Neutrophil cytoplasts induce Th17 differentiation and skew inflammation toward neutrophilia in severe asthma

Supplementary Materials

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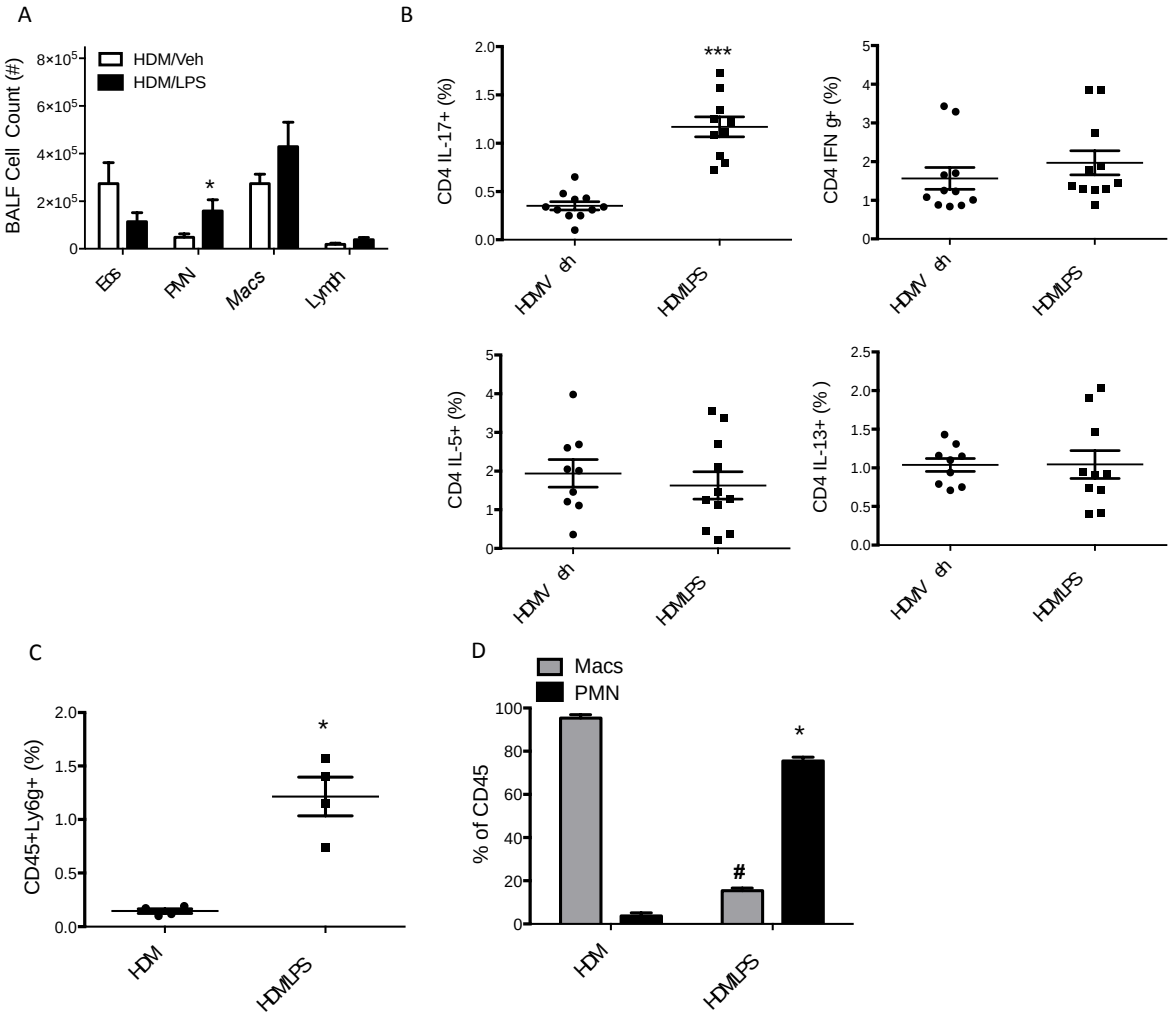
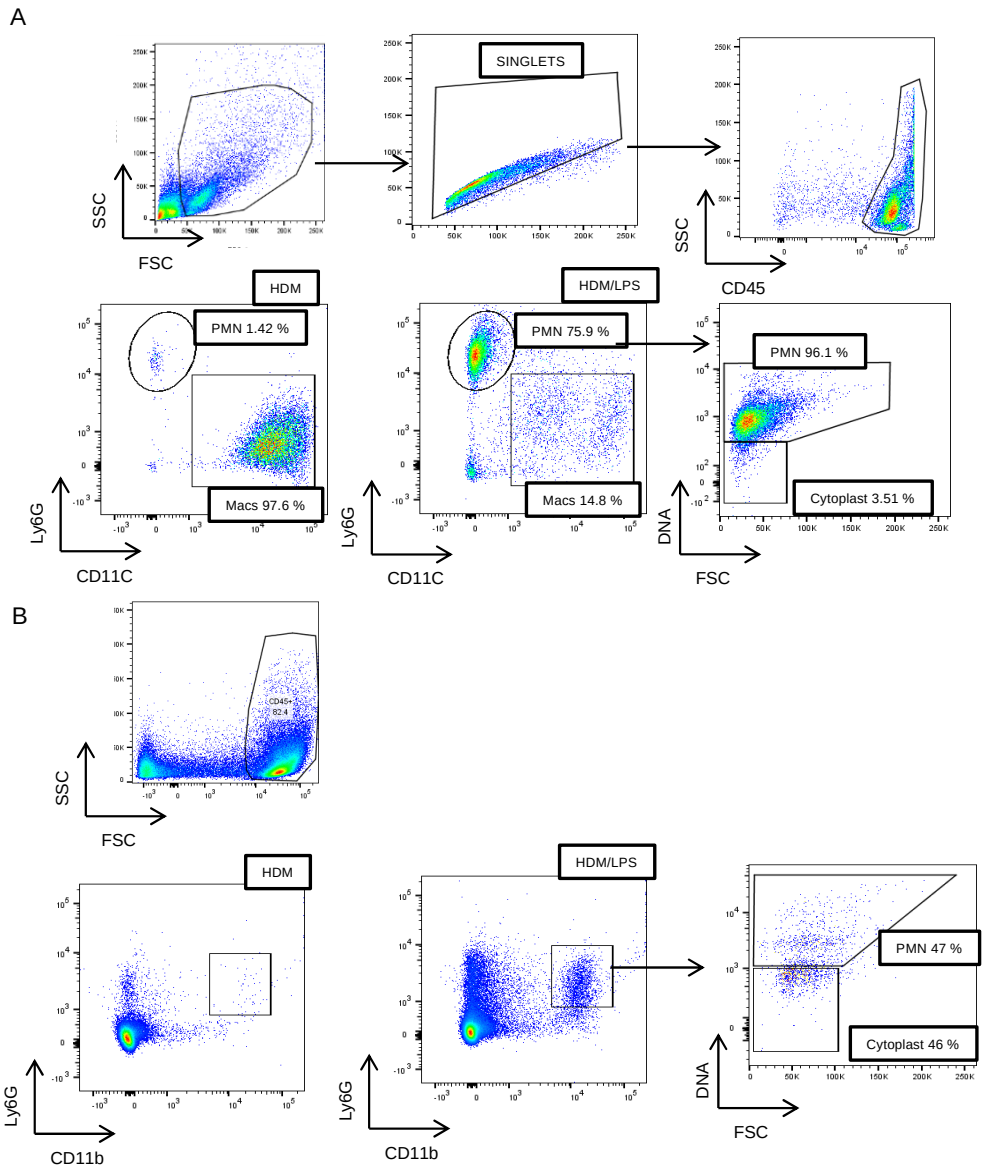


