Allergen-specific IgE and IgG4 patterns among patients with different allergic diseases

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Abstract

Background: In addition to allergen-specific IgE (sIgE), allergen-specific IgG4 (sIgG4) antibodies are also involved in the immune response resulting from an allergen exposure. The aim of our study was to analyze sIgE and sIgG4 patterns in the most common allergic disorders: bronchial asthma, upper airway disorders and atopic dermatitis.

Methods: In this study a screening analysis of blood serum samples from 673 patients aged from 6 months to 17 years with different allergic entities was performed on microarrays. sIgE and sIgG4 levels to the most common allergens were estimated.

Results: sIgE response to most pollen allergens is more strongly associated with respiratory diseases than with atopic dermatitis, while sIgE responses to cat and dog dander are more strongly associated with bronchial asthma than with atopic dermatitis and upper airway disorders such as rhinosinusitis and allergic rhinitis. A lower prevalence of sIgG4 to pollen allergens in cases of atopic dermatitis is observed compared with that in cases of asthma and upper airway disorders. Analyzing all the allergic disorders, one can see that sIgG4 response to inhalant allergens is strongly associated with sensitization to the corresponding allergen.

Conclusion: Allergen-specific IgE and IgG4 patterns that are relevant to concrete allergic diseases differ by sIgE and sIgG4 prevalences to defined allergens.

Keywords: Allergy diagnostics, IgE, IgG4, Microarrays, Sensitization, Asthma, Dermatitis, Rhinitis

Background

Bronchial asthma, allergic rhinitis and atopic dermatitis are the most common allergic reactions. The pathologies individually and their comorbidities are often diagnosed among many atopic patients in different periods of life [1]. Along with genetics and adverse environmental factors, allergens as triggering factors represent major aspects of the pathogenic pathways for the mentioned entities [2–4].

Currently, allergen-specific IgE is considered the only notable serological marker of type 1 hypersensitivity. A number of studies [5, 6] have focused on sIgE responses among children and adults with different allergic pathologies. However, most of the studies have assessed only one particular pathology or only one particular group of allergens [7, 8].

Allergen-specific IgG, including subclass G4, participates in the development of allergic reactions. The analysis of allergen-specific IgG4 is mainly performed in studies that are related to allergen-specific immunotherapy (ASIT) [9], and a change in the sIgG4 level is one of the indicators of the efficiency of ASIT, during which allergen tolerance occurs [10]. However, in some cases, similar processes of tolerance induction with the involvement of sIgG4, as a part of the whole pool of allergen-specific IgG, occur among subjects not treated with ASIT [11]. The most representative example is the development of tolerance to food allergens while growing among children and adults with different allergic pathologies.
up [12]. However, the slgG4 response among atopic pa-
tients of different age, states and diseases was less studied,
and IgG4 antibodies are regarded as minor party in hyper-
sensitivity reaction manifestation because of low serum
concentrations.

The aim of our study was to analyze the slgE and
slgG4 responses to allergens that are most relevant to
the chosen allergic pathologies. We determined slgE and
slgG4 levels in blood serum samples from 673 patients
diagnosed with bronchial asthma, upper airway disorders
or atopic dermatitis using microarrays with immobilized
protein extracts of 31 allergens. Profiles of the groups
with different allergic pathologies were defined, and the
correlation between slgE and slgG4 occurrence was

Methods
Participants and samples

Patients from the Filatov Moscow City Pediatric Clinic
No. 13 aged from 6 months to 17 years were enrolled in
the study (Table 1). The recruitment was carried out
from September, 2016, to September, 2017. Participants
chosen for the study had three different diagnosis ac-
cording to clinical history and medical examination: 1) atopic patients without dermatitis symptoms with lower
airway disease or bronchial asthma (145 patients), 2)
atopic patients with upper airway disorders (rhinosinusitis and allergic rhinitis) without asthma and dermatitis
symptoms (194 patients), 3) participants with atopic
dermatitis without symptoms of airway disorders (334
patients) (Table 1).

