in vivo neutralization of IL-17 demonstrated that
43 both IL-4 and IL-17 are responsible by the exacerbated airway inflammation and AHR observed in NOD
44 mice. Overall, our findings indicate that autoimmune diabetes-prone NOD mice might become useful
45 as a new HDM-induced asthma model to elucidate allergic dysimmune mechanisms involving Th2 and
46 Th17 responses that could better mimic some asthmatic endotypes.

47

48 **Introduction**

49 Asthma is a heterogeneous immune pathology characterized by wheeze, cough, shortness of breath,
50 chest tightness and variable degrees of airflow limitation. These symptoms are associated with
51 different patterns of inflammation (1-5). Commonly, asthma is distinguished in two types: allergic and
52 non-allergic. In the first case, inflammation is primarily caused by type 2 immune responses mediated
53 through the Th2 cytokines IL-4, IL-5, and IL-13 and associated with increased Th2 cells and eosinophils
54 in the airways (6,7). By contrast, non-allergic asthma is mainly triggered by an inflammatory response
55 to viral infections with a major neutrophilic component (7). There is mounting evidence that
56 neutrophilic forms of murine and human asthma are associated with IL-17A (hereafter referred to as
57 IL-17) (8-10).

58 Th2 and Th17 inflammatory pathways in asthma are currently in investigation (11-13). It was reported
59 that therapeutic targeting of Th2 and Th17 cytokines resulted in the amplification of activity of the
60 opposing pathway (14). The cross-talk between these pathways is complex and further analysis are
61 required to better understand this complexity.

62 We have previously reported using a classical asthma protocol induced by sensitization and challenge
63 with ovalbumin (OVA) that Non Obese Diabetic (NOD) mice, which spontaneously develops insulin-
64 dependent diabetes (15, 16), presented an exacerbated Th2-mediated airway inflammation and AHR
65 (17). Further, we reported that NOD mice were prone to produce pro-Th17 cytokines and that IL-17-
66 producing iNKT (iNKT17) cells (18,19) were overrepresented in these mice (20). These observations led
67 us to examine in more detail whether both Th2 and Th17 inflammatory pathways were implicated in
68 the exacerbated airway inflammation in NOD mice. Here we used a more relevant allergic asthma
69 allergen, the house dust mite (HDM) extracts, and analyzed the airway inflammation by comparing
70 NOD and the Th2 prone BALB/c mice. We show that both IL-4 and IL-17 are critically implicated in the
71 exacerbated AHR observed in NOD mice.

72

73 **Materials and Methods**

74 *Mice.*

75 Eight to ten-week-old specific pathogen-free NOD and BALB/c mice were bred in our facility. All animal
76 experiments were carried out according to the guidelines for care and use of animals approved by the
77 French Institutional Committee (APAFIS#4105-201511171831592).

78

79 *Airway allergen sensitization and challenge model.*

80 Mice were immunized by intra nasal (i.n.) injection of 100 µg of HDM extracts (Greer laboratories, USA)
81 in 0.2 ml saline solution. Mice were then challenged on days 7, 11 and 17 with i.n. HDM (50 µg/mouse)
82 or saline solution. Twenty-four hours after the last challenge, mice were anesthetized with a mixture
83 of ketamine (150 mg/kg) and xylazine (400 µg/kg) and their tracheas were cannulated (tracheostomy
84 with ligation). A FlexiVent apparatus (SCIREQ) was used to measure airway-specific resistance (R_n, tidal
85 volume of 10 ml/kg at a respiratory rate of 150 breaths/min in response to increasing doses of
86 aerosolized acetyl-β-methylcholine chloride (methacholine; Sigma-Aldrich). Assessments were
87 performed at least three times and the maximum R value obtained after each dose of methacholine
88 was used for the measure.

89 Airway inflammation was assessed on cytospin preparations of cells from bronchoalveolar lavage fluid
90 (BALF, 3 x 0.5 mL washes with PBS) that were stained with May-Grünwald/Giemsa (Merck). For some
91 experiments, BALF cells were also analysed by flow cytometry.

92 Serum was collected and total IgE and HDM-specific IgG1 were measured by ELISA.

93

94

95 *Lung histology.*

96 Lungs were fixed with 10% formalin via the trachea, removed and stored in 10% formalin. Lung tissues
97 were embedded into paraffin and 3 µm sections were stained with periodic acid Schiff (PAS) using
98 standard protocols and examined with a light microscope.

99

100 *Flow cytometry.*

101 BALF or lung mononuclear cells were stained at 4°C in staining buffer (1X PBS, 2% FCS, 2mM EDTA), in
102 the presence of Fc block (2.4G2; BD Biosciences) and analyzed by flow cytometry.

103 Cells were incubated with CD1d-PBS57-APC tetramers and/or the specific antibodies listed below. For
104 intracellular staining, cells were further fixed with 4% PFA, washed, and permeabilized with 0.5%
105 saponin (Sigma-Aldrich), and then incubated with the anti-cytokine antibodies. The cells were washed
106 and fluorescence was detected using a LSRFortessa (Becton Dickinson). Data were analysed using the
107 FlowJo 10.4.1 software (Tree Star). **Figure S1** represents the gate strategy used.

