CORRELATED DYNAMICS OF SERUM IGE AND IGE$^+$ CLONOTYPE COUNT WITH ALLERGEN AIR LEVEL IN SEASONAL ALLERGIC RHINITIS

Mikelov AI[1,2], Staroverov DB[2], Komech EA[2,3], Lebedev YB[2,3], Chudakov DM[1,2,3], Zvyagin IV[2,3] ✉

[1] Skoltech, Moscow, Russia

[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia

[3] Pirogov Russian National Research Medical University, Moscow, Russia

Mechanisms of maintenance of immunological memory in the chronic course of seasonal allergic rhinitis remain poorly understood. The detailed understanding of these mechanisms is required for design of new approaches for allergy treatment. It is known that the level of allergen-specific IgE antibodies (sIgE), which play a key role in the development of the disease, is increased in patients with seasonal allergic rhinitis during pollination season. This study aimed to investigate the dynamics of serum IgE levels and characteristics of the clonal repertoire of IgE-secreting lymphocytes depending on the intensity of the patient's contact with the allergen. For three patients, allergic to birch pollen (22, 22, and 28 y.o.), we measured total IgE and birch pollen specific IgE levels at 6 time points with 2 week interval during the birch pollination season. Immunoglobulin heavy chain gene (IGH) clonal repertoire data for several B-cell subpopulations at different time points were obtained for one patient. We observe growth of the sIgE level (91%, 37%, and 64% compared to the baseline) at the peak of pollination season in all three donors. Initial increase in sIgE and total IgE levels coincides with the birch pollination initiation; sIgE and total IgE levels correlate with the birch pollen air level (sIgE: $R^2 = 0.98$ at $p < 0.05$; total IgE: $R^2 = 0.95$ at $p < 0.05$). We detected IgE clonotypes only in samples obtained during the birch pollination, which indicates an increase of IGE-expressing cells concentration during this period. The frequency of IgE clonotypes was extremely low compared to that of the clonotypes of other isotypes (IgE — 0.01%, IgM — 48.4%, IgD — 14%, IgG — 17.4%, IgA — 19.8%). Hypermutation and phylogenetic analysis of the sequences from the 13 detected IgE-containing clonal groups showed that these IgE clonotypes could originate from IgG as a result of sequential isotype-switching.

Keywords: allergic rhinitis, allergen-specific IgE, birch pollen, seasonal dynamics, immunoglobulin clonal repertoire

Funding: the study was supported by the Grant Council of the President of the Russian Federation (grant MK6000.2018.4).

Acknowledgments: we are very grateful to all the donors who participated in the study.

Author contribution: Mikelov AI — ELISA, preparation of IGH cDNA libraries, sequencing data and results analysis, research design, drafting the manuscript; Staroverov DB — cell subpopulations isolation (flow cytometry); Komech EA — blood samples collection, cell subpopulations isolation (flow cytometry); Lebedev YB — results analysis and discussion, advisory support; Chudakov DM — results analysis and discussion, advisory support (cDNA libraries preparation); Zvyagin IV — preparation of IGH cDNA libraries, sequencing data and results analysis, research design, drafting the manuscript, research organization.

Compliance with ethical standards: the study was conducted in full compliance with the requirements of the 2013 Helsinki Declaration; all donors signed a voluntary informed consent to participate in the study. Blood samples were taken by qualified medical personnel in the certified clinic ("Invitro").

✉ **Correspondence should be addressed:** Ivan V. Zvyagin
Miklukho-Maklaya, 16/10, Moscow, 117997; izvyagin@gmail.com

Received: 09.10.2019 **Accepted:** 23.10.2019 **Published online:** 31.10.2019

DOI: 10.24075/brsmu.2019.072

СОГЛАСОВАННАЯ ДИНАМИКА СЫВОРОТОЧНОГО IGE И ЧИСЛЕННОСТИ IGE$^+$-КЛОНОТИПОВ С УРОВНЕМ ПЫЛЬЦЫ В ВОЗДУХЕ ПРИ ПОЛЛИНОЗЕ

А. И. Микелов[1,2], Д. Б. Староверов[2], Е. А. Комеч[2,3], Ю. Б. Лебедев[2,3], Д. М. Чудаков[1,2,3], И. В. Звягин[2,3] ✉

[1] Сколковский институт науки и технологий, Москва, Россия

[2] Институт биоорганической химии имени М. М. Шемякина и Ю. А. Овчинникова, Москва, Россия

[3] Российский национальный исследовательский университет имени Н. И. Пирогова, Москва, Россия

Механизмы поддержания иммунологической памяти, обусловливающей хроническое течение сезонного аллергического ринита у человека, слабо изучены. Рациональный дизайн новых подходов к терапии аллергических заболеваний требует детального понимания этих механизмов. Известно, что уровень аллергенспецифичных антител класса IgE (sIgE), являющихся ключевым агентом в развитии данного заболевания, повышен у пациентов с поллинозом в период цветения. Целью работы было изучить динамику сывороточного уровня IgE и характеристик клонального репертуара IgE-секретирующих лимфоцитов в зависимости от интенсивности контакта пациента с аллергеном. Для трех пациентов (22, 22 и 28 лет) с аллергией на березовую пыльцу были измерены уровни общего IgE и sIgE к березовой пыльце в 6 временных точках с интервалом в 2 недели, включая период пыления березы. Для одного донора получены данные о клональном репертуаре генов тяжелых цепей иммуноглобулинов (IGH) субпопуляций B-клеточного ряда. Прирост уровня sIgE в период пыления составил 91%, 37% и 64% по сравнению с исходным у трех доноров. Момент начала роста уровня sIgE и общего IgE и его динамика согласуются с ростом концентрации аллергена (sIgE: $R^2 = 0{,}98$ при $p < 0{,}05$; IgE общий: $R^2 = 0{,}95$ при $p < 0{,}05$). IgE-клонотипы обнаружены только в образцах, взятых в период цветения березы, что свидетельствует о росте их концентрации в этот период. Доля IgE-клонотипов оказалась крайне низкой по сравнению с клонотипами других изотипов (IgE — 0,01%, IgM — 48,4%, IgD — 14%, IgG — 17,4%, IgA — 19,8%). Оценка числа гипермутаций и филогенетический анализ последовательностей внутри 13 обнаруженных IgE-содержащих клональных групп показали возможность происхождения IgE-клонотипов из IgG в результате смены изотипа.

Ключевые слова: аллергический риноконъюнктивит, аллергенспецифичный IgE, пыльца березы, сезонная динамика, клональный репертуар иммуноглобулинов

Финансирование: исследование выполнено при поддержке Совета по грантам Президента Российской Федерации (грант МК6000.2018.4).

Благодарности: выражаем большую признательность всем донорам, принявшим участие в исследовании.

Информация о вкладе авторов: А. И. Микелов — определение уровня антител, подготовка библиотек кДНК IGH, анализ данных секвенирования и полученных результатов, дизайн исследования, подготовка рукописи; Д. Б. Староверов — выделение клеточных субпопуляций с помощью проточной цитофлюорометрии; Е. А. Комеч — сбор образцов крови, выделение клеточных субпопуляций с помощью проточной цитофлюорометрии; Ю. Б. Лебедев — анализ и обсуждение полученных результатов, консультативная поддержка; Д. М. Чудаков — анализ и обсуждение полученных результатов, консультативная поддержка подготовки библиотек кДНК; И. В. Звягин — подготовка библиотек кДНК IGH, анализ данных секвенирования и полученных результатов, дизайн исследования, подготовка рукописи, организация исследования.

Соблюдение этических стандартов: исследование проведено в полном соответствии с требованиями Хельсинской декларации 2013 г.; все доноры подписали добровольное информированное согласие на участие в исследовании. Забор образцов крови выполнен квалифицированным медицинским персоналом в сертифицированных кабинетах компании «ИНВИТРО».