The patients included in groups with airway disorders
(bronchial asthma and upper airway disorders) had not
demonstrated symptoms of allergic skin reaction for a
period of 1 year before the study. The patients in the
group with atopic dermatitis had not demonstrated
symptoms of airway diseases for a period of 1 year before the study. The children with well controlled
and/or well-medicated asthma were not included in the
group with atopic dermatitis.

Surplus blood serum samples that remained after rou-
tine diagnostic procedures were not performed. The study was

approved by the local ethics committee of the Filatov
Moscow City Pediatric Clinic No. 13.

Microarray design and manufacturing

Microarray design and manufacturing technologies were
described in full in the previous works [13, 14]. Briefly,
the microarray is a matrix of semispherical hydrogel ele-
ments (0.1 nl in volume) that contain one of the immo-
ibilized allergens: 28 allergen extracts and 3 individual
isolated proteins (components of cow milk) (for the list
of allergens see Fig. 1). The allergens for immobilization
were purchased from GREER (Lenoir, NC, USA) and
were diluted to working concentrations (from 1 to 5 mg/
ml depending on the allergen) according to the manu-
facturer’s recommendations. The list of allergens in-
cudes most common allergens in Central Russia [14];
this list largely overlaps with the widespread allergens
in Central and Northern Europe.

Concentrations of the allergens were chosen in such a
way that the results obtained from the microarrays most
accurately coincided with the results obtained by the ref-
ence methods (Specific IgE REAST (ALLERG-O-LIQ)
and Specific IgG4 ELISA (Dr. Fooke Laboratorien
GmbH, Germany)) [14]. The slgE levels were determined
in the range of 0.35–100 IU/ml, and the slgG4 levels were determined in the range of 100–2500 ng/ml.

Analysis of allergen-specific IgE and IgG4

The analysis of slgE and slgG4 on the microarrays in-
cludes 4 stages: 1) incubation of the microarray with
the serum sample; 2) washing with a washing buffer
containing detergent; 3) incubation with the develop-
ing antibodies, a mixture of anti-human IgE and
anti-human IgG4 antibodies conjugated with Cy5 and
Cy3, respectively; 4) washing with a washing buffer
containing detergent [14].

After the analysis, fluorescent signals from the gel pads
with immobilized allergens are detected using a micro-
array analyzer with laser illumination and a device for
speckle suppression [15]. Fluorescent signals are calcu-
lated by referencing piecewise linear calibration curves
constructed on the basis of the signals from the gel pads
with immobilized IgE and IgG4 to obtain the slgE and
slgG4 concentrations in IU/ml and ng/ml, respectively.

Patients were considered sensitized to the allergen if
the slgE level for the allergen exceeded the minimum
cutoff of 0.35 IU/ml. Nonsensitized, monosensitized
and polysensitized patients were enrolled in the study.

Data evaluation

slgE prevalence was determined as the ratio of patients
who exhibit slgE above the cutoff of 0.35 IU/ml to the
total number of patients in the group of interest. slgG4
prevalence was determined as the ratio of patients who

| Allergic disorders | Male | Female |
|-------------------|------|--------|
|                   | 0–6 years | 7–12 years | 13–17 years | 0–6 years | 7–12 years | 13–17 years |
| Asthma            | 28    | 37      | 36        | 9        | 21      | 14        |
| Upper airway disorders | 26    | 44      | 36        | 28       | 30      | 30        |
| Dermatitis        | 104   | 38      | 11        | 134      | 35      | 12        |
exhibit sIgG4 above the value of 100 ng/ml to the total number of patients in the group of interest.

sIgE prevalence and sIgG4 prevalence were adjusted by age with the average population as a standard [16] using the Epitools package [17] in R [18]. Relationships between allergic sensitization and diseases were analyzed using a logistic regression model. To compare sIgE and sIgG4 prevalences among patients with different diseases, adjusted odds ratios (aORs) and corresponding 95% confidence intervals (CIs) were calculated via multinomial logistic regression analysis with the age and gender as covariates using IBM SPSS Statistics 23.0.0.0.