108 Antibodies from BD Biosciences: anti-CD3-FITC (145-2C11), anti-CD45-APC-Cy7 (30F11). Antibodies
109 from BioLegend: anti-CD4-Brilliant Violet 605 (RM4-5), anti-CD69-FITC (H1.2F3), anti-CD8a-Brilliant
110 Violet 785 (53-6.7), anti-TCR V γ 1/Cr4-PE (2.11) (Tonegawa 1986 nomenclature, 21). Antibodies from
111 eBioscience: anti-CD44-eFluor450 (IM7), anti-TCRb-AlexaFluor 700 (H57-598), anti-IL-13-PE-eFluor610
112 (eBio13A), anti-IL-17A-PerCP-Cy5.5 (eBio17B7), anti-IL-4-PE-Cy7 (BVD6-24G2), anti-TCR δ -eFluor450
113 and -PE-Cy7 (GL3) and Fixable viability dye eFluor 506.

114

115 *Leucocytes from lung tissue.*

116 After measurement of airway resistance and collection of BALF, lungs were perfused with PBS, lung
117 tissues were cut into pieces using a GentleMACS Dissociator (Miltenyi Biotec) and treated with

118 collagenase type 4 (Thermo Fischer Scientific) plus DNase I (Roche). The lymphocyte-enriched fraction
119 was collected at the 35-70% interface of Percoll gradients (GE Healthcare). Cells were immediately
120 stained or stimulated for 4 h with with 10^{-8} M PMA and $1 \mu\text{g ml}^{-1}$ ionomycin, in the presence of $10 \mu\text{g}$
121 ml^{-1} brefeldin A (all from Sigma-Aldrich).

122

123 *Measurement of total IgE and HDM-specific IgG1 by ELISA*

124 Total IgE was measured in the serum by using a mouse IgE ELISA set (BD Biosciences) according to
125 supplier's recommendations. An indirect ELISA method was used to measure HDM-specific IgG1 levels
126 in serum samples as previously described by Trompette *et al.* (22). Briefly, 96-well microtiter plates
127 were coated overnight with $100 \mu\text{l}$ of HDM at $10 \mu\text{g/ml}$ in PBS. The next day, $200 \mu\text{l}$ of blocking solution
128 (1% BSA in PBS) was added to the plate for 2 hours at room temperature. Subsequently, $100 \mu\text{l}$ of
129 serum sample diluted 1:100 and 1:500 in blocking buffer was added to the plate at 4°C overnight
130 followed by goat-anti-mouse IgG1 (Southern Biotech) for 1 hour. Then HRP-donkey anti-goat IgG
131 (Santa Cruz) was added to the plate for 1 hour at room temperature, followed by the substract TMB.
132 Absorbance was measured at 450 nm using a microplate reader (VersaMax microplate reader,
133 Molecular Devices). No HDM-specific IgG1 was detected in control mice.

134

135 *mRNA expression.*

136 RNAs were extracted using the RNeasy Plus Minikit (Qiagen) including a DNase treatment. Then RNA
137 was reverse transcribed using the High Capacity RNA-to-cDNA Kit (ThermoFisher Scientific), according
138 to the manufacturer's instructions. Primers and probes for real-time PCR were provided by
139 ThermoFisher Scientific under references: beta-2 microglobulin: Mm00437762_m1; interleukin 5:
140 Mm00439646_m1; interleukin 13: Mm00434204_m1; interleukin 17A: Mm00439618_m1; Mucin 5b
141 (Muc5b): Mm00466391_m1. All reactions were performed in triplicate with TaqMan® Fast Advanced

142 Master Mix according to the supplier's instructions for a Step One Plus apparatus (ThermoFisher
143 Scientific). All data were normalized to the internal standard, namely beta-2 microglobulin expression
144 in each sample, and expressed as relative expression using the $\Delta\Delta C_t$ method versus the reference
145 sample.

146

147 *Statistics.*

148 Data are expressed as means \pm SEM. The AHR values were analysed with 2-way repeated measures
149 ANOVA followed by Bonferroni correction as a post-hoc test. All other values were analysed with
150 Mann-Whitney U test. Results were considered significant at a *P* value of 0.05 or less (**p*<0.05;
151 ***p*<0.01; ****p*<0.001). Data were analysed using GraphPad Prism version 6 (GraphPad Software).

152

153

154 **Results**

155 *NOD mice display exacerbated airway inflammation in response to HDM challenge.*

156 Here, we used a HDM-induced asthma model, consisting in intra-nasal (i.n.) immunization followed by
157 three i.n. challenges on days 7, 11 and 17. No adjuvant was added. Mice were sacrificed 24h later. We
158 found that total IgE tended to be higher in NOD compared to the Th2-prone BALB/c mice, but the
159 difference was not statistically significant (1.55 ± 0.13 and $2.12 \pm 0.62 \mu\text{g/ml}$ mean \pm s.e.m. for BALB/c
160 and NOD, respectively). However, circulating HDM-specific IgG1 was enhanced in NOD compared to
161 BALB/c mice (**Figure 1A**). Another cardinal feature of asthma, the percentage and the numbers of
162 airway eosinophils were also augmented in NOD mice (**Figure 1B**). No eosinophils, neutrophils or
163 lymphocytes were observed in BALF from BALB/c or NOD control mice treated with saline solution.
164 Further, we found that NOD mice presented a higher airway hyperreactivity (AHR) as compared to
165 BALB/c mice (**Figure 1C**). Of note, the HDM-induced asthma protocol used here barely induced AHR in
166 BALB/c mice (**Figure 1C**) clearly showing that NOD mice strongly reacted to low doses of HDM
167 sensitization and challenge.