✉ **Для корреспонденции:** Иван Владимирович Звягин
ул. Миклухо-Маклая, д. 16/10, г. Москва, 117997; izvyagin@gmail.com

Статья получена: 09.10.2019 **Статья принята к печати:** 23.10.2019 **Опубликована онлайн:** 31.10.2019

DOI: 10.24075/vrgmu.2019.072

Seasonal allergic rhinoconjunctivitis (SAR), or seasonal allergic rhinitis, is one of the most common allergies. According to various studies, 17 to 28% of the population of Europe suffer from this disease [1]. A significant part of SAR patients (8–16% of the total population) react to tree pollen, and birch pollen is the most common tree-produced allergen [2]. Same as other types of allergy, SAR is a chronic disease for most patients, and the probability of development of concomitant pathologies, such as asthma and food allergies, is quite high. While the most widely used therapeutic agents only aim to alleviate the symptoms (antihistamine and glucocorticosteroid drugs, mast cell membrane stabilizers), allergen-specific immunotherapy is the only currently practiced approach to SAR treatment that is designed to bring a long-term change in the chronic course of the disease. This is a long-term therapy that requires strict patient compliance and yields complete disappearance of the symptoms only in some patients [3]. Therefore, development of the new therapeutic approaches designed to prevent SAR from turning chronic is an extremely urgent task.

Rational design of such approaches requires a deep understanding of the mechanisms of maintenance of immunological memory that determine chronic course of allergy. A number of recent unsuccessful clinical trials of the allergic rhinitis treatment modalities demonstrate that the currently available information about these mechanisms is insufficient [4, 5]. Basic research efforts implying deeper investigation of the mechanisms of immunological memory preservation, which turns the allergy chronic, is a necessary basis both for the development of the new approaches to SAR treatment and improvement of the existing therapies.

It is known that IgE, a class E immunoglobulin, is one of the key agents in the development of an allergic response. However, there is little information about cell subpopulations producing IgE in allergic rhinitis patients: their localization, lifespan and required survival factors are not clear. Recently, using a mouse model of food allergy it was demonstrated, that shortly after sensitization IgE-secreting plasma cells can be found in the bone marrow and have a limited lifespan (up to 1 year) [6]. IgE class antibodies can remain bound at mast cell membranes for up to 100 days. It was also shown that at least some of the long-term immunological memory that turns allergy chronic is maintained by the subpopulation of IgG1$^+$ allergen-specific memory B-lymphocytes, which serves as a "reservoir" from which the population of IgE producers is replenished in case of contact with the allergen [6, 7].

This study aimed to investigate the dynamics of serum IgE levels in greater detail compared to the previous research efforts [8–10]. We enrolled patients allergic to birch pollen and studied the dependence of the aforementioned dynamics on the intensity of their contact with the allergen that is the derivative of the pollen concentration in air. As part of the study, we sought to identify the time point that, relative to the moment of contact with the allergen, marks replenishment of the IgE secreting cell fraction. We also aimed to characterize the subpopulation of IgE-secreting plasmablast and plasma cells aand describe their relationship to other isotypes and memory B-cell population during seasonal exacerbation of the disease.

METHODS

Patient cohort and collection of serum samples

The studied cohort included three patients (22, 22, and 28 years old). The inclusion criteria were: 1) any gender; 2) previously diagnosed seasonal birch pollen allergic rhinitis; 3) no bronchial asthma; 4) no other chronic inflammatory, autoimmune, oncological and infectious diseases. The exclusion criteria were: 1) ongoing or recently finished allergen-specific immunotherapy course; 2) vaccination during the study period. Venous blood samples were taken in medical laboratories and by medical personnel of the Invitro CDL chain of labs; the personnel used the Vacuette Serum Gel Z and Vacuette K3EDTA tubes (BD Biosciences; USA). Further experimental work was carried out in the research laboratory of the Institute of Bioorganic Chemistry, Russian Academy of Science.

To measure the total level of IgE and the level of birch and alder pollen-specific IgE we collected the patients' blood samples every fortnight from March to May 2019, thus taking 6 samples from each donor. From one of the donors (28 years old) we also collected four additional serum samples collected in March and May 2017, and in March and May 2018.

ELISA

We followed the protocol provided by the tube manufacturer (BD Biosciences; USA) to isolate blood serum. To establish the total IgE level and the level of birch and alder pollen-specific IgE we used the commercially available ELISA kits (Alkor Bio; Russia) according to the manufacturer's protocol. Hidex Sense reader (Hidex Oy; Finland) was used to measure the optical density. Each serum sample was measured in two independent replicates.

Isolation of subpopulations of memory B-lymphocytes, plasmablasts and plasma cells

For one of the donors (28 years old), we obtained samples of three cell B-cell subpopulations: memory B-lymphocytes, plasmablasts and peripheral blood plasma cells. These samples were collected at three time points, March and May 2017 and March 2018.

Peripheral blood mononuclear cells from whole blood were isolated using standard Ficoll density gradient protocol: centrifugation at 310g for 20 min, Eppendorf 5804 centrifuge (Eppendorf; Germany). Then, the cells were stained with the set of fluorescently-labeled antibodies: anti-CD19-APC, anti-CD20-VioBlue, anti-CD27-VioBright FITC, anti-CD138-PE-Vio770 (Myltenyi Biotec; USA). Next, using the fluorescence-activated sorting equipment (BD FacsAria III, BD Biosciences; USA) we isolated the target B-cell subpopulations: memory B-lymphocytes (CD19$^+$ CD20$^+$ CD27$^+$), plasmablasts (CD20$^-$ CD19$^+$ CD27^{++} CD138$^-$) and plasma cells (CD20$^-$ CD19$^{low/-}$ CD27$^+$ CD138$^+$). At each time point, we collected two samples of memory B-lymphocytes (50,000 cells), plasmablasts and plasma cells (1000 and 500 cells, respectively).

Sequencing and analysis of B-cell receptor repertoires

Preparing the immunoglobulin (IgH) heavy chain cDNA libraries, we followed the previously published protocol [11] with some modifications. At the reverse transcription stage we introduced the adapter with a unique molecular identifier (UMI) and sample-specific barcode (Table 1). Further amplification was done in two stages using the primers described in Table 1. For sequencing we used Illumina Hiseq 2000/2500 (Illumina; USA) in pair-end mode with 310 + 310 read length.

MIGEC software [12] was used for raw data set demultiplexing and consensus assembling on the basis of unique molecular identifier and sample-specific barcode sequences [12]. MIXCR software [13] was used to annotate the V-, D-, J-, and C-segments,

to identify the unique clonal IGH sequences (clonotypes) and to assess the level of hypermutations. To assemble the IGH clonal sequences, we used the IGH cDNA sequences supported by at least two reads based on the UMIs analysis. Clonotypes were defined by the nucleotide sequence of the immunoglobulin heavy chain starting from V segment framework region 1 (FR1) to the end of the J segment, with respect to the antibody isotype determined by the 5' fragment of the C segment 15–16 nucleotides long. R programming language [14] was used for IGH clonal repertoire analysis, statistical processing of the results and regression analysis; the Figures were prepared using the ggplot2 package [15]. Change-O software [16] was used to identify the clonal groups on the basis of the following criteria: one clonal group contains IGH sequences which have the same CDR3 length and IGH V-segment, and at least 85% of CDR3 nucleotide sequence identity 15% R. Phylogenetic analysis was performed using MEGA 7 software [17] (maximum likelihood phylogenetic trees, evolutionary GTR model).

Regression analysis

Regression model was used to test whether there is a relation between the birch pollen sIgE serum level dynamics and the allergen air level. Table 2 shows the data used for the model. Regression equation:

$$sIgE\_level = b + k1 \times pollen\_level + k2 \times donorMRK + k3 \times donorLK,$$

where sIgE_level is a dependent variable, representing serum level of the birch pollen-specific IgE; pollen_level — predictor variable, representing average level of birch pollen in the air (class) registered through the two weeks before sampling blood to measure the serum IgE level; donor MRK — dummy variable, can be 1 (MRK donor) and 0 (not MRK donor); donorLK — dummy variable, can be 1 (donor LK) and 0 (donor not LK); b is the intercept of the regression model that reflects the basic level of the dependent variable, relating to MS donor; $k1$, $k2$, $k3$ are the regression coefficients ($k1$ reflects the change in the dependent variable when the pollen level in the air changes by one pollination class; $k2$ reflects the difference in the basic level of birch pollen-specific IgE between MRK and MS donors; $k3$ reflects the difference in the basic level of birch pollen-specific IgE between LK and MS donors.