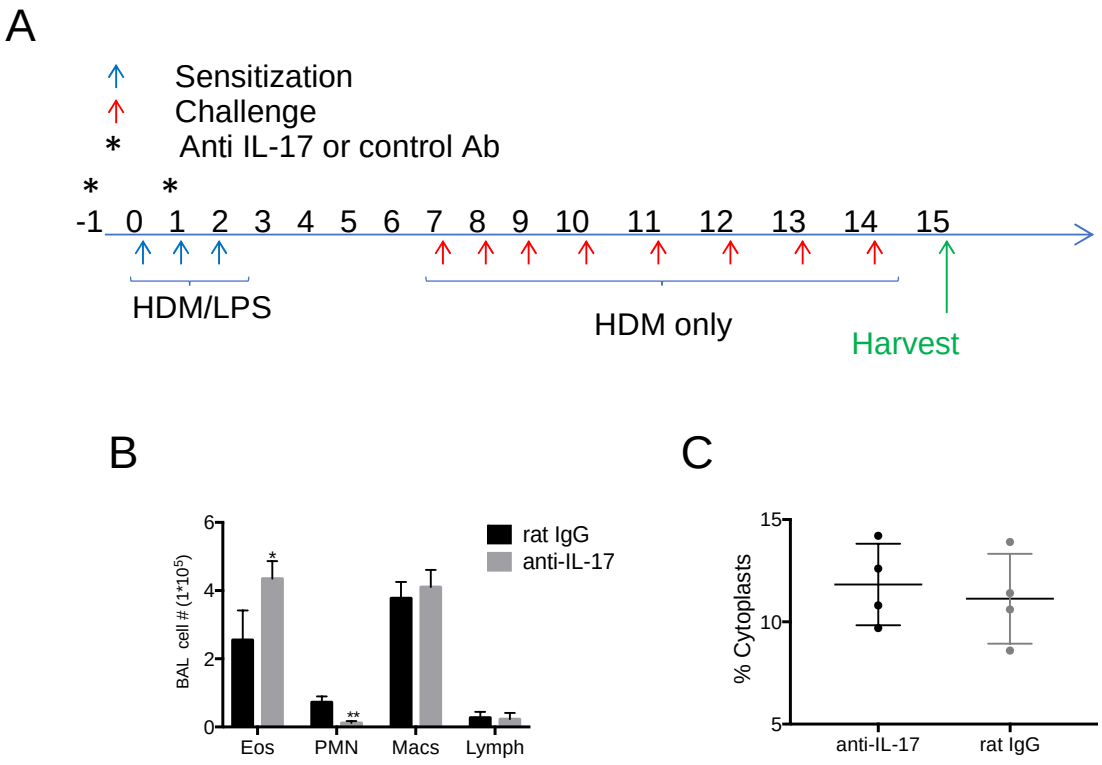
Supplementary Figure S1. A mouse model of mixed granulocytic inflammation from HDM airway challenge results from neutrophil recruitment and Th17 activation during sensitization**.**