The statistical significance of the differences in sIgG4 response for sensitized and non-sensitized patients was estimated according to the results of the Fisher's exact test calculated by MedCalc. The differences were considered statistically significant for pairwise comparisons with \( p < 0.05 \).

The diagrams were plotted using Microsoft Excel 2010.

**Results**

In the current study, sIgE and sIgG4 levels of 673 blood serum samples from patients aged from 6 months to
Table 2 Adjusted ORs (aORs) for sIgE and sIgG4 prevalences among patients diagnosed with bronchial asthma, upper airway disorders (such as rhinosinusitis and rhinitis) or atopic dermatitis for inhalant allergens

| Allergens                | Asthma versus Upper Airway Disorders | Asthma versus Dermatitis | Upper Airway Disorders versus Dermatitis |
|--------------------------|--------------------------------------|--------------------------|------------------------------------------|
|                          | aOR       | 95% CI          | aOR       | 95% CI          | aOR       | 95% CI          |
| sIgE to inhalant allergens |          |                |          |                |          |                |
| Pollen allergens         |          |                |          |                |          |                |
| Alder                    | 0.809    | (0.521;1.257)  | 2.980    | (1.863;4.766)  | 3.682    | (2.384;5.684)  |
| Birch                    | 1.015    | (0.653;1.578)  | 2.705    | (1.725;4.242)  | 2.664    | (1.764;4.032)  |
| Hazelnut (p)             | 0.889    | (0.571;1.385)  | 3.700    | (2.251;6.081)  | 4.160    | (2.617;6.611)  |
| Oak                      | 0.921    | (0.568;1.494)  | 3.086    | (1.714;5.555)  | 3.349    | (1.928;5.815)  |
| Wormwood                 | 0.849    | (0.487;1.478)  | 1.253    | (0.669;2.349)  | 1.477    | (0.832;2.629)  |
| Mugwort                  | 0.856    | (0.518;1.415)  | 1.395    | (0.82;2.434)   | 1.629    | (0.979;2.712)  |
| Dandelion                | 0.668    | (0.37;1.208)   | 1.031    | (0.522;2.041)  | 1.542    | (0.844;2.819)  |
| Bermuda grass            | 1.460    | (0.873;2.44)   | 5.192    | (2.569;10.491) | 3.556    | (1.792;7.055)  |
| Orchard grass            | 1.265    | (0.772;2.074)  | 3.740    | (1.999;6.997)  | 2.956    | (1.622;3.585)  |
| Meadow fescue            | 1.146    | (0.705;1.865)  | 3.179    | (1.754;7.61)   | 2.773    | (1.577;4.875)  |
| Perennial rye grass      | 1.139    | (0.701;1.852)  | 3.075    | (1.706;5.543)  | 2.698    | (1.543;4.718)  |
| Timothy grass            | 1.123    | (0.697;1.81)   | 3.405    | (1.904;6.091)  | 3.032    | (1.747;5.262)  |
| Cultivated rye           | 1.145    | (0.685;1.913)  | 2.601    | (1.389;4.686)  | 2.272    | (1.249;4.133)  |
| Indoor allergens         |          |                |          |                |          |                |
| Cat dander               | 1.808    | (1.154;2.833)  | 2.344    | (1.461;3.761)  | 1.411    | (0.897;2.221)  |
| Dog dander               | 2.225    | (1.365;3.628)  | 2.401    | (1.451;3.975)  | 1.079    | (0.646;1.803)  |
| *D. pteronyssinus*       | 1.390    | (0.735;2.629)  | 1.478    | (0.723;3.032)  | 1.063    | (0.522;2.165)  |
