SUPPLEMENTARY MATERIAL

Supplemental methods

Study participants

The study included 67 individuals between 15-41 years old in a 3-5 year follow-up of the MIDAS cohort (Minimally Invasive Diagnostic Procedures in Allergy, Asthma, or Food hypersensitivity), MIDASII. Data from MIDASII have been reported before.[1] Of the 67 participants, 39 subjects had asthma and 28 did not have an asthma, were healthy and constituted the control group. The controls were non-atopic (<0.35 kU_A/L in Phadiatop and fx5; ImmunoCAP). All subjects gave their written informed consent to participate in the study, which was approved by the Uppsala Regional Ethics Review Board (Dnr 2012/420).

Clinical analysis

Asthma control was determined by the Asthma Control Test (ACT). Lung function testing included spirometry according to ATS/ERS guidelines, [2] and the highest value of three acceptable measurements for FEV_1 (forced expiratory volume in one second), FVC (forced vial capacity) and PEF (peak expiratory flow). Hedenström reference values were used for the majority of the subjects i.e.18 years or older, [3] whereas Solymar reference values were used for the subjects less than 18 years old (13 subjects). FeNO (fraction of exhaled nitric oxide) was analysed following the American Thoracic Society/European Respiratory Society recommendations, [4] using a chemiluminescence analyser (NIOX Flex; Aerocrine AB, Solna, Sweden). Blood cells were counted at the Department of Clinical Chemistry and Pharmacology at Uppsala University Hospital using a routine method (Cell-Dyn Sapphire, Abbott, Illinois, USA) for 62 out of 67 subjects.

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Serum and plasma analyses

Total serum IgE, Phadiatop and fx5 were quantified using the ImmunoCAP system (Immunodiagnostics, Thermo Fisher Scientific, Uppsala, Sweden). The levels of 180 different proteins in EDTA plasma were quantified by the proximity extension and ligation method using the Inflammation (www.olink.com/products/inflammation/biomarkers/) and Immune response (www.olink.com/products/immune-response-panel/biomarkers/) panels from Olink Proteomics (Uppsala, Sweden).[5] Samples from 61 and 63 subjects passed the sample quality control for the Inflammation panel and the Immune response panel, respectively, and were further analysed.

Preparation of PBMCs and Flow cytometry

Blood samples collected in EDTA-treated tubes (12-15 ml; BD Vacutainer, BD Bioscience, Franklin Lakes, NJ, USA) or buffy coats from anonymized blood donors at Uppsala University hospital (used for phenotyping of FcεRI$^+$ blood monocytes) were used to enrich peripheral blood mononuclear cells (PBMC) using Ficoll-Paque Premium (ρ=1.076 g/ml) (GE Healthcare, Little Chalfont, UK) in SepMate™-50 tubes (Stemcell Technologies, Vancouver, Canada). Platelets were removed by centrifugation (2 x 200g, 10 min). To quantify FcεRI$^+$ monocytes, 10x10^6 enriched mononuclear cells were incubated in PBS, pH 7.4 with 2% heat-inactivated fetal calf serum (Sigma-Aldrich, St. Louis, MO, USA) with the following fluorescent-conjugated antibodies from BD Bioscience and eBioscience, San Diego, CA, USA. The following antibody targets (clone names) were used for analysis: CD4 (RPA-T4), CD8 (RPA-T8), CD13 (WM15), CD14 (M5E2), CD19 (HIB19), CD34 (581), CD117 (104D2) and FcεRI (AER-37). The flow cytometry was performed on a LSRII, LSRFortessa or a FACSAria III (BD Biosciences). Data analysis was performed using FlowJo software version 9.8.

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Data analysis

Statistical analysis was made using Prism 7.0c (GraphPad software Inc., San Diego, CA) or R (version 3.4.3). Single comparison was assessed by unpaired, two-tailed Student's t-test, and multiple comparisons were performed by one-way ANOVA followed by Tukey's test. Means are shown ±SEM in figures and text. Associations between the proportion of FcεRI^{+} monocytes among all monocytes and continuous variables were analysed with Spearman rank correlation test. All graphs were prepared using Prism. A p-value equal to or less than 0.05 was considered significant.

Method references

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Supplemental Table 1. Subjects characteristics. The results are presented as number and percentage or mean ± SEM, except for total IgE which is given as GeoMean ± GeoSEM and blood eosinophils and FeNO which are given as the median.

	Asthma (n = 39)	Controls (n =28)	p-value
Female Gender	22 (56%)	17 (60%)	0.72
Age	26 ± 1	24 ± 1	0.25
Height (cm)	172 ± 1.4	175 ± 1.9	0.13
Weight (kg)	68.9 ± 2	71.9 ± 3.5	0.44
BMI	23.4 ± 0.7	23.2 ± 0.8	0.86
ACT	21 ± 0.5	NA †	-
ICS	7	0	-
ICS+LABA	23	0	-
LTRA	5	0	-
Total IgE (kU/L)	245 ± 1	13 ± 1	< 0.0001
Blood eosinophils (x10^9/L)	0.2	0.1	0.0003
FeNO (ppb)	23.1	11	0.0015
Phadiatop > 0.35 kU_A/L	37 (95%)	0 (0%)	-
Fx5 > 0.35 kU_A/L	15 (38%)	0 (0%)	-

BMI = Body mass index, ACT = Asthma control test, ICS = inhaled corticosteroid, ICS+LABA = inhaled corticosteroid + long-acting beta agonists, LTRA = leukotriene receptor antagonists. FeNO = fraction of inhaled nitric oxide. † = not applicable. Comparisons were done by two-tailed unpaired Student's t-test, except for gender which was tested by Chi-squared test.

Supplemental Table 2. Asthma patients with > 50 % or < 50 % FcεRI$^+$ monocytes have similar lung function and disease control.

	p value
FEV1†	0.48
PEF†	0.49
FeNO†	0.56
ACT	0.35
Age	0.43
BMI	0.43

Pairwise comparison using unpaired Student's t-test. † = % of predicted.

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