Calculated coefficients: $b = 207.813$ ($p < 0.01$); $k1 = 6.474$ ($p < 0.05$); $k2 = -194.4$ ($p < 0.01$); $k3 = -208.15$ ($p < 0.01$). Adjusted $R^2 = 0.98$; $p < 0.01$; F test.

Similarly, we used a regression model to analyze the relation between the serum IgE level dynamics and the air level of birch pollen. Total IgE serum level was selected as the dependent variable; predictor variables were the same as in the described model for sIgE. For the model where the total IgE serum level is the dependent variable, the coefficients were as follows: $b = 1299.05$ ($p < 0.01$); $k2 = -1056.4$ ($p < 0.01$); $k1 = 51.45$ ($p < 0.05$); $k3 = -1313.65$ ($p < 0.01$). Adjusted $R^2 = 0.95$; $p < 0.01$; F test.

Pollen monitoring data

Pollen monitoring data for Moscow were obtained from open sources (Allergophone). Table 3 is the pollination classification table that shows the number of pollen grains per cubic meter of air for each class.

Table 1. Sequences of oligonucleotide primers used for IGH cDNA libraries

Primer name	Description	Sequence
cDNA synthesis		
SmartMK	Template-switch adapter for cDNA synthesis. *U* = dU, rG-riboG, *N* — A/G/C/T nucleotides	CAGUGGUAUCAACGCAGAGUACNNNNNNUTGAAAUNNNNNNUCTT(rG)4
hIGG_r1	Isotype-specific primer, IgG	GAAGTAGTCCTTGACCAGGCA
hIGM_r1	Isotype-specific primer, IgM	GTGATGGAGTCGGGAAGGAAG
hIGA_r1	Isotype-specific primer, IgA	GCGACGACCACGTTCCCATCT
hIGD_r1	Isotype-specific primer, IgD	GGACCACAGGGCTGTTATC
hIGE_r1	Isotype-specific primer, IgE	AGTCACGGAGGTGGCATTG
PCR amplification, step 1		
M1ss	Forward primer	AAGCAGTGGTATCAACGCA
hIGG_r2	Isotype-specific reverse primer, IgG/IgE	CCAGGGGGAAGACCGATG
hIGA_r2	Isotype-specific reverse primer, IgA	CTCAGCGGGAAGACCTTG
hIGM_r2	Isotype-specific reverse primer, IgM	ACGAGGGGGAAAAGGGTTG
hIGD_r2	Isotype-specific reverse primer, IgD	CCTGATATGATGGGGAACAC
hIGE_r2	Isotype-specific reverse primer, IgE	GTCAAGGGGAAGACGGATG
PCR amplification step 2		
M1s_long_i7	Forward primer *	CAAGCAGAAGACGGCATACGAGAT(i7)GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGTATCAACGCAGAG
hIGD_r2_long_i5	Isotype-specific reverse primer, IgD *	AATGATACGGCGACCACCGAGATCTACAC(i5)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATATGATGGGGAACAC
hIGM_r2_long_i5	Isotype-specific reverse primer, IgM *	AATGATACGGCGACCACCGAGATCTACAC(i5)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGGGGAAAAGGGTTG
hIGA_r2_long_i5	Isotype-specific reverse primer, IgA *	AATGATACGGCGACCACCGAGATCTACAC(i5)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGCGGGAAGACCTTG
hIGGE_r2_long_i5	Isotype-specific reverse primer, IgG/IgE *	AATGATACGGCGACCACCGAGATCTACAC(i5)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGARGGGGAAGACSGATG

Note: * (i5) and (i7) — sequences 8 nucleotides in length, which are sample-specific barcodes.

RESULTS

The total and birch pollen-specific IgE serum levels increased as the birch pollen level grew in the air

We measured total and birch pollen-specific serum IgE levels at 6 time points before and after the start of birch pollination season. The absolute values reflecting serum levels of both specific (Fig. 1A) and total IgE (Fig. 1B) significantly differ among patients, and the individual differences significantly exceed differences between IgE levels of the same donor at different time points. In all donors, the absolute values of both birch pollen sIgE and total IgE increased between time point 1 and time point 6. The level of birch pollen-specific IgE correlates with the total IgE level in each donor; it exceeds the reference level at all time points (Fig. 1).

The change in the levels of birch-pollen sIgE and total IgE registered through the period of the study is significantly smaller than the differences in the total and specific IgE serum levels between donors (coefficient of variation between all time points, one donor, birch pollen-sIgE: MS — 0.130, MRK — 0.228, LK — 0.282; total IgE: MS — 0.189, MRK — 0.154, LK — 0.485; the smallest variation coefficient between donors, sIgE — 1.39, total IgE — 1.17). Taking this into account, in order to characterize the seasonal dynamics of total IgE and sIgE in serum we considered these levels with regards to the initial level of total IgE and sIgE, measured before the birch pollination season had started (time point 1). For each donor, each total and birch pollen-specific IgE level measurement was normalized to the corresponding values of total and birch pollen-specific IgE level at time point 1.

Our findings are consistent with the previously published research on the seasonal dynamics of serum levels of IgE specific to other pollen allergens [8–10]: birch pollen-sIgE level grows significantly between the off-season time point (1) and the season peak time point (6). The dynamics of birch pollen sIgE correlates with the level of birch pollen in the air (Fig. 2). The maximum increase of sIgE was observed at the peak of the birch pollen level, between time points 3 and 5: the increase reached 90.7% for donor LK, for MRK — 63.7%, for MS — 36.9%.

A linear regression model was developed to statistically test the hypothesis that the level of birch pollen in the air and the serum level of birch pollen-specific IgE are related. According to the model, a high proportion of variance of the dependent variable (sIgE level) depends on the selected predictors (adjusted R^2 = 0.98), i.e. pollen air level and donor identifier (the latter accounts for significant differences in the absolute sIgE level between donors). All coefficients are significantly not equal to 0, ($p < 0.05$ for all coefficients), and the coefficient at the predictor-variable reflecting birch pollen air level is greater than 0 ($k1$ = 6.47), which serves as a statistical confirmation of the consistency of serum sIgE level dynamics with the birch pollen air level. Similarly, the regression model for total IgE level also demonstrates that its level grows together with the growth of the allergen's concentration in the air (adjusted R^2 = 0.95; $p < 0.05$ for each of the coefficients).