| *D. farinae*             | 1.802    | (0.947;3.431)  | 1.760    | (0.873;3.549)  | 0.976    | (0.474;2.012)  |
| Alternaria tenuis        | 1.212    | (0.552;2.665)  | 1.243    | (0.525;2.942)  | 1.025    | (0.452;2.326)  |
| Cockroach, German        | 0.218    | (0.025;1.909)  | 0.886    | (0.048;16.465) | 4.065    | (0.406;40.654) |
| sIgG4 to inhalant allergens |          |                |          |                |          |                |
| Pollen allergens         |          |                |          |                |          |                |
| Alder                    | 1.102    | (0.679;1.787)  | 3.311    | (1.845;5.943)  | 3.006    | (1.728;5.229)  |
| Birch                    | 0.864    | (0.557;1.338)  | 2.379    | (1.505;3.76)   | 2.754    | (1.807;4.199)  |
| Hazelnut (p)             | 0.941    | (0.547;1.618)  | 2.643    | (1.358;5.146)  | 2.810    | (1.505;5.248)  |
| Oak                      | 0.925    | (0.381;2.246)  | 4.063    | (1.204;13.71)  | 4.391    | (1.412;13.652) |
| Wormwood                 | 0.942    | (0.432;2.058)  | 2.963    | (1.048;8.383)  | 3.144    | (1.193;8.288)  |
| Mugwort                  | 1.147    | (0.624;2.109)  | 1.938    | (0.973;3.873)  | 1.690    | (0.875;3.263)  |
| Dandelion                | 0.339    | (0.132;0.878)  | 0.811    | (0.275;2.388)  | 2.390    | (1.057;5.407)  |
| Bermuda grass            | 0.446    | (0.119;1.67)   | 1.371    | (0.301;6.245)  | 3.076    | (0.972;9.735)  |
| Orchard grass            | 1.140    | (0.337;3.854)  | 2.593    | (0.598;11.248) | 2.274    | (0.566;9.139)  |
| Meadow fescue            | 1.189    | (0.473;2.992)  | 5.462    | (1.322;22.598) | 4.593    | (1.168;18.064) |
| Perennial rye grass      | 1.745    | (0.645;4.721)  | 4.491    | (1.215;16.598) | 2.573    | (0.693;9.556)  |
| Timothy grass            | 0.926    | (0.395;2.171)  | 2.673    | (0.855;8.358)  | 2.888    | (0.998;3.358)  |
| Cultivated rye           | 0.395    | (0.081;1.952)  | 2.725    | (0.345;21.526) | 6.896    | (1.303;36.492) |
| Indoor allergens         |          |                |          |                |          |                |
| Cat dander               | 1.496    | (0.913;2.453)  | 1.671    | (0.992;2.814)  | 1.117    | (0.673;1.852)  |
| Dog dander               | 1.067    | (0.607;1.876)  | 1.813    | (0.975;3.372)  | 1.699    | (0.948;3.045)  |
Additional file 1. Sensitization rates to each selected allergen in different age groups are presented in Table 2. By age and gender are shown in Fig. 1. The most significant differences between sIgE prevalences in different diseases are observed for inhalant allergens. For these allergens, ORs adjusted between sIgE prevalences in different diseases are observed.

Patients with airway disorders (bronchial asthma and upper airway disorders) are mostly sensitized to pollen allergens. Notably, the adjusted sIgE prevalence for most pollen allergens distinguishes less than 10% for the patients with asthma and with upper airway disorders (Fig. 1). Sensitization to the majority of pollen allergens is more associated with airway diseases than with atopic dermatitis (i.e. aOR is significantly higher than 1, see Table 2).

