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Direct versus sequential immunoglobulin switch in allergy and antiviral responses

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ABSTRACT

Allergy is characterized by IgE production to innocuous antigens. The question whether the switch to IgE synthesis occurs via direct or sequential pathways is still unresolved. The aim of this work was to analyze the distribution of immunoglobulins (Ig) to house dust mite *D. farinae* and *A. alternata* fungus in allergic children with primarily established diagnosis and compare it to Epstein-Barr antiviral (EBV) response in the same patients. In allergy patients the only significant difference was found in allergen specific IgE, likely mediated by a direct isotype switch, while antiviral response was dominated by EBV specific IgG and low level of concordant IgA and IgG4 production consistent with a minor sequential Ig switches. Taken collectively, we concluded that sequential isotype switch is likely to be a much rarer event than a direct one.

1. Introduction

Type I allergy is characterized by the formation of class E immunoglobulins to innocuous nonreplicating microorganisms, food and insect proteins or fungal conidia which penetrate the body through the epithelial barrier of lungs, skin, or gastrointestinal tract. Currently there are two main concepts of the mechanisms leading to type I allergic response. The first one considers allergy as a result of adaptive immune response activation mediated by type 2 T-helpers (Th2) which produce allergy-associated interleukins (IL) 4, 5, and 13 [1]. The role of IL-4 and IL-13 as trigger factors in B-cell switching to IgE synthesis is well established [2–5]. IgE production in IL-4 or IL-13 knock-out mice is significantly diminished [6].

The second concept considers the formation of allergy as a result of innate cells activation. In 2013 Licona-Limón et al. demonstrated that IL-4, IL-5, IL-13, and IgE were produced after parasitize invasion in Rag −/− mice, which do not have T-cells [7]. Earlier several research groups demonstrated the existence of ancient type lymphoid cells named nuocytes or innate lymphoid type 2 cells (ILC2) [8–12]. These cells are found in low quantities in lymphoid clusters of fatty tissue, lymph nodes, lungs, spleen and skin [9]. Their main characteristic is the release of IL-4, 5, and 13, the cytokines earlier considered to be specific exclusively to Th2, in response to alarmins IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) which are secreted by damaged epithelium cells [8–12]. Alarmin gene knockouts block IgE response [6]. The role of ILC2 in allergic response is modernly widely discussed [13–15].

Immunoglobulin class switch recombination (CSR) in B-cells occurs via the excision of the DNA located between the two Ig switch regions. It was shown in vitro that human B cell CSR directed by IL-4 can be accomplished either by direct mu to epsilon switching; sequential mu-gamma-epsilon switching; or double sequential mu-alpha-gamma-epsilon switching [16]. Xiong et al. demonstrated in *N. brasiliensis* parasite infection in mice an independent formation of two IgE + B-cell populations by direct and sequential CSR accordingly, one of which with low affinity was found only in germinal centers of lymph nodes while the other one formed high affinity memory and plasma IgE + cells [17]. In contrast to the results of Xiong et al., Talay et al. demonstrated that both germinal centers and plasma IgE + cells originated from B-cells which undergone a direct mu to epsilon switch [18].

The question whether mu to epsilon switching occurs in germinal centers of lymph nodes is also unresolved. Takhar et al. as well as Cameron et al. demonstrated local CSR in the nasal mucosa of patients with allergic rhinitis [19,20]. The germline and epsilon circle transcripts were found in nasal mucosa of grass pollen-allergic subjects but not of healthy controls [19]. Both direct and sequential DNA switch circles were produced [20]. The authors concluded that allergen stimulates local CSR to IgE.

IgE type allergy is often a life-long disease. It is interesting to compare CSR type in primary sensitization and after multiple sensitizations. Niederberger et al. demonstrated a direct mu to epsilon switching in

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90% of 1–7 years old children allergic to birch and/or grass pollen [21]. At the same time Resch Y et al. showed both IgE and IgG4 production in house dust mite (HDM) sensitized children [22]. A direct comparison of IgE and IgG levels showed significantly higher IgE titers in children with allergic asthma but fewer asthmatic children showed IgG reactivity than nonasthmatic children [22] showing that IgG and IgE responses can be independent. Close data were found by Jarvis D et al. in European Community Respiratory Health Survey which included 22 European centers and 2780 patients and studied IgE, IgG and IgG4 levels in the sera of cat and HDM sensitized patients. There was no evidence that high levels of allergen specific IgG or IgG4 were associated with a decreased risk of IgE sensitization or respiratory symptoms [23]. At the same time Miranda DO et al. found out that serum IgE and IgG4 levels to *D. pteronyssinus* major allergens Der p 1 and Der p 2 were higher in allergic children whereas serum IgA levels were higher in nonallergic children [24]. Moreover IgE levels positively correlated with IgG4 and IgA to all allergens in allergic children, while IgA levels negatively correlated with IgG4 to Der p1 in nonallergic children [24].

The type of allergic response can depend on the allergen. HDM allergy is induced by inhaled mite feces which completely innocuous and cannot replicate. Contrary to HDM allergy, immune reaction to fungal conidia can differ due to the ability of conidia to germinate in the bronchial airways. Allergy to *A. alternata* is one of the most often found even in one year-old infants [25]. Direct comparison of HDM and Alt a allergy can provide a deeper understanding of the mechanisms of allergy formation.