168 HDM-sensitized and -challenged NOD (hereafter referred to as NOD HDM) mice expressed increased
169 levels of IL-4, IL-5, IL-13 and mucin mRNA compared to HDM-sensitized and -challenged BALB/c
170 (BALB/c HDM) mice (**Figure 1D**). In addition, HDM challenge in NOD mice led to increased PAS⁺ goblet
171 cell metaplasia (**Figure 1E**).

172 *Activation and cytokine production by lung ILT and conventional T cells in HDM-treated BALB/c versus*
173 *NOD mice.*

174 The distinct cytokine profiles generated during allergic asthma result from activation of both
175 conventional and nonconventional T, also named innate-like T (ILT), cells, such as invariant Natural
176 Killer T (iNKT) and $\gamma\delta$ T. Our previous studies demonstrated that iNKT and $\gamma\delta$ T cells contribute to the
177 development of major asthma hallmarks in experimental models (23, 24). Here we provide evidence

178 for reduced iNKT cell frequency in the lung of NOD HDM mice, relative to those recovered from BALB/c
179 mice (**Figure 2A**). The few iNKT cells that did remain in the lung produced less IL-4 than their BALB/c
180 counterpart, but promptly secreted IL-17) (**Figure 2B**), thereby revealing an overrepresentation of IL-
181 17 producers (iNKT17) at the expense of IL-4 producers. These results confirm previous reports
182 showing quantitative iNKT cell deficiency in thymus and spleen of NOD mice (25, 26). Further, our data
183 also confirm that the remaining iNKT cells in NOD mice are mainly iNKT17 (20).

184 In contrast to iNKT cells, total $\gamma\delta$ T cells as well as the $V\gamma 1^+$ $\gamma\delta$ T subset, were increased in the lung of
185 NOD HDM mice (**Figure 2C and 2D**). However, they were similarly biased in favor of a pro-Th17 profile,
186 since total $\gamma\delta$ T cells (**Figure 2C**) as well as the $V\gamma 1^+$ $\gamma\delta$ T subset (**Figure 2D**) generated low IL-4 and $V\gamma 1^+$
187 $\gamma\delta$ T subset high IL-17 levels in the lung of NOD HDM compared to BALB/c HDM mice (**Figure 2D**).

188 Analysis of conventional $TCR\alpha\beta^+CD4^+$ T cells revealed that they were more activated in BALF from NOD
189 HDM than from BALB/c HDM mice, as assessed by a higher expression of the CD69 marker (**Figure 2E**).

190 In contrast to nonconventional iNKT and $\gamma\delta$ T cells, both Th2 and Th17 profiles were increased among
191 conventional $TCR\alpha\beta^+CD4^+$ T cells infiltrating the lung of NOD mice, as demonstrated by a higher
192 proportion of IL-4⁺, IL-13⁺ as well as IL-17⁺ subsets, by comparison with their BALB/c counterpart
193 (**Figure 2F**). These findings indicated an inherent pro-Th2 and pro-Th17 potential in NOD HDM mice.

194 *Reduced airway inflammation in NOD IL-4KO mice.*

195 Knowing that HDM model is globally considered as IL-4 dependent in BALB/c mice (27-29), we first
196 assessed the implication of this cytokine in the severe airway inflammation observed in NOD HDM
197 mice. We addressed the role played by IL-4-producing cells in aggravating asthma, using NOD IL-4KO
198 mice sensitized and challenged with HDM (NOD IL-4KO HDM). In the absence of IL-4, these mice were
199 unable to mount high airway eosinophilia but presented a higher frequency of neutrophils in BALF
200 (**Figure 3A and 3B**). Further, their expression of mucin mRNA and of PAS⁺ goblet cells were decreased,
201 compared to NOD HDM mice (**Figure 3C and 3D**). It is noteworthy that AHR was lower in NOD IL-4KO
202 HDM than in NOD HDM mice (**Figure 3E**). However, AHR observed in NOD IL-4KO HDM remained higher

203 than in control saline treated NOD or NOD IL-4KO mice, suggesting the implication of additional
204 mechanisms.

205 *IL-4 and IL-17 are required for the development of airway inflammation in NOD mice.*