Indoor allergens also have a significant impact, especially the animal epithelium allergens of cat dander and dog dander. sIgE sensitization to these allergens is more common for patients with bronchial asthma than for patients affected by only upper airway disorders or atopic dermatitis (Fig. 1). For these cases, as shown in Table 2, when comparing bronchial asthma and upper airway disorders for cat dander aOR = 1.808, 95% CI (1.154;2.833), for dog dander aOR = 2.225, 95% CI (1.365;3.628); when comparing bronchial asthma and atopic dermatitis for cat dander aOR = 2.344, 95% CI (1.461;3.761), for dog dander aOR = 2.401, 95% CI (1.451;3.975).

The adjusted prevalence of sensitization to food allergens of animal origin is higher in asthma and atopic dermatitis patients than in patients with upper airway disorders. However, this difference is most commonly not statistically significant (i.e. 95% CI for aORs contains 1, Table 3). Sensitization to a number of plant food allergens (hazelnut, carrot, peach and apple) is observed mostly for patients sensitized to tree pollens. It is strongly influenced by pollen sensitization, because mentioned allergens contain major components homologous to major tree pollen proteins from Bet v1-like family [19, 20].

slgG4 responses to food allergens are observed in patients with all allergic diseases (Fig. 2). Among inhalant allergens, the highest adjusted slgG4 prevalence is observed for the allergens most responsible for sensitization, i.e. detectable slgE production (birch pollen, alder pollen, cat dander, and dog dander). Figure 3 indicates that detectable slgG4 levels to inhalant allergens and some plant food allergens are common in sensitized patients with detectable slgE levels, whereas no reliable difference is observed in rates of patients with slgG4 to food allergens of animal origin between sensitized and non-sensitized groups.

Significant differences in adjusted slgG4 prevalence are observed between the groups with atopic dermatitis and with airway disorders for a variety of inhalant (mostly pollen) and food allergens (Tables 2 and 3).

**Discussion**

This study is aimed at the slgE and slgG4 responses among atopic patients from 6 months to 17 years of age with allergic diseases: bronchial asthma, upper airway disorders or atopic dermatitis. The age range was chosen as 0 to 17 years because it is known that there are noticeable changes in immunoglobulin patterns among allergic patients during this period of life [21, 22]. After the age of 18–20 years the slgE profiles do not change significantly [23].

Although genetic predispositions to asthma and upper airway disorders are associated with different genetic polymorphisms, both diseases have common profiles of inflammatory mediators and cell-mediated responses involving eosinophils in the allergic inflammation [24]. Subsequently, the corresponding similarity in the physiological processes affects the prevalence of slgE to the allergens involved.

For all explored allergens, with few exceptions, namely, cat and dog dander, considerable differences between slgE profiles for patients with asthma and upper airway disorders are not observed (Fig. 1). Daniel J. Stoltz et al. [25] reported that increased asthma risk at the age of 6 years is more strongly associated with
sensitization to dog and cat dander at 1 and 3 years than with the absence of sensitization at younger age. Similar interrelation is not observed for allergic rhinitis; that is why the authors suggested that sensitization to perennial allergens is more closely linked to asthma development.

Our investigations are in agreement: sIgE sensitization to cat or dog dander are more strongly associated with asthma then with upper airway disorders (for cat dander – aOR = 1.808, 95% CI (1.154;2.833); for dog dander – aOR = 2.225, 95% CI (1.365;3.628)).

Our data on sIgE sensitization partly coincides with the results observed among adult population (22–86 years old) in the study [26]; there were no significant differences in sIgE prevalences between patients with asthma and rhinitis not only for pollen allergens but for cat and dog dander too.

Unlike upper airway disorders, atopic dermatitis and bronchial asthma have a strong connection with food allergens [27, 28], which agrees with our results: sIgE prevalences for food allergens are higher for these pathologies, though the difference is not statistically significant, probably because of the insufficient sampling size.