It should be noted that a relatively small (compared to the maximums registered through the observation period) birch pollen sIgE level increase occurs before the start of pollination season or when the allergen concentration is low (time points 1–3). The possible reasons could be a random fluctuation in birch pollen sIgE level (two out of three donors exhibited the absolute level increase below 1 IU/ml between time points 1–2 and 2–3) or a contact of the patient with birch pollen that occurred early, before pollen level growth was registered by pollen monitoring stations. Such an increase in the birch pollen sIgE level can also be the result of its cross-reactivity to alder pollen antigens as alder pollination season precedes birch pollination season and there is a high level of structural similarity between birch and alder pollen antigens. To assess the contribution of cross-reactive IgE we measured the level of alder pollen sIgE in all collected serum samples (Fig. 3). In all donors, the level of alder

Table 2. Data used to build the regression models. Birch pollen-specific and total IgE levels, averaged level of birch pollen in the air between corresponding time points

Time point	Donor	Birch pollen sIgE serum level, IU/ml	Total IgE serum level, IU/ml	Av. level of birch pollen in the air, class
1	MS	178.4	1066.3	0.00
2	MS	192.4	1148.9	0.00
3	MS	202.7	1275.9	0.07
4	MS	232	1419.7	1.07
5	MS	243.1	1469.4	3.83
6	MS	244.3	1779.9	2.13
1	MRK	15.7	273.7	0.00
2	MRK	17.4	245.9	0.00
3	MRK	17.6	288.5	0.07
4	MRK	23	293.1	1.07
5	MRK	27.1	358.3	3.83
6	MRK	25.7	362.2	2.13
1	LK	5.4	26.2	0.00
2	LK	6.4	31.2	0.00
3	LK	6.1	28.2	0.07
4	LK	6.2	44.8	1.07
5	LK	9.6	69.1	3.83
6	LK	10.3	78.7	2.13

Table 3. Pollination level — pollination classes according to the volume of pollen in the air

Amount of pollen grains per 1 m^3 of air	0	1–10	11–100	101–1000	1001–5000	> 5000
Class	0	1	2	3	4	5

pollen sIgE followed birch pollen air level more than alder pollen air level. Alder pollen sIgE peaked between time points 4 and 5, when the concentration of birch pollen in the air was at its maximum, but not at time points 1 and 2, when alder pollen level showed most of its growth. Therefore, we can assume a high level of birch pollen sIgE cross-reactivity to alder pollen antigens in all participating donors.

In 2 donors (MRK, LK), birch pollen sIgE grew slightly (<1 IU/ml) while alder pollen sIgE went down. Thus, the insignificant increase in birch pollen sIgE level shown for MRK and LK donors cannot be explained by its cross-reactivity to alder pollen sIgE. In MS donor, we registered growing alder pollen sIgE between time points 1 and 2, which may explain the increase in birch pollen sIgE level before the beginning of the birch pollination season.

The abundance of IgE-expressing cells in peripheral blood B-cell subpopulations is relatively low and increases during seasonal exacerbation of allergies

Based on the data obtained from the mouse model of food allergy, we can assume that birch pollen sIgE serum level and total IgE level grow with the increase of the number of IgE-producing cells. In order to assess the seasonal dynamics of IgE$^+$ cell abundance in different B-cell subpopulations, we analyzed the immunoglobulin heavy chain (IGH) clonal repertoires of memory B-cells, plasmablasts and plasma cells from peripheral blood at three time points (1_2017, 3_2018 — time points outside the birch pollination season, 2_2017 — season peak).

For all three time points, sequencing yielded a total of 50,550,291 reads representing 1,616,747 unique IGH cDNA molecules. To eliminate the majority of errors in determining the clonal sequence, we included only those IGH cDNA sequences which were supported by at least two independent reads. A total of 116,437 IGH clonotypes were identified (a clonotype is defined as unique nucleotide sequence of IGH starting from the framework region (FR1) of the V segment to the end of the J segment, with antibody isotype determined on the basis of 5' fragment of C segment). IgE clonotypes were extremely rare (~0.01% out of all detected clonotypes). For comparison, the frequency of IgM clonotypes was 48.4%, IgD — 14%, IgG — 17.4%, IgA — 19.8%.

All IGE$^+$ clonotypes were detected in IGH repertoires of memory B-lymphocytes, plasmablasts and long-lived plasma cells exclusively in samples obtained during the birch pollination

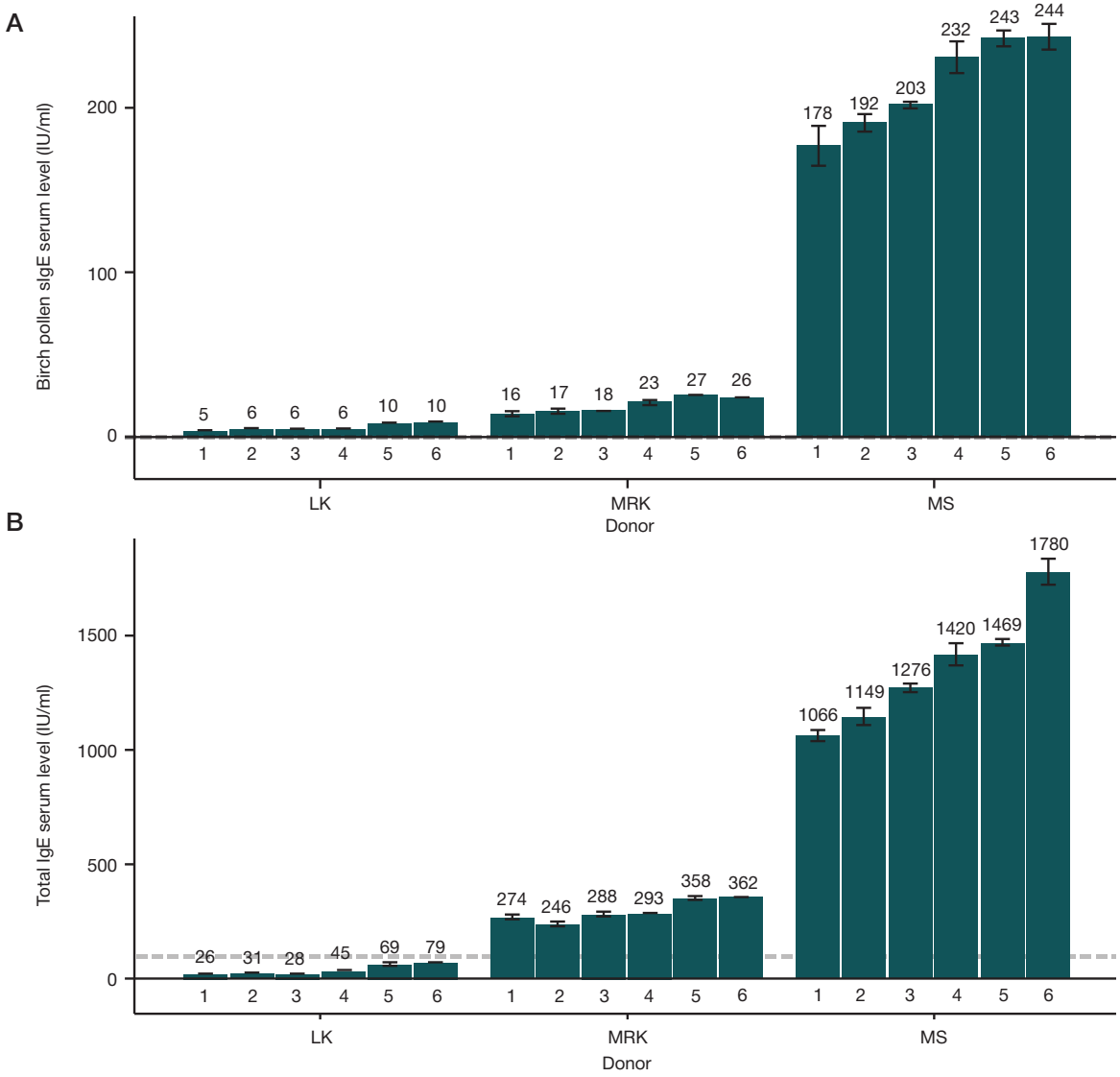

Fig. 1. Level of birch pollen-specific IgE (**A**) and total IgE (**B**) for three donors (LK, MRK, MS) with seasonal allergic rhinoconjunctivitis at six time points (1–6). Horizontal dashed line represents the reference level of corresponding antibodies in a healthy adult donor's serum (<0.35 IU/ml for sIgE, <100 IU/ml for general IgE). Error bars reflect difference between minimum and maximum measurement values

season (Fig. 4), while the sample size (in the number of cells) for each subpopulation does not differ between time points. This result demonstrates the increase in abundance of IGE$^+$ cells in the patient's peripheral blood during birch pollination season.