206 Bearing in mind that IL-17 is involved in certain severe cases of asthma likely by regulating neutrophilic
207 inflammation (10) together with our present observation that iNKT, total $\gamma\delta$ T, $V\gamma 1$ and $CD4^+$ T cells
208 from the lung of NOD HDM mice produced high levels of this cytokine (**Figure 2**), and that NOD IL-4KO
209 HDM mice presented higher levels of neutrophils in the airway (**Figure 3A and 3B**), we further
210 examined whether IL-17 contributed to the airway inflammation and AHR observed in NOD IL-4KO
211 HDM mice. IL-17 mRNA expression was enhanced in the lung of NOD IL-4KO HDM compared to NOD
212 HDM (**Figure 4A**) supporting the possible implication of IL-17 in the residual inflammation observed in
213 NOD IL-4KO HDM mice. To assess IL-17 implication, NOD IL-4KO HDM mice were treated *in vivo* with a
214 neutralizing anti-IL-17 mAb. These animals developed lower airway eosinophilia and neutrophilia
215 compared to Ig treated NOD IL-4KO HDM mice (**Figure 4B**). Additionally, anti-IL-17 treatment
216 significantly decreased IL-5 and tended to decrease IL-13 mRNA expression in the lung of NOD IL-4KO
217 HDM mice (**Figure 4C**). Finally, AHR was decreased in NOD IL-4KO HDM mice when compared to both
218 NOD IL-4KO HDM Ig and NOD HDM WT (**Figure 4D**). No significant difference was observed between
219 NOD IL-4KO HDM treated with anti-IL-17 and NOD control, indicating that both IL-4 and IL-17 were
220 required for increasing AHR. These results agree with previous observations showing that anti-IL-17
221 treatment could dampen neutrophil influx in BALF and airway hyperreactivity in mice (30).

222

223 **Discussion**

224 Autoimmunity and allergy are two major examples of dysimmune diseases. They are both caused by
225 an uncontrolled immune response against self or non-self-antigens involving Th1 or Th2 mechanisms,
226 respectively. This notion has become more complex since it turned out that Th17 cells could also play

227 a part in this process. NOD mouse spontaneously develops insulin-dependent diabetes, a prototypic
228 Th1-mediated autoimmune disease (31, 32). These animals are also prone to produce pro-Th17
229 cytokines (20). These observations led us to examine in more detail the inflammatory response of NOD
230 mice in a typical Th2-mediated disease.

231 Here we demonstrated that NOD mice presented enhanced airway inflammation and AHR in response
232 to HDM sensitization and challenge when compared to the Th2-prone BALB/c mice. Both IL-4 and IL-
233 17 were required for the severity of the symptoms. Our previous report showed that NOD mice
234 developed a more pronounced Th2-mediated inflammatory response to OVA-alum sensitization and
235 challenge compared to BALB/c mice (17). Using an HDM-induced asthma protocol, where HDM
236 extracts were administrated intranasally and without adjuvants in mice, we could get inside the
237 mechanisms implicated in the exacerbated airway inflammation and AHR observed in NOD mice. In
238 fact, we demonstrated, by using NOD IL-4KO mice and by blocking IL-17 in vivo, that both Th2- and
239 Th17-mediated immune responses were implicated.

240 Conventional CD4⁺ T cells were the major source of Th2 cytokines while iNKT, V γ 1⁺ and CD4⁺ produced
241 IL-17 in the lung of NOD mice. We previously reported that iNKT cells were implicated in airway
242 eosinophilia observed in NOD mice sensitised and challenged with OVA but the mechanisms remained
243 to be determined (17). Here we showed that the frequency and the IL-4-producing capacity of iNKT
244 cells in the lung of NOD mice were impaired. The ability of remaining iNKT cells to produce high levels
245 of IL-17 suggest that iNKT17 cells could contribute to airway inflammation, as described (18), and AHR
246 observed in NOD mice. It is noteworthy that both iNKT and V γ 1⁺ T cells, in contrast to conventional
247 CD4⁺ T cells, do not recognize peptides (33-35). These ILT cells can promptly produce cytokines
248 following TCR-dependent or -independent activation (33-35). Taken these findings into account, we
249 could consider that the exacerbated airway inflammation and AHR observed could result from a
250 simultaneous and combined activation of conventional and ILT cells in the lung of NOD mice.

251 Previous studies reported that Th2 and Th17 inflammatory pathways are reciprocally regulated in
252 asthma (14). Here we confirm these results since NOD IL-4KO HDM presented higher IL-17 mRNA
253 expression and airway neutrophilia than NOD HDM animals indicating that in the absence of IL-4, IL-
254 17 could induce a compensatory airway inflammation in NOD mice. Further, IL-17 blockage sufficed to
255 reduce airway neutrophilia, IL-5 and IL-13 lung mRNA expression and AHR in NOD IL-4KO HDM mice.
256 It was already described that IL-17 blockage could impair both neutrophilia and airway smooth muscle
257 contraction in response to HDM sensitization and challenge in BALB/c mice (30). Our findings clearly
258 indicate that Th2 and Th17 inflammatory responses contribute to asthmatic airway inflammation and
259 AHR observed in NOD mice. However, further studies are required to better determine whether Th2
260 and Th17 responses act together or independently to induce huge airway inflammation and AHR in
261 NOD mice.

262 Studies in patients indicated positive associations between asthma and type 1 diabetes (36, 37).
263 However, the mechanisms implicated are still unclear. The co-occurrence of asthma and type 1
264 diabetes is in direct opposition to the proposed inhibitory model where Th1 and Th2-mediated
265 immune responses could be exclusive. Our present findings support the idea that pathologies
266 associated with pro-Th1, pro-Th2 and pro-Th17 immune responses could co-exist since NOD mice,
267 which spontaneously develop autoimmune diabetes, presented high Th2 and Th17 airway
268 inflammatory responses. Therefore, NOD mice could represent a unique model to better understand
269 the key mechanisms implicated in the association between allergic airway inflammation and
270 autoimmune diabetes. Future studies are required to determine for instance, as reported in humans
271 (37), whether the asthma protocol using HDM sensitization and challenge could modify the incidence
272 of diabetes in NOD mice.