As the study has not included healthy controls, we have compared sIgE prevalence in our study with the

| Table 3 Adjusted ORs (aORs) for sIgE and sIgG4 prevalences among patients diagnosed with bronchial asthma, upper airway disorders (such as rhinosinusitis and rhinitis) or atopic dermatitis for food allergens |
|-----------------|-----------------|-----------------|-----------------|
| Allergens       | Asthma versus Upper Airway Disorders | Asthma versus Dermatitis | Upper Airway Disorders versus Dermatitis |
|                 | aOR              | 95% CI           | aOR              | 95% CI           | aOR              | 95% CI           |
| **sIgE to food allergens** | | | | | | |
| **Animal food allergens** | | | | | | |
| Egg white       | 1.782 (0.977;3.25) | 1.693 (0.976;2.939) | 0.950 (0.55;1.64) |
| Cow milk        | 1.526 (0.518;4.499) | 0.645 (0.253;1.646) | 0.423 (0.168;1.066) |
| α-lactalbumin, cow milk | 1.057 (0.289;3.866) | 0.527 (0.165;1.686) | 0.499 (0.181;1.374) |
| β-lactoglobulin, cow milk | 3.264 (0.286;37.209) | 0.852 (0.183;3.96) | 0.261 (0.033;2.049) |
| Casein, cow milk | 1.227 (0.363;14.149) | 0.875 (0.293;26.19) | 0.714 (0.268;1.962) |
| Codfish         | 3.560 (1.215;10.432) | 2.646 (1.039;6.739) | 0.743 (0.237;2.329) |
| **Plant food allergens** | | | | | | |
| Wheat flour     | 1.442 (0.464;4.83) | 1.935 (0.645;8.5) | 1.342 (0.472;3.811) |
| Peanut          | 1.455 (0.528;4.01) | 1.290 (0.494;3.67) | 0.887 (0.345;2.78) |
| Hazelnut (f)    | 2.874 (1.059;7.798) | 1.626 (0.687;3.847) | 0.566 (0.202;1.587) |
| Carrot          | 1.075 (0.587;1.969) | 3.089 (1.449;6.584) | 2.873 (1.388;5.946) |
| Apple           | 0.789 (0.506;1.23) | 3.164 (1.927;5.195) | 4.011 (2.535;6.346) |
| Peach           | 0.772 (0.179;3.327) | 1.863 (0.392;8.66) | 2.413 (0.609;9.561) |
| **sIgG4 to inhalant allergens** | | | | | | |
| **Animal food allergens** | | | | | | |
| Egg white       | 0.297 (0.091;0.973) | 2.305 (1.065;5.014) | 7.762 (2.666;22.6) |
| Cow milk        | 1.001 (0.557;1.803) | 1.493 (0.841;2.63) | 1.490 (0.878;2.531) |
| α-lactalbumin, cow milk | 0.865 (0.537;1.395) | 1.262 (0.789;2.02) | 1.458 (0.942;2.64) |
| β-lactoglobulin, cow milk | 1.105 (0.688;1.776) | 1.765 (1.106;2.818) | 1.597 (1.048;2.437) |
| Casein, cow milk | 1.213 (0.761;1.939) | 2.037 (1.273;3.262) | 1.678 (1.125;2.6) |
| Codfish         | 1.288 (0.623;2.663) | 3.841 (1.613;9.151) | 2.981 (1.285;6.92) |
| **Plant food allergens** | | | | | | |
| Wheat flour     | 1.164 (0.75;1.809) | 1.960 (1.262;3.044) | 1.683 (1.128;2.512) |
| Peanut          | 0.974 (0.622;1.524) | 1.823 (1.134;2.93) | 1.871 (1.206;2.905) |
| Hazelnut (f)    | 0.919 (0.594;1.425) | 1.971 (1.257;3.093) | 2.144 (1.418;3.24) |
| Carrot          | 1.261 (0.682;3.338) | 2.137 (1.084;2.32) | 1.694 (0.878;3.269) |
| Apple           | 1.090 (0.667;1.781) | 2.004 (1.171;3.43) | 1.838 (1.112;3.041) |
| Peach           | 1.348 (0.774;2.351) | 2.593 (1.428;7.11) | 1.923 (1.088;3.4) |