The technology applied to prepare immunoglobulin heavy chain cDNA libraries allows estimating the number of hypermutations along the entire length of the IGH sequence, from the FR1 region to 5' fragment of the constant region. For each clonotype, we identified the number of hypermutations and compared it between clonotypes with different isotypes. As expected, the lowest level of hypermutations was detected among IgD and IgM clonotypes (median — 2.3 and 3.7 bp per 100 bp of the V segment sequence, respectively), while a higher level — among IgG and IgA clonotypes (median — 6.8 and 7.1 bp per 100 bp of the V segment sequence, respectively) (Fig. 5), which are typically expressed in B-lymphocytes that matured in germinal centers. The average number of hypermutations of IgE clonotypes was not lower (median — 7.4 bp per 100 bp of the V segment sequence) compared to that of IgG and IgA clonotypes. Similar level of hypermutations between IgE and IgG clonotypes may reflect the origination of the IgE clonotypes of antibody-secreting cell subpopulations from memory IgG$^+$ B lymphocytes, but also does not exclude the independent isotype switching and accumulation of hypermutations in IgE$^+$ and IgG$^+$ B lymphocytes.

To analyze the relationship between IgE clonotypes in immunoglobulin heavy chain repertoires and clonotypes of other isotypes in greater detail we applied the previously described approach for identification of sets of clonotypes most likely derived from a single precursor and having a similar but not necessarily identical nucleotide sequence encoding a variable domain of the B-cell receptor heavy chain (clonal groups) [16]. A total of 13 IgE-containing clonal groups were identified; they included 154 clonotypes. In 4 of them we have identified clonotypes of other isotypes: IgG — 82, IgA — 19, IgM — 4 and IgD — 1. Phylogenetic analysis that we did for clonotypes of each of these clonal groups reveals a close relationship between IgE and IgG clonotypes (Fig. 6): for most IgE clonotypes, the closest neighbour in the phylogenetic tree was an IgG clonotype. It should be noted that in each of the considered IgE-containing clonal groups there were IgG-clonotypes representing the IgG$^+$ fraction of memory B-lymphocytes.

DISCUSSION

Previously published studies describe an increase in the serum level of total and allergen-specific IgE during the seasonal allergic rhinitis exacerbation [8–10]. However, these studies present the serum levels seasonal dynamics data

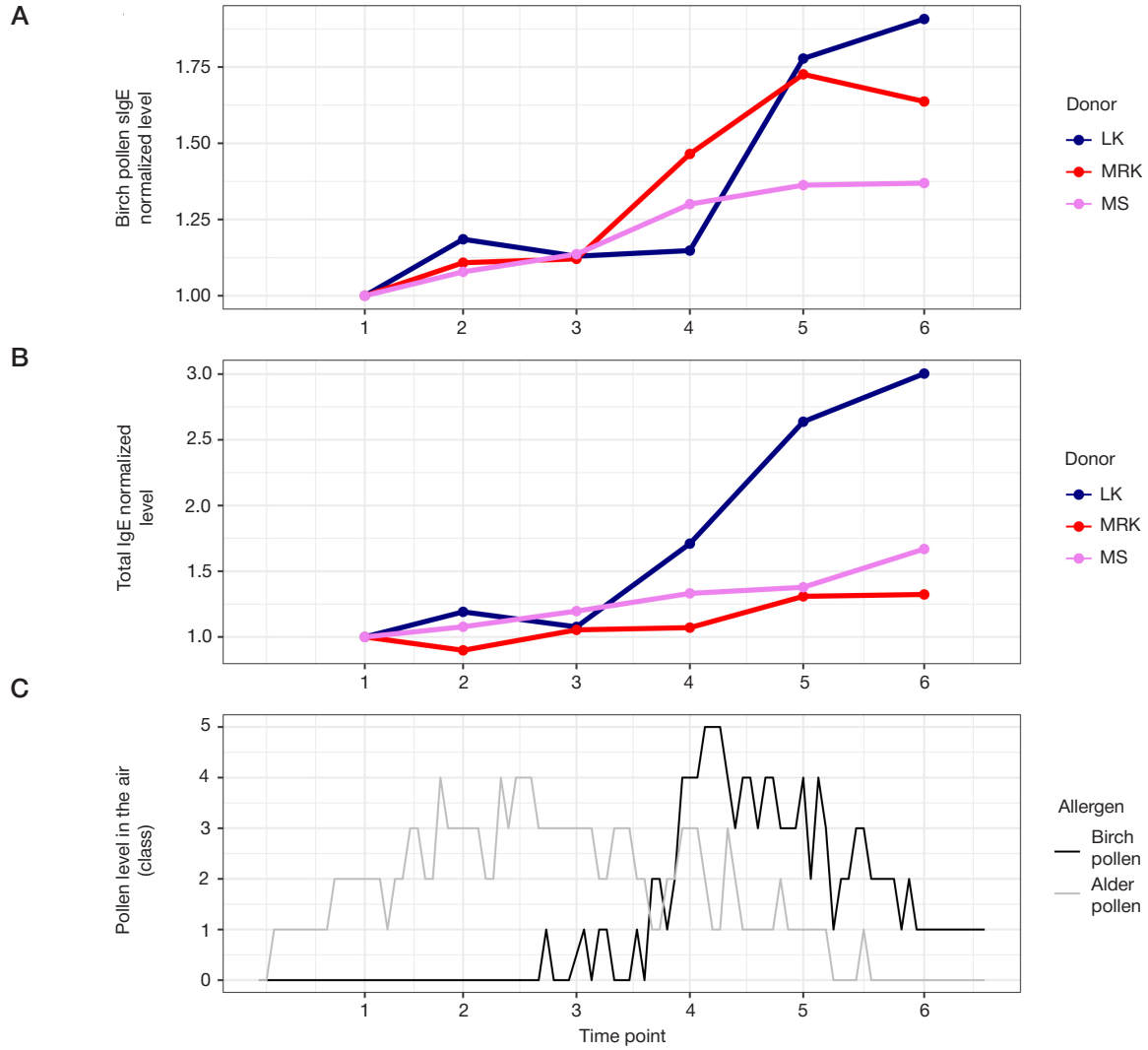

Fig. 2. Serum IgE level and birch pollen air level dynamics. **A**. Birch pollen sIgE serum level at each time point, normalized to the level of birch pollen sIgE at time point 1 for the corresponding donor. **B**. Total sIgE serum level at each time point, normalized to the level of total sIgE at time point 1 for the corresponding donor. **C**. Birch pollen level in the air

that were collected once every several months. Moreover, no connection was made to the air level of pollen that causes the allergy. In this work, we measured serum levels of total IgE and birch pollen/alder pollen-specific IgE with a higher time resolution (immunoglobulin levels were measured once a fortnight) during a period including the birch pollination commencement. Collation of the immunoglobulin levels and air level of the pollen at each time point allowed to demonstrate a correlation between birch pollen concentration in the air and level of birch pollen-specific and total IgE. Interestingly, all donors had the alder pollen sIgE growing together with the concentration of birch pollen in the air, but not with the level of alder pollen. Most likely, this is due to the birch pollen sIgE's cross-reactivity to alder pollen antigens; this fact may indicate that the donors that participated in our study lack specific antibodies to unique alder epitopes. However, since we did not register the alder pollen sIgE level before alder pollination season (it was a little over a week away from the beginning of sIgE level registration, and within this week the alder pollen air concentration was as low as 1–10 grains per 1 m^3), we cannot exclude the possibility of alder pollen sIgE peaking during this season (time points 1–3).

In all donors, birch pollen sIgE and total IgE levels began growing at the same time when the concentration of birch pollen in the air started to increase. This simultaneity may be the result of the growing count of IgE-secreting plasmablasts and plasma cells differentiating from memory B-lymphocytes in response to stimulation by the allergen. Analysis of the IGH repertoire dynamics and properties of sequences of identified IGH clonotypes generally confirms this assumption. The data obtained show that the abundance of peripheral blood IgE-expressing plasmablasts and plasma cells grows when birch starts pollinating, which reflects the active differentiation process of B-lymphocytes. The high level of hypermutations observed in IgE clonotypes is the sign of a long history of receptor selection in germination centers, which suggests that these IgE-secreting cells may originate from memory B-lymphocytes.