273

274 **Conclusion**

275 In summary, we show that NOD mice, which spontaneously develop autoimmune diabetes, undergo
276 more severe allergic asthma airway inflammation and hyperreactivity than pro-Th2 BALB/c mice upon
277 HDM sensitization and challenge. Our data support the conclusion that increased secretion of both IL-
278 4 and IL-17 by pulmonary conventional CD4⁺ and innate-like T lymphocytes is the major cause of this
279 exacerbated airway inflammation leading to increased severity in NOD mice. We identified iNKT, V γ 1⁺
280 and CD4⁺ T cells as sources of IL-17 and Th2 cells as IL-4 producers in the lung of HDM NOD mice. In
281 our NOD HDM model, both eosinophils and neutrophils were recruited into the airways. It has already
282 been reported that asthmatic patients whose airways are infiltrated with both eosinophils and
283 neutrophils suffer from more severe symptoms than those recruiting either eosinophils or neutrophils
284 (3,4,7). It is our belief that autoimmune diabetes-prone NOD mice might become useful as a new HDM-
285 induced asthma model to elucidate allergic dysimmune mechanisms involving Th2 and Th17 responses
286 that could better mimic some asthmatic endotypes observed in patients in association or not with
287 autoimmune diseases.

288

289

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398 **LEGEND TO FIGURES**

399 **Figure 1.** Severe allergic-induced asthma phenotype in NOD mice. **(A)** Specific IgG1 in HDM-treated
400 BALB/c and NOD mice (n=9). **(B)** Percentage of eosinophils and macrophage (Mac), eosinophil (Eos),
401 neutrophil (Neu) and lymphocyte (Lym) counts were determined in BALF of HDM-treated BALB/c and
402 NOD mice (n=15). No eosinophils were detected in the lung of control mice. **(C)** Lung resistance was
403 measured 24h after the last challenge with HDM or saline (n=10). **(D)** IL-4, IL-5, IL-13 and Mucin mRNA
404 expression assessed by quantitative RT-PCR in the lung of HDM-treated BALB/c and NOD mice (n=9).
405 **(E)** Representative PAS-stained lung histology sections of saline controls and HDM-treated BALB/c and
406 NOD mice (N=5). Statistical significance was determined between HDM-treated BALB/c and NOD mice.
407 * $p < 0.05$, ** $p < 0.01$. Scale bars: 50 μ m.

408 **Figure 2.** Activation and cytokine production by lung ILT and conventional T cells in HDM-treated
409 BALB/c versus NOD mice. **(A, B)** Percentage of lung iNKT cells among gated T cells (A) and of IL-4⁺ and
410 IL-17⁺ cells among gated iNKT cells (B). **(C)** Percentage of lung $\gamma\delta$ T or V γ 1⁺ $\gamma\delta$ T cells among gated T
411 cells. **(D)** Percentage of lung IL-4⁺ and IL-17⁺ cells among gated V γ 1⁺ $\gamma\delta$ T cells. **(E)** Percentage of
412 CD44⁺CD69⁺ among gated CD4⁺ T cells in BALF from HDM-treated mice. No CD4⁺ T cells were observed
413 in BALF from control mice. **(F)** Percentage of lung IL-4⁺, IL-13⁺ and IL-17⁺ cells among gated CD4⁺ T cells.
414 Light circles and squares represent BALB/c and NOD control mice, respectively. Dark circles and
415 squares represent BALB/c HDM and NOD HDM mice, respectively

416 **Figure 3.** Reduced airway inflammation in NOD IL-4KO mice. **(A, B)** Percentage and number of
417 macrophages (Mac), eosinophils (Eos), neutrophils (Neu) and lymphocytes (Lym) in BALF of HDM-
418 treated NOD and NOD IL-4KO mice (n=7). **(C)** Representative PAS-stained lung histology sections of
419 HDM-treated NOD and NOD IL-4KO mice (N=5). **(D)** Mucin mRNA expression assessed by quantitative
420 RT-PCR in lung of HDM-treated NOD and NOD IL-4KO and NOD control mice (n=9). **(E)** Lung resistance
421 was measured 24h after the last challenge with HDM challenge or saline (n=10). * $p < 0.05$, *** $p <$
422 0.001. Scale bars: 50 μ m.

423 **Figure 4.** IL-4 and IL-17 are required for the development of airway inflammation in NOD mice. **(A)** IL-
424 17 mRNA expression assessed by quantitative RT-PCR in lung of HDM-treated NOD and NOD IL-4KO
425 mice (n=4 to 6). **(B)** Percentage of eosinophils and neutrophils in BALF of HDM-treated NOD IL-4KO
426 mice treated with anti-IL-17 or Ig control (n=6 to 9). **(C)** IL-5 and IL-13 mRNA expression assessed by
427 quantitative RT-PCR in lung of HDM-treated NOD IL-4KO mice treated with anti-IL-17 or Ig control (n=4
428 to 6). **(B)** Lung resistance was measured 24h after the last HDM challenge or controls (NaCl) (n=6 to 8).
429 * $p < 0.05$, ** $p < 0.01$, $p < 0.001$.

