

IgA⁺ memory B cells are significantly increased in patients with asthma and small airways dysfunction

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Online Data Supplement

Materials and methods

Study design, methods, and definition of clinical variables

Study visits were only scheduled if a patient was without respiratory tract infections and asthma exacerbations for at least 4 weeks prior to the study visit. During each visit comprehensive questionnaire data was collected regarding asthma and rhinitis symptoms, medication, asthma control, exacerbation and quality of life. Lung function tests including spirometry with reversibility testing, body plethysmography and impulse oscillometry (IOS) were performed using a Masterscreen Body and IOS (Vyaire Medical, Germany) according to established guidelines [1-4]. Lung function parameters were expressed as z-scores [3]. Specific IgE against 36 aero- and food allergens were analysed centrally by Immunoblot (EuroImmun AG, Lübeck, Germany), differential blood counts were assessed in local laboratories. Induced sputum was obtained using an established protocol [5].

Definitions of clinical variables used in the analysis are specified in supplementary Table S1.

TABLE S1. Definition of clinical variables

Variable	Definition
Asthma severity	Defined as mild-moderate or severe asthma according to ERS / ATS guideline 2014 [6]
Asthma control	Assessed by Asthma Control Test (ACT) [7], Asthma Control Questionnaire (ACQ-7) [8] or defined as controlled, partly controlled or uncontrolled according to GINA guideline [9]
Asthma related quality of life	Assessed by the Asthma Quality of Life Questionnaire (AQLQ) [10]
Severe exacerbation	Three days of oral corticosteroids (OCS) treatment or increase of regular OCS dose over a period of at least three days
Atopy	Sensitization against at least one allergen with a specific IgE $\geq 0,7$ kU/l from a panel of 36 aero- and food allergens

Sum of specific IgE [kU/l]	Sum of 36 allergen-specific IgE measurements /36
Sputum inflammation [11]	Paucigranulocytic (eosinophils < 2%, neutrophils < 40%)
	Eosinophilic (eosinophils ≥ 2%, neutrophils < 40%)
	Neutrophilic (eosinophils < 2%, neutrophils ≥ 40%)
	Mixed (eosinophils ≥ 2%, neutrophils ≥ 40%)
Inhaled corticosteroids (ICS)	Expressed as fluticasone propionate equivalent
Smoking status	Never or former smokers <10PY
	Current or former smokers ≥10PY
Positive bronchodilator response (BDR)	Increase of FEV ₁ ≥ 12% or 200ml after inhalation of 400µg salbutamol
Adult onset	Asthma onset at adult age (≥ 18 years)
Body mass index (BMI)	weight (Kg) / [height (m)] ²
Small airway dysfunction (SAD)	Defined as R5-R20 (IOS) above the upper limit of normal (95 th centile) using age, sex, weight and height adapted reference equations of a German cohort of healthy adults [12]

GINA, Global Initiative for Asthma; PY, pack-years; FEV₁, forced expiratory volume in 1 second; R5–R20, resistance at 5 Hz – resistance at 20 Hz [kPa/l/s]; OCS, oral corticosteroids; IgE, Immunoglobulin E.

B cell characterization

PBMCs were isolated from heparinized blood by Biocoll (Biochrom, Berlin, Germany) density-gradient centrifugation. Until further use cells were stored in freezing medium (90% FBS, 10% DMSO; Biochrom; Sigma-Aldrich, Steinheim, Germany, respectively) in liquid nitrogen. For phenotypic analyses of B cell subpopulations, isolated PBMCs were blocked with normal rat and mouse serum, followed by incubation with suitable antibodies. Dead cells were excluded by Live/Dead staining according to the manufacturer's instructions (supplementary table S2). B cell subsets were measured on a FACSCanto II flow cytometer (BD, Heidelberg, Germany) and analyzed by FlowJo software (TreeStar, Ashland, OR, USA). Gating strategies are shown in supplementary figure S1. B cell populations were always presented as percentage of total live CD19⁺ B cells.

TABLE S2. Anti-human antibodies used for flow cytometric analyses of B cell subsets in peripheral blood

Marker / Dye	Fluorophore	Clone	Company
LIVE/DEAD Fixable Dead Cell Stain	amcyan		Invitrogen, Carlsbad, CA, USA
CD19	PE Cy7	HIB19	BioLegend, San Diego, CA, USA
CD19	PerCP Cy5.5	HIB19	BioLegend, San Diego, CA, USA
CD24	FITC	ML5	BD, Franklin Lakes, NJ, USA
CD27	Pacific Blue	M-T271	BioLegend, San Diego, CA, USA
CD27	APC	O323	eBioscience, San Diego, CA, USA
CD38	PerCP Cy5.5	HIT2	BioLegend, San Diego, CA, USA
IgM	Pacific Blue	MHM-88	BioLegend, San Diego, CA, USA
IgG	PE Cy7	G18-145	BD, Franklin Lakes, NJ, USA
IgA	PE	IS11-8E10	MACS Miltenyi Biotec, Bergisch Gladbach, Germany

Statistical analysis

Clinical variables and B cell populations included in the association analysis are specified in figure 1 and supplementary table S1. Categorical variables comprised gender, smoking status (never or former smoker <10 pack-years/ current or former smoker \geq 10 pack-years), GINA control status (controlled/ partly controlled/ uncontrolled), positive bronchodilator response defined as increase of FEV₁ \geq 12% or 200ml after inhalation of salbutmatol (yes/no), asthma severity according to ERS/ATS Guideline 2014 (mild-moderate/ severe), regular oral corticosteroid intake (yes/no), sputum inflammation type (neutrophilic/ eosinophilic/ mixed/ paucigranulocytic). Continuous variables comprised age, BMI [Kg/m²], age at first asthma diagnosis, FEV₁ [z-score], FEV₁/FVC [z-score], FEF₂₅₋₇₅ [z-score], reactance area [kPa/l/s], R5-R20 [kPa/l/s]; blood neutrophils [1000/ μ l], blood eosinophils [1000/ μ l], specific IgE (sum of 36 specific IgE against allergens/36), and severe exacerbations. The dataset version used for the analysis was 20180731_V2-1.

B cell subsets were always displayed as percentage of total B cells (CD19⁺ B cells) and included naïve B cells (CD19⁺CD27⁻CD24^{low}CD38^{low}), early transitional 1 B cells (T1 B cells, CD19⁺CD27⁻CD24^{high}CD38^{high}) and late transitional 2 B cells (T2 B cells, CD19⁺CD27⁻CD24^{high}CD38^{med}), unswitched CD27⁺IgM⁺ memory B cells, class-switched CD27⁺IgG⁺ and CD27⁺IgG⁺ as well as CD27⁺IgA⁺ and CD27⁺IgA⁺ memory B cells.

The same variables were included into the linear regression model with age and regular systemic corticosteroid intake as co-variables.

Some variables have missing data: Sputum cell counts are missing in n=5 healthy controls and n=21 asthma patients; FEF₂₅₋₇₅ is missing in n=30 asthma patients, number of severe exacerbations in n=9, asthma quality of life questionnaire in n=8 asthma patients.

The multivariate regression model included SAD defined by the 95th centile of R5-R20 and percentage of CD27⁺IgA⁺ memory B cells, regular OCS intake (yes/ no), blood eosinophils [1000/ μ l], sputum eosinophils [%], FeNO [ppb], BMI [Kg/m²], gender, age, sum of 36 allergen-specific IgE and smoking [pack-years].

Supplement References

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