Overall, the obtained results allow assuming that the long-term immunological memory maintenance model proposed for food allergies [6] may also be valid for seasonal allergic rhinitis. In this model, the long-lived IgE-secreting plasma cells have a limited life span (~100 days in the mouse model), while memory IgG1 B-lymphocytes serve as the long-term "reservoir" from which the pool of IgE-secreting plasma cells is replenished. The replenishment occurs when the patient contacts the allergen, the process itself implies rapid proliferation, isotype switching, and differentiation of memory IgG1 B-lymphocytes under the action of Th2-associated cytokines (IL4, IL13, etc.). The previously published study of the B-cell receptor repertoires in respiratory allergies [18] did not allocate clonotypes to specific subpopulations, however, there were indications of clonal connections between IgG and IgE clonotypes in the peripheral blood immunoglobulin heavy chain repertoires, which is an indirect confirmation of correctness of the model suggested by R. Jiménez-Saiz and co-authors. The phylogenetic analysis of IgE-containing clonal groups, which was part of our study, demonstrates that the IgE-secreting plasma cells may originate from memory IgG^+ B lymphocytes in allergic rhinoconjunctivitis patients. However, the analysis does not exclude other IgE-producers origination patterns.

Our results also do not exclude the possibility of serum IgE level increase being caused by the boost in production and secretion of antibodies by existing IgE-secreting plasma cells. The average lifespan of IgE^+ plasma cells in human red bone marrow remains unexplored. Allergic rhinitis patients exhibit high IgE concentration in serum even outside the pollination season, which suggests the lifespan exceeding the time between two pollination seasons. However, taking into account the previously published data on correlation between

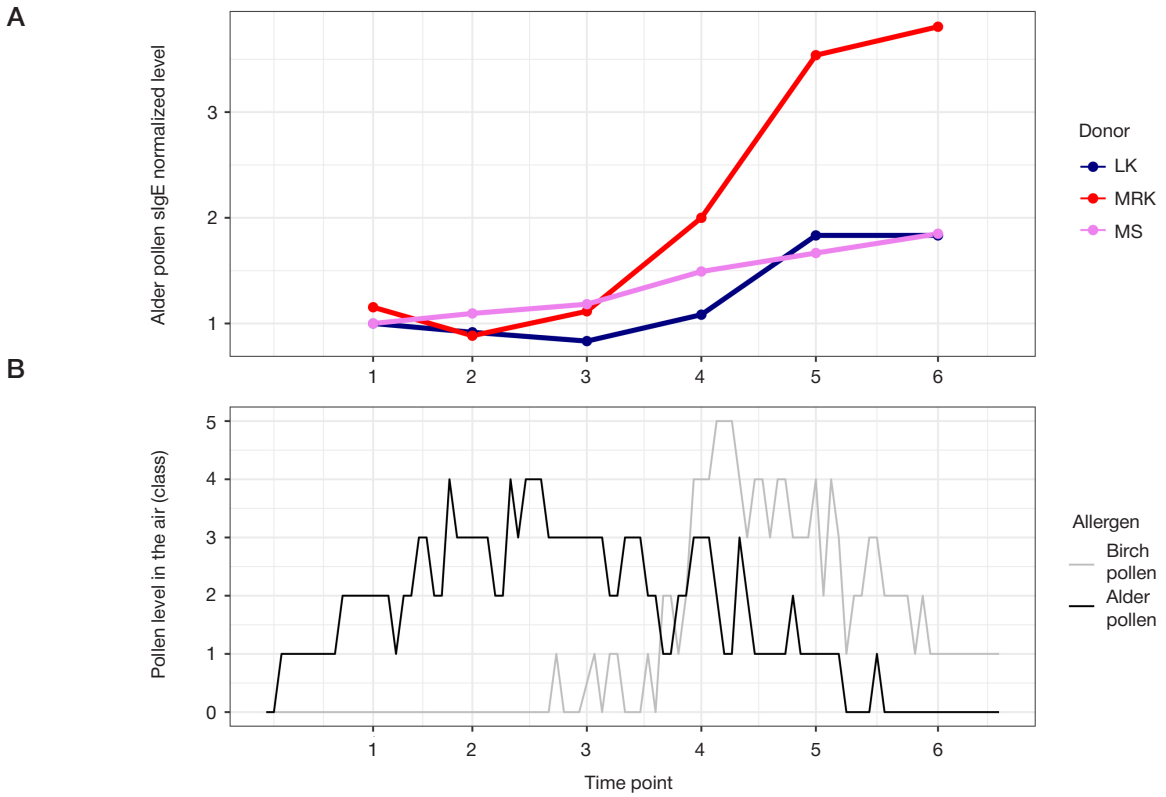

Fig. 3. Dynamics of alder pollen-specific IgE level and concentration of pollen in the air. **A**. Alder pollen sIgE serum level at each time point, normalized to the level of alder pollen sIgE at time point 1 for the corresponding donor. **B**. Pollen level in the air

serum IgE and peripheral blood IgE$^+$ plasma cells count [19], the growing number of IgE-secreting cells seems to be a more likely explanation for growth of IgE and sIgE levels, which is consistent with the results of this work.

CONCLUSIONS

Compared to the previously published study, we monitored the level of antibodies in serum at a higher temporal resolution, which allowed to demonstrate that level of sIgE starts to increase at the same time with the increase of birch pollen in air for patients with seasonal birch pollen allergy. The dynamics of total and allergen-specific IgE correlates with the level of birch pollen in the air.

Combined with the data obtained by other researchers in a mouse model of food allergy, our results of high-throughput sequencing and analysis of repertoires of cells of B-cell lineage suggest that this growth is most likely caused by the growing abundance of IgE-secreting plasma cells. The analysis of IGH nucleotide sequences of IgE-containing clonal groups of different B cell subpopulations showed a high similarity of IgE and IgG clonotypes and presence of IgG clonotypes representing the IgG$^+$ memory B lymphocyte fraction in these groups, which suggests that IgE-secreting cells could originate from a pool of IgG$^+$ B lymphocytes.

The small sample size does not allow to generalize the revealed characteristics to all patients since there is an alleged variety in endotypes of allergic rhinoconjunctivitis. The results we obtained in our study highlight similarities in the long-term immune memory maintenance mechanisms for SAR and food allergies. To validate this assumption, it is necessary to conduct

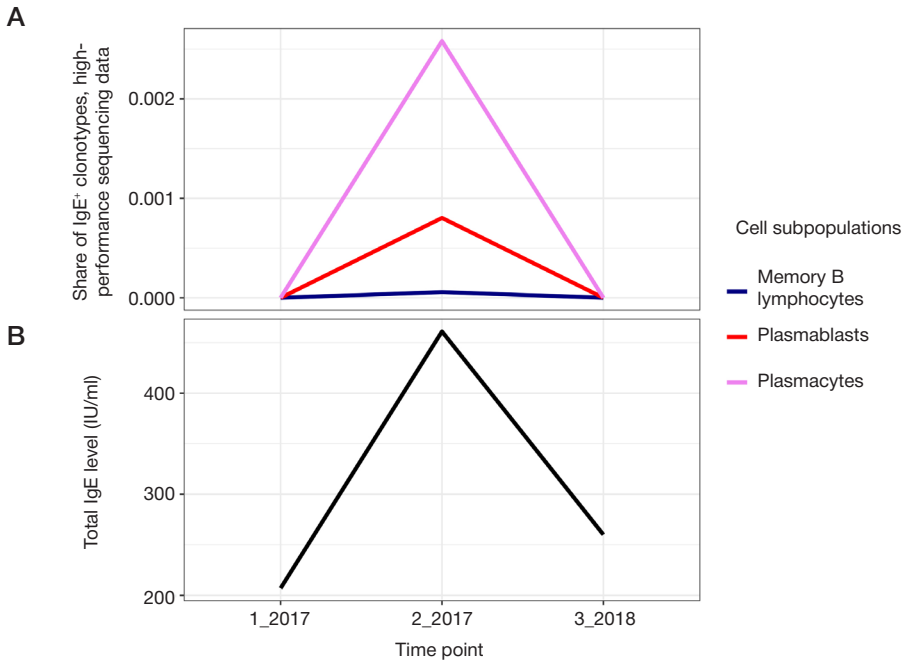

Fig. 4. B cell clonal repertoire dynamics. **A**. Abundance of IgE-clonotypes as revealed by high-throughput sequencing of immunoglobulin heavy chain repertoires for three subpopulations of peripheral blood cells: memory B lymphocytes, plasmablasts and plasma cells. **B**. Serum level of total IgE at the corresponding time points