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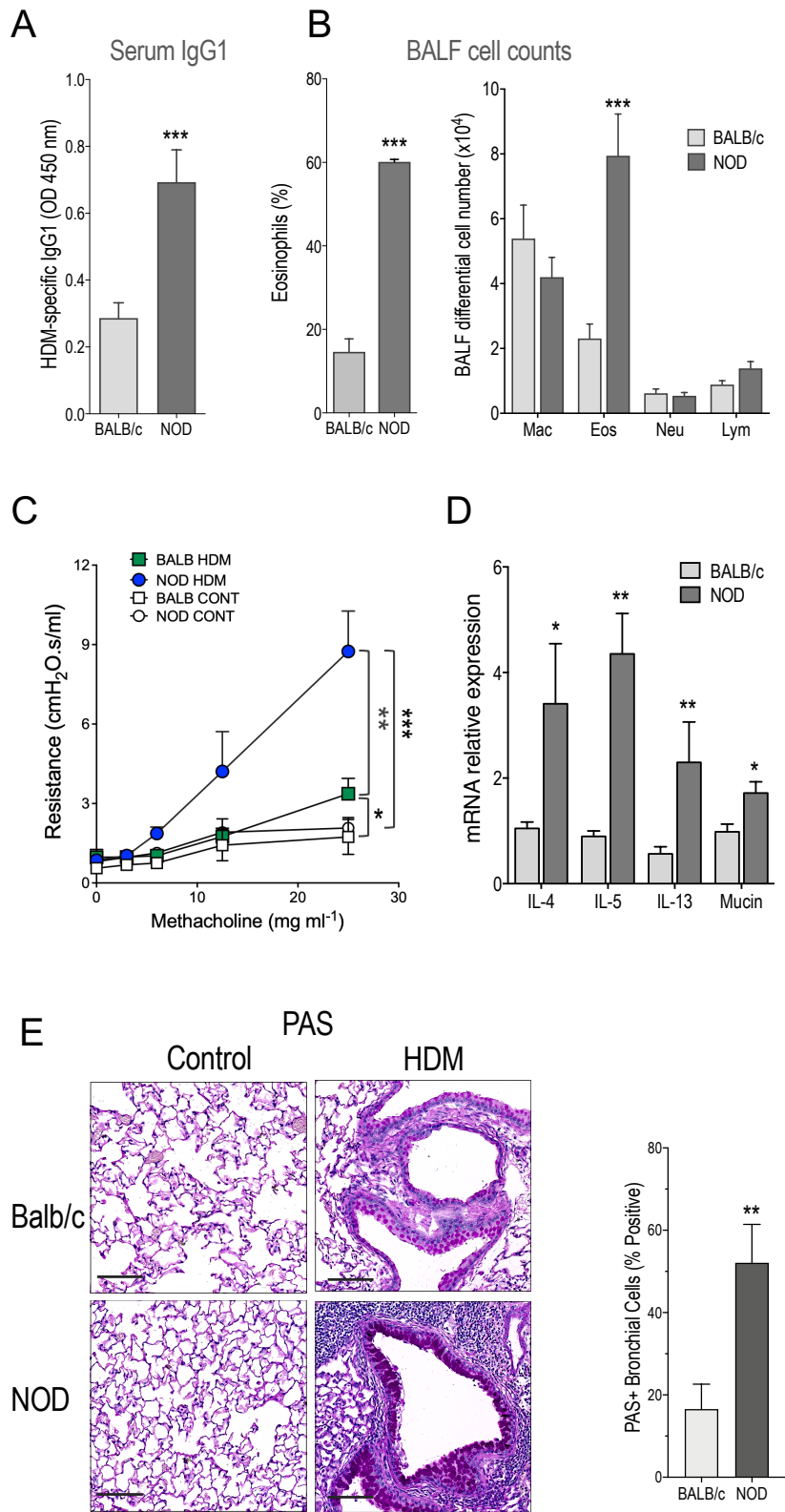