(A) Differential BAL cell count on day 15. (B) Mediastinal lymph nodes (MLN) were harvested on day 7 and dissociated cells were restimulated with PMA and ionomycin. $CD4^+$ T cells expressing IL-17, IFN-g, IL-5 and IL-13 expressed as % of CD45+ cells (n = 10 mice, Student's *t* test). (C and B) BAL fluid and MLN were harvested at the end of sensitization (Day 3). Representative graphs showing BALF leukocyte differential (C) and MLN (D) on protocol day 3 from HDM/Veh or HDM/LPS sensitized mice expressed as % of CD45+ cells. Data are representative of two independent experiments with $n \geq 3$. *$p < 0.05$, $^{\#}p < 0.01$, ***$p < 0.001$ by two-tailed unpaired Student's *t* test.



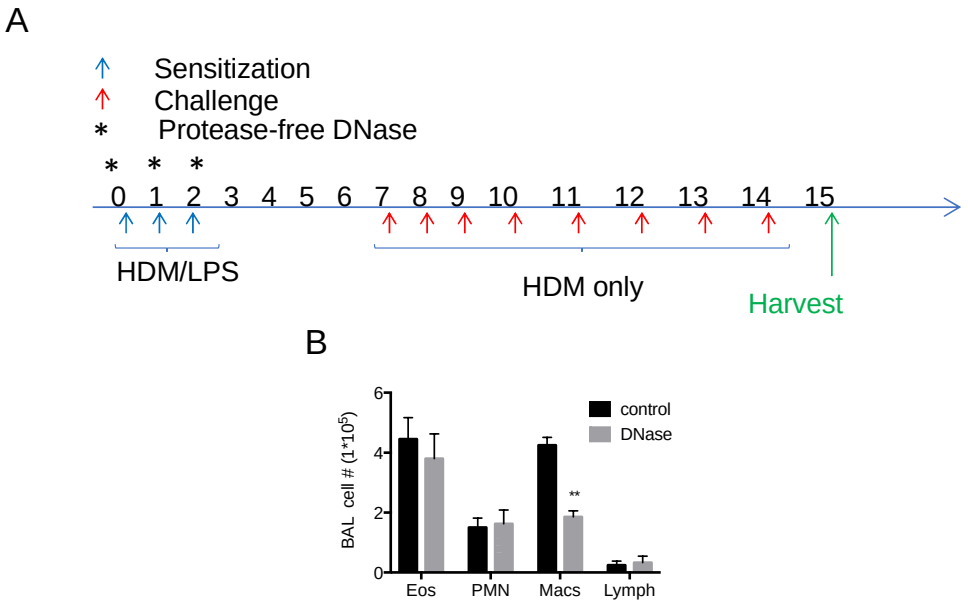
Supplementary Figure S2. Gating strategy to identify neutrophils and cytoplasts.

BALF and MLN were harvested on protocol day 3 from HDM/LPS mice. $CD45^{+}CD11b^{+}Ly6g^{+}DNA^{+}$ neutrophils and $CD45^{+}CD11b^{+}Ly6g^{+}DNA^{-}$ cytoplasts were identified from (A) BALF and (B) MLN



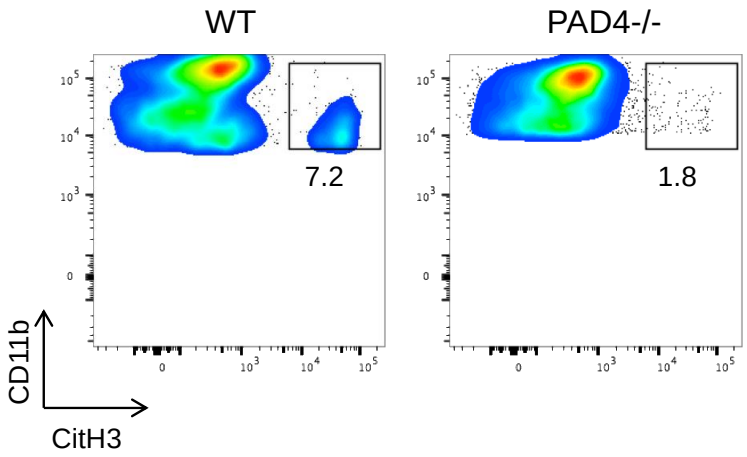
Supplementary Figure S3. Anti-IL-17 administration during the sensitization phase blunts neutrophilia without impacting cytoplast generation.

(A) Schematic diagram showing anti-IL-17 or control antibody administration during allergen sensitization. (B) Differential BALF cell count on day 15. (C) Cytoplast generation from the BALF was evaluated($CD45^+CD11b^+Ly6g^+DNA^-$) on day 3. Data are representative of two independent experiments with $n \geq 3$. *$p < 0.05$, **$p < 0.01$ by two-tailed unpaired Student's *t* test.