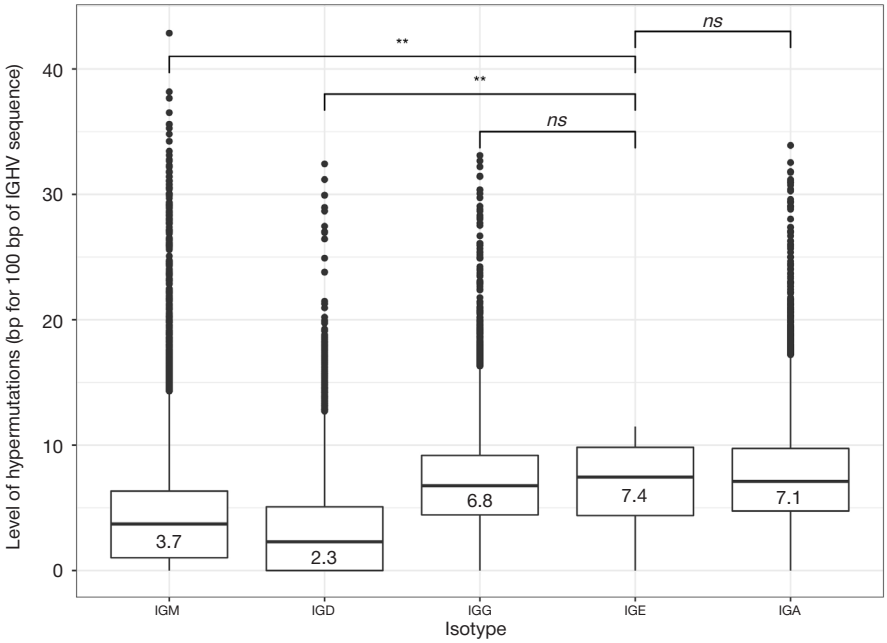

Fig. 5. Level of hypermutations (per 100 bp of IGHV sequence) calculated from high-throughput IGH repertoire sequencing data. IGM (n = 55 865), IGD (n = 15 834), IGG (n = 20 321), IGE (n = 13), IGA (n = 23 955). (** — $p < 0.01$; *ns* — not significant; Wilcoxon rank-sum test)

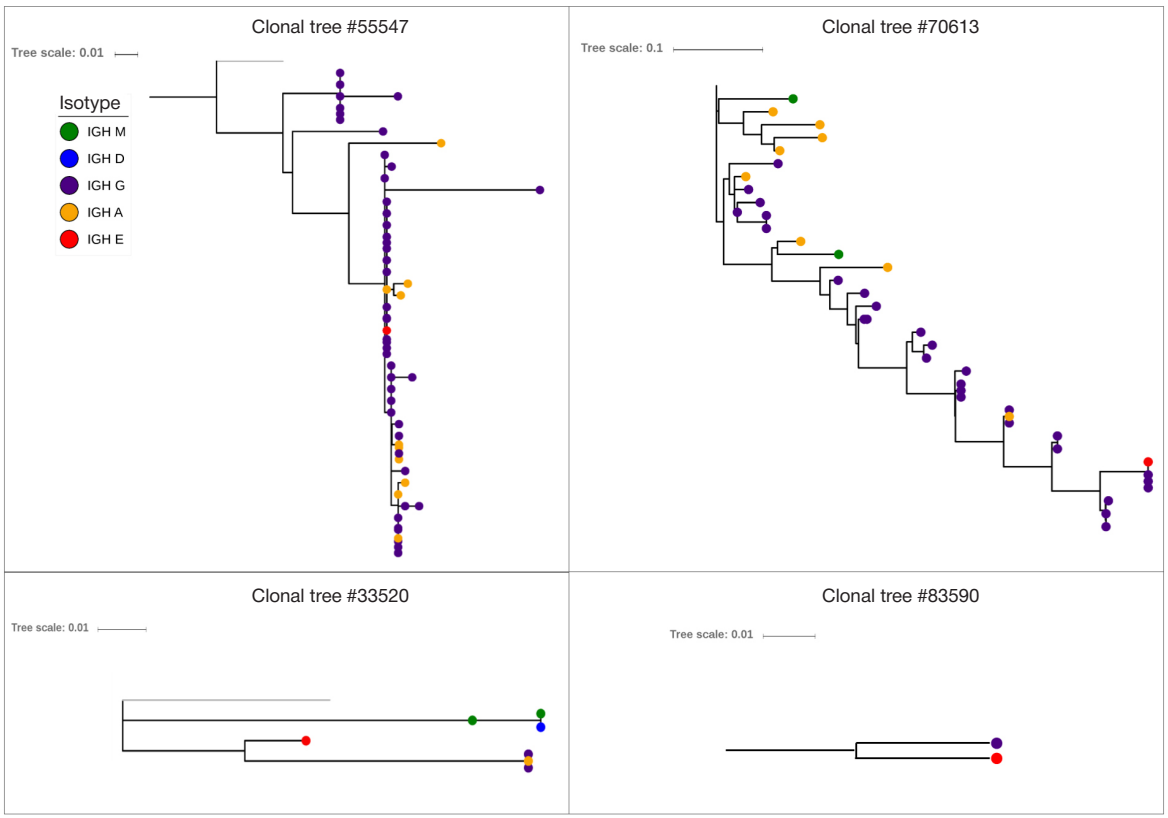

Fig. 6. Phylogenetic trees for IgE-containing clonal groups with clonotypes of different isotypes. Each dot represents a unique IGH clonotype. Clonotypes of different isotypes are color coded (dark blue — IgG, red — IgE, orange — IgA, green — IgM, blue — IgD). Length of the branches reflects distance between nodes according to the number of mutations between sequences. "Tree scale" reflects the scale used to build the tree

further research with larger cohorts and more elaborate clinical characterization of patients. Such research efforts are needed to develop new therapies aimed at breaking the allergen-specific immunological memory. For example, such new therapy may imply blocking isotype switching to IgE during differentiation of IgG^+ allergen-specific memory B cells, which is achievable through suppressing the effect of Th2 T cell cytokines by using a monoclonal antibody to IL4R. The obtained information on the dynamics of serum antibodies level with changing the allergen air concentration facilitates further investigation of the seasonal dynamics of concentration of IgE-secreting allergen-specific cells.