Figure 1

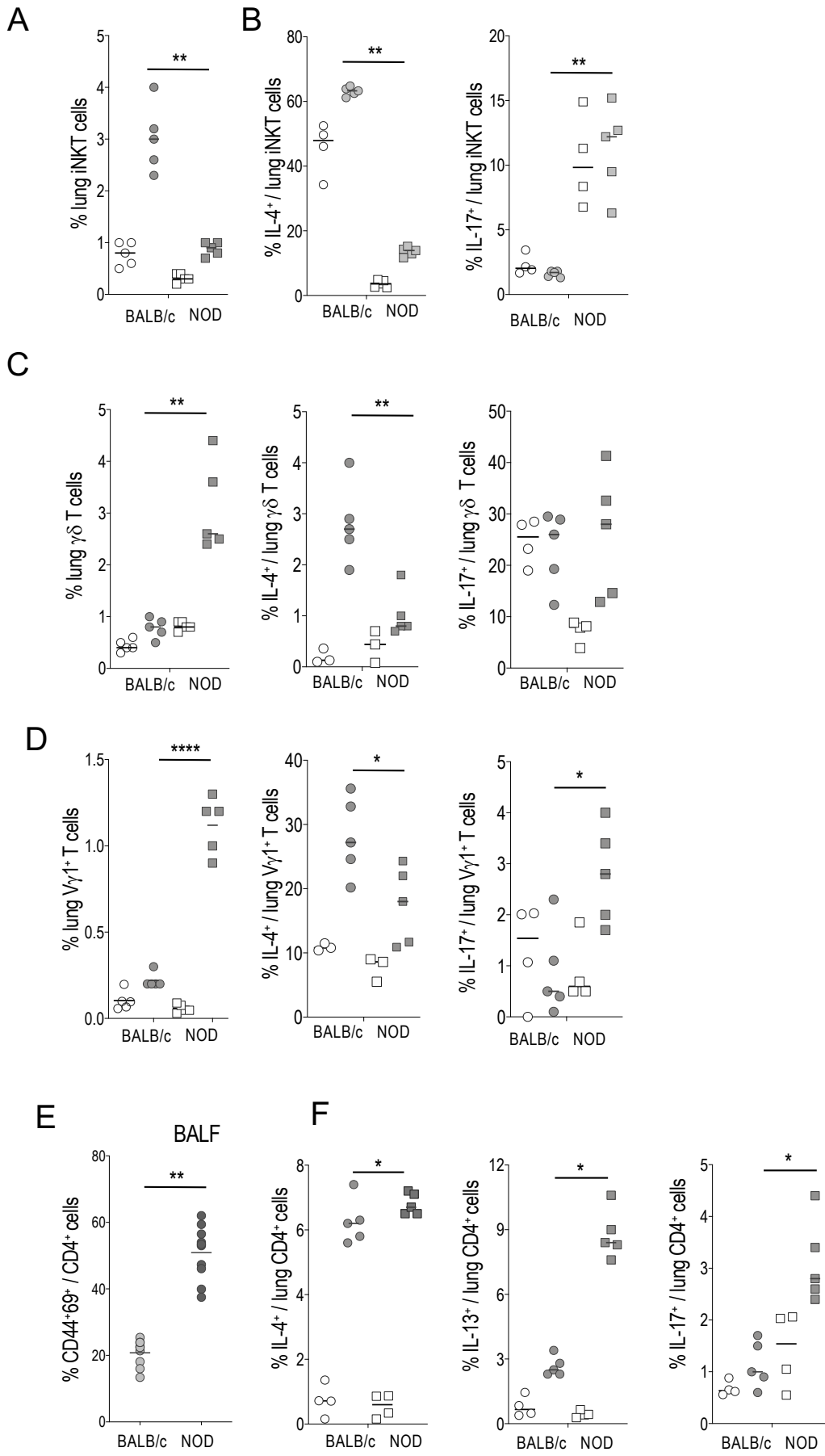


Figure 2

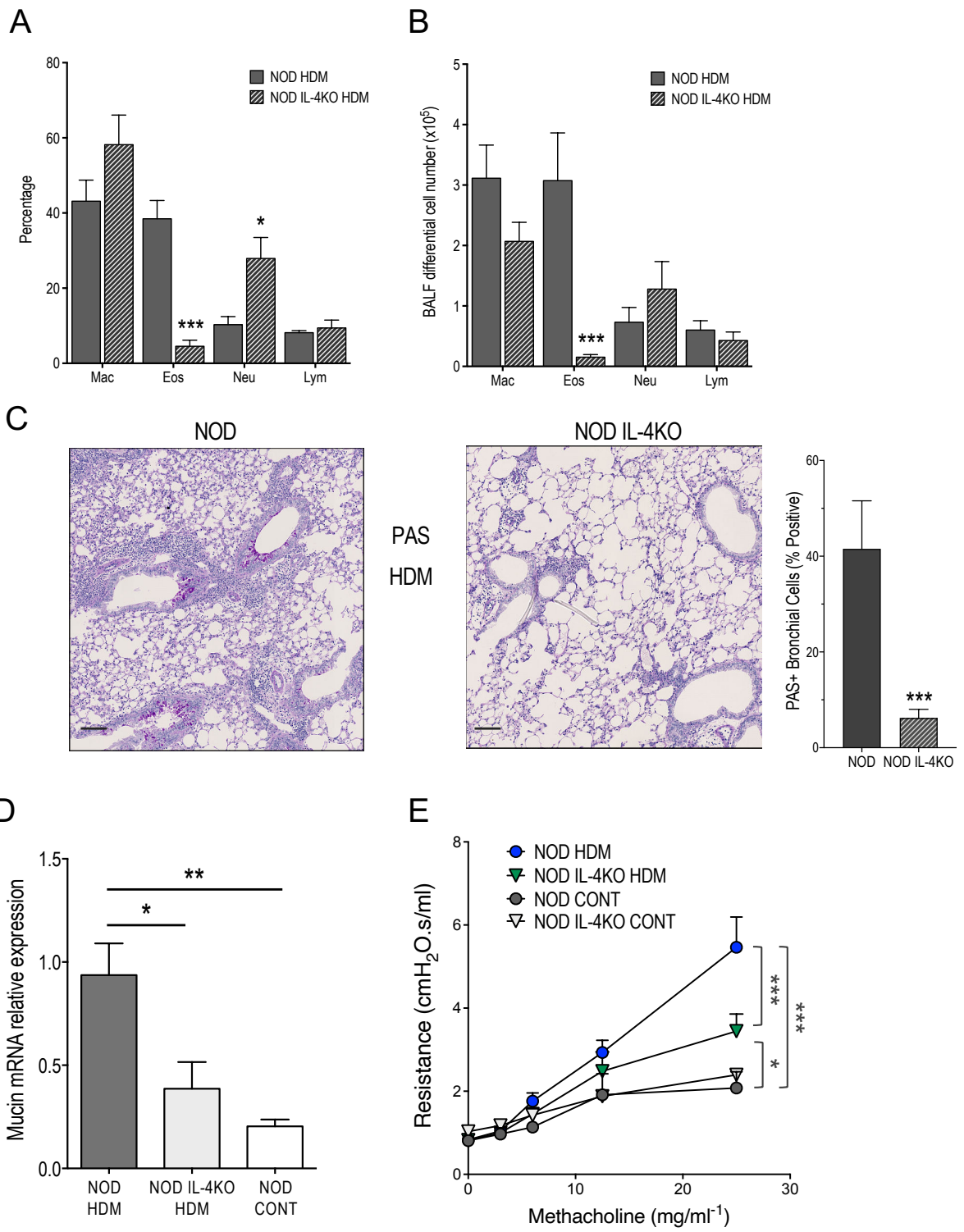