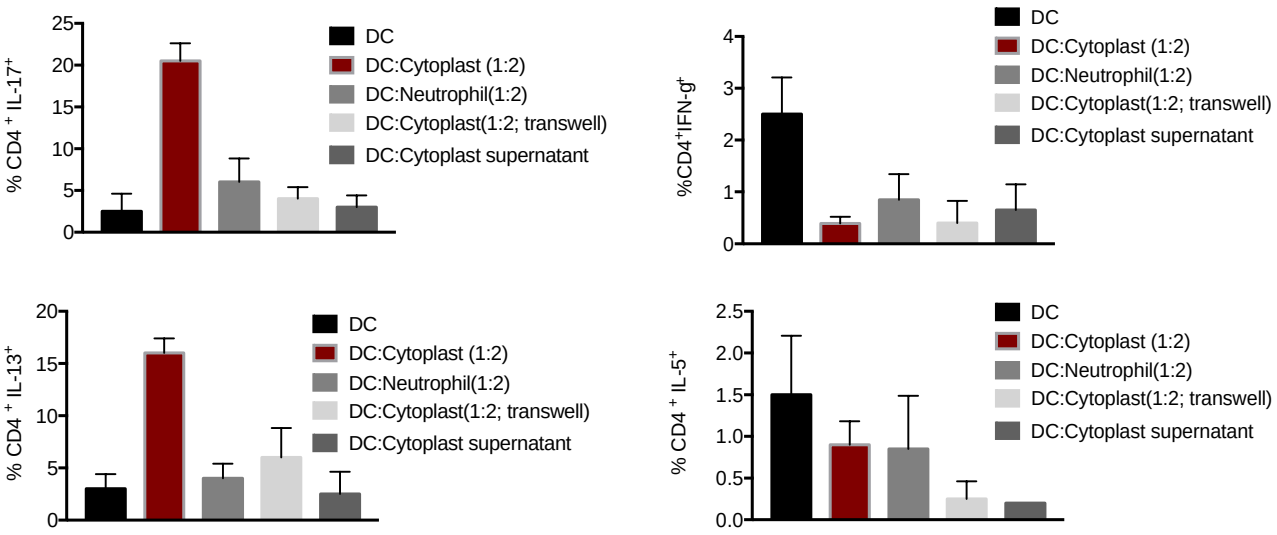
Supplementary Figure S4. Protease free-DNAse does not impact neutrophilia.

(A) Schematic diagram showing administration of Protease free-DNAse during allergen sensitization. (B) Differential BALF cell count on day 15. Data are representative of two independent experiments with n≥3. ** $p < 0.01$ by two-tailed unpaired Student's *t* test.