aORs were calculated using logistic regression analysis with the age and gender as covariates.
slgE prevalence in random population from Russian Karelia, that was analyzed in the study of Ruokolainen L et al. [29] (Additional file 2 in Supplementary materials). From the Additional file 2 one can see that sensitization rate for analyzed inhalant allergens in a random population is lower than normalized sensitization rate in the groups with atopic patients from our study. As the random population from the study of Ruokolainen L et al. included some atopic patients (5.1% with atopic eczema, 1.2% with asthma) we assume that the sensitization rate in the group containing only healthy donors would be even lower, so the mentioned tendency would be correct for the control group of healthy donors too.

In contrast to IgE, the functions of IgG antibodies of different subclasses in the allergic pathologic process are still being discussed. The food-specific IgG response of different subclasses is mostly considered as part of the normal reaction to natural exposure to food products [11, 30]. Food allergens are the most common antigens that lead to the production of slgG, and its subclass slgG4 accordingly [31]. The data regarding slgG4 obtained in our study confirm these findings (Fig. 2). Previously, we have studied the changes in slgG4 prevalence among pediatric patients depending on the age and our results showed that the prevalence of slgG4 to food antigens increases throughout the age [22]. According to a

| Inhaled Allergens     | Prevalence (%) |
|-----------------------|----------------|
| Alder                 | 10             |
| Birch                 | 12             |
| Hazelnut (p)          | 5              |
| Oak                   | 10             |
| Wormwood              | 5              |
| Mugwort               | 10             |
| Dandelion             | 5              |
| Bermuda grass         | 5              |
| Orchard grass         | 10             |
| Meadow fescue         | 15             |
| Perrenial rye grass   | 5              |
| Timothy grass         | 20             |
| Cultivated rye        | 10             |

| Indoor Allergens      | Prevalence (%) |
|-----------------------|----------------|
| Cat dander            | 5              |
| Dog dander            | 10             |
| D. pteronyssinus      | 15             |
| D. farinae            | 20             |
| Alternaria tenuis     | 5              |
| Cockroach, German     | 10             |

| Food Allergens        | Prevalence (%) |
|-----------------------|----------------|
| Egg white             | 10             |
| Cow milk              | 15             |
| a-lactalbumin, cow milk| 20            |
| b-lactoglobulin, cow milk| 15          |
| Casein, cow milk      | 5              |
| Codfish               | 10             |
| Wheat flour           | 20             |
| Peanut                | 15             |
| Hazelnut (f)          | 10             |
| Carrot                | 5              |
| Apple                 | 10             |
| Peach                 | 5              |

In Fig. 2 Age-adjusted slgG4 prevalences (the rate of patient who exhibit slgG4 above 100 ng/ml) among the patients diagnosed with bronchial asthma, upper airway disorders (such as rhinosinusitis and rhinitis) or atopic dermatitis.
number of studies [12, 32], this increase might be the hallmark of tolerance induction.

Notably, IgG4 represents a small portion of total IgG. Thus, the evolution of IgG in the young population is not associated with the evolution of IgG4 but with the IgG1 subclass, which is the main fraction of IgG [33]. Xinyuan Huang et al. [31] demonstrated that the production of the total IgG specific to inhalant allergens correlates with sensitization to the antigen in question. Schwarz A et al. [30] showed that sIgG to inhalant allergens as well as sIgG4 subclass to a variety of food allergens are observed mostly among sensitized patients at the age of 2 years. In [30] sIgG4 to inhalant allergens was not observed due to the extremely low sIgG4 prevalence to these antigens (< 5%) at the studied age.

The median age of the participants in our study is higher than that in [30], which corresponds to the increased sIgG4 prevalence in our cohort; it explains noticeable sIgG4 prevalence to inhalant allergens observed in the present experiments. Our study is focused only on sIgG4, however, the present analysis of sIgG4 alone is also of interest because subjects with the prevailing sIgG4 response are usually present along with the subjects with the prevailing sIgG1 response [34].