Figure 3

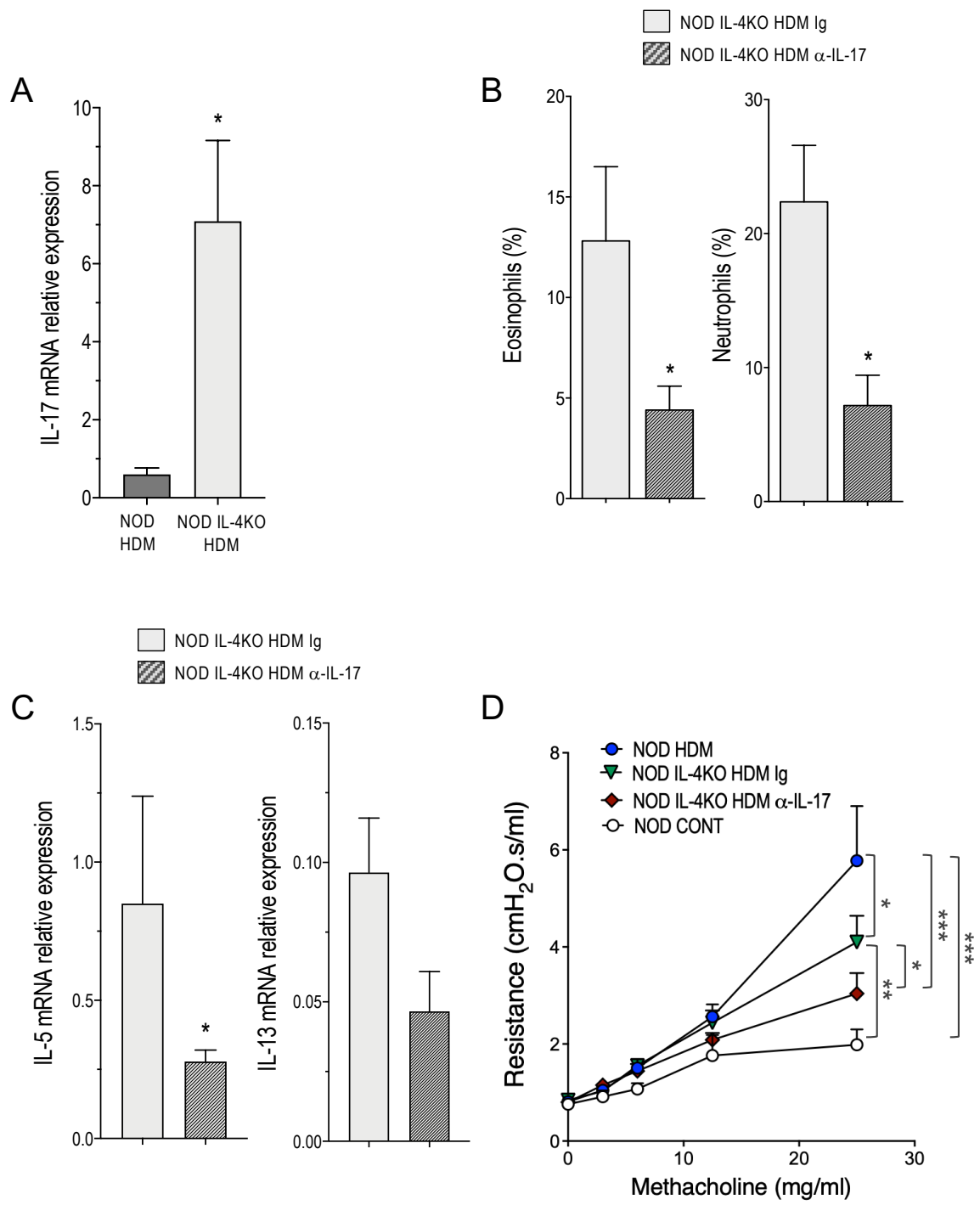


Figure 4