References

1. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. European Respiratory Journal. 2004; 24 (5): 758–64. https://doi.org/10.1183/09031936.04.00013904.
2. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. Allergy. 2019; 74 (7): all.13758. https://doi.org/10.1111/all.13758.
3. Penagos M, Eifan AO, Durham SR, Scadding GW. Duration of Allergen Immunotherapy for Long-Term Efficacy in Allergic Rhinoconjunctivitis. Current Treatment Options in Allergy. 2018; 5 (3): 275–90. https://doi.org/10.1007/s40521-018-0176-2.
4. Balbino B, Conde E, Marichal T, Starkl P, Reber LL. Approaches to target IgE antibodies in allergic diseases. Pharmacology & Therapeutics. 2018; (191): 50–64. https://doi.org/10.1016/j.pharmthera.2018.05.015.
5. Rudulier CD, Tonti E, James E, Kwok WW, Larché M. Modulation of CRTh2 expression on allergen specific T cells following peptide immunotherapy. Allergy. 2019; all.13867. https://doi.org/10.1111/all.13867.
6. Jiménez-Saiz R, Chu DK, Mandur TS, Walker TD, Gordon ME, Chaudhary R, et al. Lifelong memory responses perpetuate humoral TH2 immunity and anaphylaxis in food allergy. Journal of Allergy and Clinical Immunology. 2017; 140 (6): 1604–15. https://doi.org/10.1016/j.jaci.2017.01.018.
7. Jiménez-Saiz R, Bruton K, Koenig JF, Waserman S, Jordana M. The IgE memory reservoir in food allergy. The Journal of Allergy and Clinical Immunology. 2018; 142 (5): 1441–3. https://doi.org/10.1016/j.jaci.2018.08.029.
8. Beeh K, Beier J, Buhl R. Seasonal variations of serum-IgE and potential impact on dose-calculation of omalizumab (rhuMab-E25, anti-IgE). Pneumologie. 2004; 58 (08): 546–51. https://doi.org/10.1055/s-2004-818483.
9. Sato K, Nakazawa T, Sahashi N, Kochibe N. Yearly and Seasonal Changes of Specific IgE to Japanese Cedar Pollen in a Young Population. Annals of Allergy, Asthma & Immunology. 1997; 79 (1): 57–61. https://doi.org/10.1016/S1081-1206(10)63085-2.
10. Gleich GJ, Jacob GL, Yunginger JW, Henderson LL. Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. Journal of Allergy and Clinical Immunology. 1977; 60 (3): 188–98. https://doi.org/10.1016/0091-6749(77)90123-3.
11. Turchaninova MA, Davydov A, Britanova OV, Shugay M, Bikos V, Egorov ES, et al. High-quality full-length immunoglobulin profiling with unique molecular barcoding. Nature Protocols. 2016; 11 (9): 1599–616. https://doi.org/10.1038/nprot.2016.093.
12. Shugay M, Britanova OV, Merzlyak EM, Turchaninova MA, Mamedov IZ, Tuganbaev TR, et al. Towards error-free profiling of immune repertoires. Nature Methods. 2014; (11): 653. http://dx.doi.org/10.1038/nmeth.2960.
13. Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nature Methods. 2015; (12): 380.

https://doi.org/10.1038/nmeth.3364.
14. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. 2013. URL http://www.R-project.org/.
15. Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag, 2016. Retrieved from https://ggplot2.tidyverse.org.
16. Gupta NT, Vander Heiden JA, Uduman M, Gadala-Maria D, Yaar G, Kleinstein SH. Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. Bioinformatics. 2015; 31 (20): 3356–8. DOI: 10.1093/bioinformatics/btv359.
17. Sudhir Kumar, Glen Stecher, Koichiro Tamura. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets, Molecular Biology and Evolution. 2016; 33 (7): 1870–4. https://doi.org/10.1093/molbev/msw054.
18. Looney TJ, Lee JY, Roskin KM, Hoh RA, King J, Glanville J, et al. Human B-cell isotype switching origins of IgE. Journal of Allergy and Clinical Immunology. 2016; 137 (2): 579–86. https://doi.org/10.1016/j.jaci.2015.07.014.
19. Horst A, Hunzelmann N, Arce S, Herber M, Manz RA, Radbruch A, et al. Detection and characterization of plasma cells in peripheral blood: Correlation of IgE$^+$ plasma cell frequency with IgE serum titre. Clinical and Experimental Immunology. 2002; 130 (3): 370–8. https://doi.org/10.1046/j.1365-2249.2002.02025.x.

Литература

1. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. European Respiratory Journal. 2004; 24 (5): 758–64. https://doi.org/10.1183/09031936.04.00013904.
2. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. Allergy. 2019; 74 (7): all.13758. https://doi.org/10.1111/all.13758.
3. Penagos M, Eifan AO, Durham SR, Scadding GW. Duration of Allergen Immunotherapy for Long-Term Efficacy in Allergic Rhinoconjunctivitis. Current Treatment Options in Allergy. 2018; 5 (3): 275–90. https://doi.org/10.1007/s40521-018-0176-2.
4. Balbino B, Conde E, Marichal T, Starkl P, Reber LL. Approaches to target IgE antibodies in allergic diseases. Pharmacology & Therapeutics. 2018; (191): 50–64. https://doi.org/10.1016/j.pharmthera.2018.05.015.
5. Rudulier CD, Tonti E, James E, Kwok WW, Larché M. Modulation of CRTh2 expression on allergen specific T cells following peptide immunotherapy. Allergy. 2019; all.13867. https://doi.org/10.1111/all.13867.
6. Jiménez-Saiz R, Chu DK, Mandur TS, Walker TD, Gordon ME, Chaudhary R, et al. Lifelong memory responses perpetuate humoral TH2 immunity and anaphylaxis in food allergy. Journal of Allergy and Clinical Immunology. 2017; 140 (6): 1604–15. https://doi.org/10.1016/j.jaci.2017.01.018.
7. Jiménez-Saiz R, Bruton K, Koenig JF, Waserman S, Jordana M. The IgE memory reservoir in food allergy. The Journal of Allergy and Clinical Immunology. 2018; 142 (5): 1441–3. https://doi.org/10.1016/j.jaci.2018.08.029.
8. Beeh K, Beier J, Buhl R. Seasonal variations of serum-IgE and potential impact on dose-calculation of omalizumab (rhuMab-E25, anti-IgE). Pneumologie. 2004; 58 (08): 546–51. https://doi.org/10.1055/s-2004-818483.
9. Sato K, Nakazawa T, Sahashi N, Kochibe N. Yearly and Seasonal Changes of Specific IgE to Japanese Cedar Pollen in a Young Population. Annals of Allergy, Asthma & Immunology. 1997; 79 (1): 57–61. https://doi.org/10.1016/S1081-1206(10)63085-2.
10. Gleich GJ, Jacob GL, Yunginger JW, Henderson LL. Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. Journal of Allergy and Clinical Immunology. 1977; 60 (3): 188–98. https://doi.org/10.1016/0091-6749(77)90123-3.
11. Turchaninova MA, Davydov A, Britanova OV, Shugay M, Bikos V, Egorov ES, et al. High-quality full-length immunoglobulin profiling with unique molecular barcoding. Nature Protocols. 2016; 11 (9): 1599–616. https://doi.org/10.1038/nprot.2016.093.
12. Shugay M, Britanova OV, Merzlyak EM, Turchaninova MA, Mamedov IZ, Tuganbaev TR, et al. Towards error-free profiling of immune repertoires. Nature Methods. 2014; (11): 653. http://dx.doi.org/10.1038/nmeth.2960.
13. Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nature Methods. 2015; (12): 380. https://doi.org/10.1038/nmeth.3364.
14. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. 2013. URL http://www.R-project.org/.
15. Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag, 2016. Retrieved from https://ggplot2.tidyverse.org.
16. Gupta NT, Vander Heiden JA, Uduman M, Gadala-Maria D, Yaar G, Kleinstein SH. Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. Bioinformatics. 2015; 31 (20): 3356–8. DOI: 10.1093/bioinformatics/btv359.
17. Sudhir Kumar, Glen Stecher, Koichiro Tamura. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets, Molecular Biology and Evolution. 2016; 33 (7): 1870–4. https://doi.org/10.1093/molbev/msw054.
18. Looney TJ, Lee JY, Roskin KM, Hoh RA, King J, Glanville J, et al. Human B-cell isotype switching origins of IgE. Journal of Allergy and Clinical Immunology. 2016; 137 (2): 579–86. https://doi.org/10.1016/j.jaci.2015.07.014.
19. Horst A, Hunzelmann N, Arce S, Herber M, Manz RA, Radbruch A, et al. Detection and characterization of plasma cells in peripheral blood: Correlation of IgE$^+$ plasma cell frequency with IgE serum titre. Clinical and Experimental Immunology. 2002; 130 (3): 370–8. https://doi.org/10.1046/j.1365-2249.2002.02025.x.