In the present study, a significant difference is observed in the rates of patients with sIgG4 response between the groups of sensitized and non-sensitized patients for most inhalant allergens assessed (Fig. 3). In contrast, significant differences for food allergens that are analogous to the food components in the study [30] (β-lactoglobulin – Bos d5, casein – Bos d8) were not affirmed. This finding can be explained by the evolution of sIgG4 as subjects age.

The observed interrelation of sIgG4 and sIgE responses for inhalant allergens combined with the differences in sIgE profiles between patients with airway disorders and those with atopic dermatitis leads to significant distinctions in the sIgG4 profiles for pollen allergens, for which significant differences in adjusted sIgE prevalence were observed while comparing different allergic diseases (Table 2).

Due to the positive correlation between the presence of sIgE and significant sIgG4 to inhalant allergens in the serum samples, we assume that lower sensitization rates to inhalant allergens in healthy donors in comparison with atopic patients lead to the lower rates of sIgG4 response to inhalant allergens in the group of healthy population. Similar observation was made by Dubakiene et al. [35] for one of the inhalant allergens, D. pteronyssinus.

Overall, this analysis of sIgE response to inhalant allergens among atopic patients shows that sIgE prevalence to pollen allergens is significantly reduced in patients with atopic dermatitis compared with those with asthma and upper airway disorders. In addition, cat and dog sensitization are more strongly associated with bronchial asthma than with atopic dermatitis and upper airway disorders. The increased sIgG4 response to pollen allergens among patients with respiratory allergic diseases can be considered a consequence of the fact that sIgG4 response is most common among patients with detectable sIgE and of the features of the sIgE profiles for these pathologies, as illustrated by experimental data analysis in our study.
Conclusions
The obtained data demonstrate that different allergic disease entities are characterized by individual features of allergen-specific IgE and IgG4 production, which leads to specific manifestations of allergic inflammation. A more comprehensive study of patients’ sIgE and sIgG4 profiles with respect to the clinical performance and functions of allergen-specific antibodies would allow more effective diagnosis and treatment of atopic patients.

Additional files

Additional file 1: Results of the determination of allergen-specific IgE and IgG4 on the microarrays. The data are shown in qualitative format: 0 - antibody concentration do not reach the cutoff of 0.35 IU/ml for sIgE or the value of 100 ng/ml for sIgG4. 1 - antibody concentration reach the cutoff of 0.35 IU/ml for sIgE or the value of 100 ng/ml for sIgG4. Spreadsheet “Age groups”: The rate of patients with sIgE > 0.35 IU/ml or sIgG4 > 100 ng/ml %. The data were normalized in each cell to the number of patients with the indicated diseases in the indicated age group. (XLSX 16 kb)

Additional file 2: Comparison of age-adjusted sIgE prevalences among the patients diagnosed with bronchial asthma, upper airway disorders (such as rhinosinusitis and rhinitis) or atopic dermatitis involved in the study with the sIgE prevalences of the random population from Russian Karelia (Ruokolainen L et al., Clin Exp Allergy. 2017). (TIF 96 kb)

Abbreviations
aOR: Adjusted odds ratio; ASIT: Allergen-specific immunotherapy; CI: Confidence interval; sIgE: Specific immunoglobulin E; sIgG4: Specific immunoglobulin G4

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Authors’ contributions
ET, TF, EA and LP contributed to the clinical biomaterial collecting and its primary characterization; OS, GF contributed to the designing of the experiments; SV and AA were the main contributors in the performing of the analysis; YL, OS, and GF participated in the data analysis; OS, A2, and YL were involved in the data interpretation; OS and GF contributed to the manuscript preparation; AZ, VB, AC, and AR contributed to the manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the local ethics committee of the Filatov Moscow City Pediatric Clinic No. 13.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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