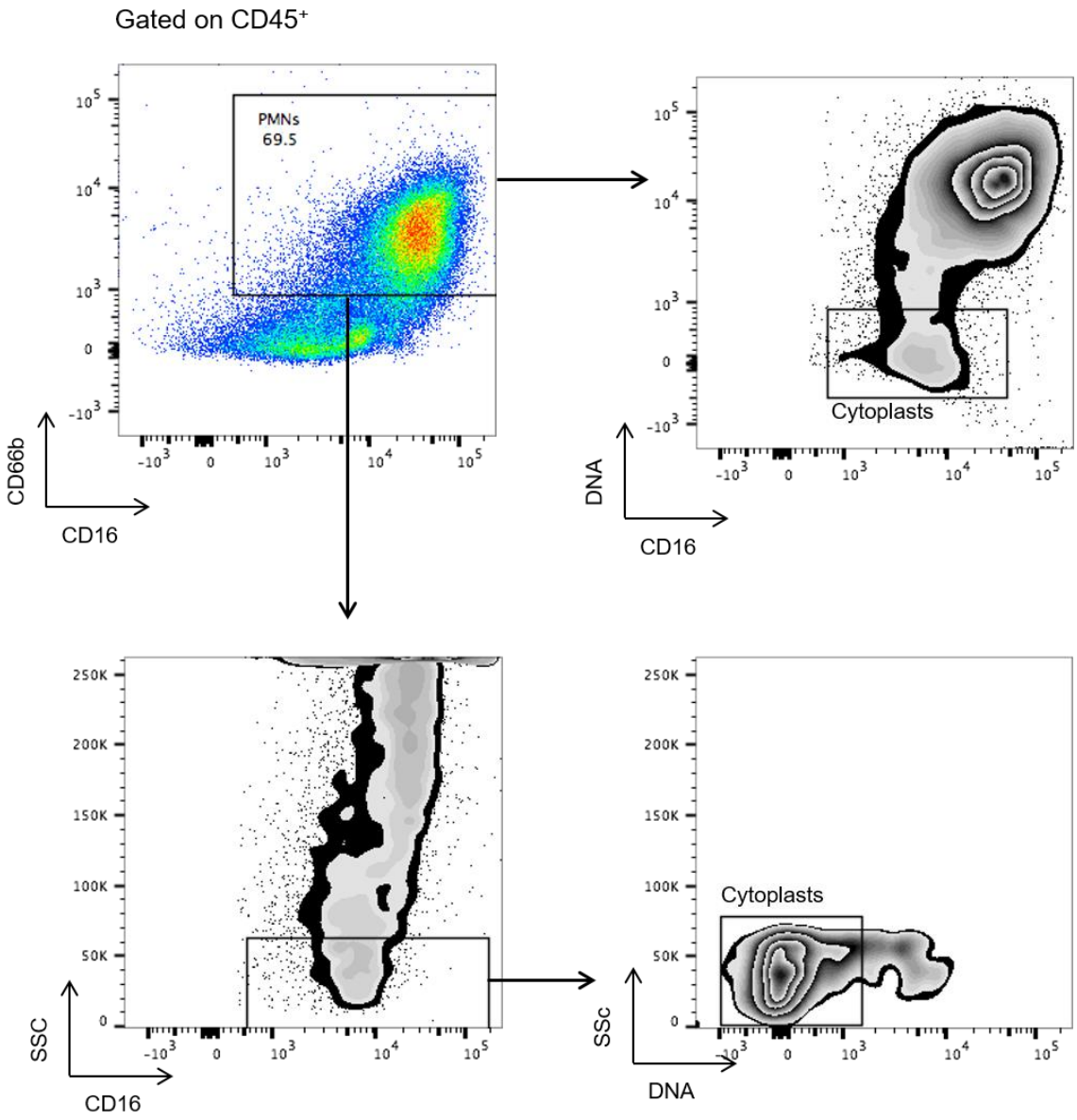
Supplementary Figure S5. Citrullinated histone identified on neutrophils from WT mice.

BALF were harvested on protocol day 3 from HDM/LPS mice. Citrullinated histone on neutrophils were identified by flow cytometry.



Supplementary Figure S6. Cytoplasts promote T cell responses via contact-dependent mechanism.

Neutrophils and cytoplasts were sorted from BALF of HDM/LPS sensitized mice and co-cultured with DC from HDM/LPS sensitized mice along with naïve T cells from DO11.10 mice. In some experimental conditions the cytoplasts were separated from the DC by a transwell. Conditioned supernatant from cytoplast culture for 24 hours was added to the DC:T cell co-culture The cytokine production from the different conditions following re-stimulation with PMA/ionomycin is graphed.



Supplementary Figure S7. Gating strategies for detection of cytoplasts in human BALF from severe asthmatics.

$CD45^{+}CD66b^{+}CD16^{+}DNA^{-}SSC^{lo}$ cytoplasts were identified from BALF of human subjects with severe asthma

Supplemental Table S1. Patient Characteristics*

	HD	NSA	SA	HD vs NSA	HD vs SA	NSA vs SA
No. of subject	25	28	41			
Clinical data						
age	41.36 +/- 2.75	38.37 +/- 2.28	42.65 +/- 2.17	ns	ns	ns
% Male	9 (38%)	9 (31%)	12 (30%)	ns	ns	ns
% African American	5 (21%)	6 (21%)	10 (25%)	ns	ns	ns
% White	17 (71%)	23 (79%)	29 (73%)	ns	ns	ns
BMI	28.28 +/- 1.13	30.12 +/- 1.36	30.45 +/- 1.11	ns	ns	ns
Symptom control						
% uncontrolled symptoms	-	20 (69%)	40 (100%)	-	-	<0.001
ACQ	-	1.16 +/- 0.17	2.05 +/- 0.17	-	-	<0.001
ACT	-	19.69 +/- 0.64	14.55 +/- 0.81	-	-	<0.001
Lung function						
FEV1 % predicted	101.86 +/- 2.19	88.02 +/- 2.82	72.52 +/- 2.77	0.005	<0.001	<0.001
FVC % predicted	102.17 +/- 2.85	97.97 +/- 3.27	86.60 +/- 2.67	ns	0.001	0.014
FEV1/FVC %predicted	99.73 +/- 1.14	90.06 +/- 1.72	83.10 +/- 1.61	<0.001	<0.001	0.005
Medication						
Inhaled corticosteroids	-	19 (66%)	38 (95%)	-	-	0.001
Oral steroids	-	0	11 (28%)	-	-	0.002
Long acting beta antagonists	-	12 (41%)	35 (88%)	-	-	<0.001
Long acting anticholinergic medication	-	0	2 (5%)	-	-	ns
Leukotriene receptor antagonists	-	6 (21%)	15 (38%)	-	-	ns
Biologics	-	0	5 (13%)	-	-	0.048
BALF leukocyte differentials						
Total cell count, millions/ml	4.70 +/- 0.63	3.87 +/- 0.53	7.79 +/- 2.70	ns	ns	ns
Lymphocytes, %	6.58 +/-0.98	5.23 +/- 0.92	8.12 +/- 1.05	ns	ns	ns
Neutrophils, %	1.72 +/- 0.47	1.49 +/- 0.30	4.78 +/- 0.93	ns	0.014	0.004
Macrophages, %	91.41 +/- 1.34	92.20 +/- 0.98	84.65 +/- 1.84	ns	0.011	0.002
Eosinophils, %	0.28 +/- 0.11	1.09 +/- 0.40	2.46 +/- 1.01	ns	ns	ns

*HD - Health Donor, NSA – Non-Severe Asthma, SA - Severe Asthma, ACQ - Asthma Control Questionnaire, ACT-Asthma Control Test, FEV1- forced expiratory volume in 1 second, FVC-forced vital capacity; Uncontrolled symptoms were defined as the occurrence of one of the following: 2 or more steroid bursts, hospitalization, intensive care unit admission, use of a ventilator wiithin the last year. FEV1 % predicted less than 80%, ACT less than 20, or self-reported worsening with tapering steroids. Values represent the mean ± SEM. One-way ANOVA for comparisons between 3 groups and unpaired Student's *t* test when only 2 groups are compared (e.g., medications).

Supplemental Table S2. Neutrophil and DNA high asthma patient symptoms, lung function and comorbidities*

	PMN^{low} DNA^{low} (n=50)	PMN^{high} DNA^{high} (n=6)	p value
Symptom Scores			
ACQ	1.5 ± 0.15	2.17 ± 0.28	NS
ACT	17.2 ± 0.69	15 ± 2.27	NS
Lung Function			
FEV1 (%predicted)	79.93 ± 2.61	83.38 ± 4.44	NS
FVC (%predicted)	92.96 ± 2.66	90.37 ± 4.40	NS
Comorbidities			
Exacerbations (≥ 4 per year)	6 (12%)	3 (50%)	0.017
Sinusitis	10 (20%)	5 (83%)	0.001
GERD	19 (40%)	5 (83%)	NS

*Asthma subjects were categorized by BAL levels of neutrophils (PMN) and DNA. PMN^{low} was defined as < 5% of total BAL cells. DNA^{low} was defined as < 0.1 mg/mL BALF. Measures of symptoms, lung function, and comorbidities were compared between the two groups and differences were assessed by Student's *t* test or chi square test as appropriate. Data are shown as mean ± SEM or number of subjects (% of total). Abbreviations: ACQ, asthma control questionnaire; ACT, asthma control test; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GERD, gastroesophageal reflux disease.

Supplementary Videos:

Video S1 (.avi format) Murine lung neutrophils were flow sorted following HDM/LPS treatment (3 days exposure) and chemotaxis to LTB_4 was monitored in a microfluidic chamber.

Video S2 (.avi format) Murine lung cytoplasts were flow sorted following HDM/LPS treatment (3 days exposure) and chemotaxis to LTB_4 was monitored in a microfluidic chamber.