Sequential CSR is a statistical process where some B-cells undergo partial mu-alpha or mu-gamma switch while the others complete mu-gamma-epsilon or mu-alpha-gamma-epsilon switching. Analysis of CSR in antiviral or antibacterial adaptive responses indeed demonstrates the presence of several Ig classes or subclasses specific to pathogens. Whitehouse et al. found circulating IgM, IgA, IgG1, and IgG3 specific to *S. aureus* [26]. The same multiple Ig distribution was found by Gahéry-Ségard et al. in the response to recombinant adenovirus immunization [27].

EBV is one of the most common viruses found in humans. Seroprevalence of EBV in children was found to be from 36 to 68% [28,29] and up to 95% of the worldwide population before an age of 40 years [29]. After primary infection EBV persists latently in memory B cells, where the only protein expressed is the EBV nuclear antigen 1 (EBNA-1) [30]. Cellular immunity is essential for EBV control, but the humoral immune response is also activated during EBV infection and different serological profiles can reflect the infection history. During EBV reactivation a coordinated IgG and IgA responses manifest sequential Ig CSR as it was shown in rheumatoid arthritis patients [31].

The aim of this study was to compare the distribution in children with first established sensitization to HDM *D. farinae* and/or *A. alternata* of Ig specific to Der f 2 or Alt a 1, major HDM and *A. alternata* allergens [24,32,33], and to Epstein-Barr virus EBNA-1 polypeptide.

2. Materials and methods

2.1. Patient selection and serum samples

Serum samples were collected from children (1–9 years old) with primarily established diagnosis of HDM or *A. alternata* (Alt a) allergy in Mechnikov's Institute of Vaccine and Sera, Moscow, Russian Federation. None of the patients had the diagnosis of allergy before. Patients' parents consent was obtained before blood collection. Specific IgE to *D. farinae* of Alt a was determined using RIDA®AllergoScreen (R-Biopharm AG, Germany). Twenty seven children with HDM allergy (average age 6 years), 35 children with Alt a allergy (average age 6 years), and 21 healthy controls (average age 7 years) were enrolled in this study (Table 1). This study was approved by the institutional review board of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS.

2.2. Production of recombinant allergens

Der f 2L was produced as described earlier [34]. DNA fragment encoding Gene encoded Alt a 1 were amplified by PCR from *Alternaria alternata* genomic DNA (forward primer: 5′-TTAGGATCCATGCAGTTC ACCACCATC-3′, reverse: 5′-CGACTGCAGTTAATCACGGATTTTACCATGG-3′); DNA fragment encoding a part of EBNA-1 protein of human herpesvirus 4 (pEBV, sequence is shown in supplemental Fig. S1) was assembled from oligonucleotides and amplified by PCR (forward primer: 5′-ATAGGATCCCCGCGTAGTCCGAGCA-3′, reverse: 5′-TTGAAGCTTCTGGC CTTC TTCGCCTTC-3′). Alt a 1 and pEBV were cloned into the pQE30 expression vector (Qiagen, USA) using restriction endonucleases *Bam*HI/*Sal*I and *Bam*HI/*Hin*dIII, respectively. Recombinant antigens Der f 2L, Alt a 1 and pEBV were expressed in *Escherichia coli* M15 strain. Der f 2L was expressed and purified as described earlier [34]. Alt a 1 and pEBV were purified from inclusion bodies in denaturing conditions by Ni-NTA agarose affinity chromatography. The concentrations of purified proteins were determined by Bradford assay, 10-μg aliquot of each protein was run on a 15% polyacrylamide gel containing sodium dodecyl sulfate under reducing conditions (Fig. S1).

2.3. Measurement of specific Igs by ELISA

Levels of antigen-specific serum IgE, IgA1, IgA2, IgG, and IgG4 were measured by ELISA. Each allergen (10 μg/mL) in phosphate-buffered saline (PBS) was coated onto microtiter plates and kept at 4 °C overnight. Plates were washed three times with PBS containing 0.05% Tween 20 (PBST) between each step. After blocking with 10% adult bovine serum in PBS, plates were incubated overnight with serum samples at different dilutions followed by incubation with biotinylated mouse anti-human IgE at 1:1000 dilution (Sorbent, Moscow, Russian Federation) for 1 h and streptavidin-peroxidase (Biolegend, USA) at 1:1500 dilution for 1 more hr. Direct anti-human IgG-HRP conjugate (SantaCruz, USA) was used at 1:2000 dilution. Mouse anti-human IgA1, IgA2, IgG4 (SouthernBiothech, Birmingham, UK) were used at 0.5 μg/mL. Anti-mouse-HRP conjugate (SantaCruz, USA) was used at 1:4000. Total IgE was determined by sandwich ELISA using anti-human IgE (SouthernBiothech, Birmingham, UK) coated plates and biotinylated mouse anti-human IgE (Sorbent, Moscow, Russian Federation). Reaction was developed using 3,3′5,5′-tetramethylbenzidine substrate solution (Sigma), blocked with 10% H_2SO_4 in water and then absorbance at 450 nm was measured. All ELISA experiments were conducted strictly in the same conditions and were repeated at least two times. The mean plus 3 SD of absorbance values in control wells was used as the cutoff to determine Ig titers. The results are shown as Ig titers which were determined as the last serum dilution above cut-off values. Sera from HDM, Alt a patients and control donors were randomly included in the same ELISA plate to increase the precision of the comparisons.

Table 1
Characterization of allergic and control patients.

	N	Age, years	Der f RIDA[a]	Alt a RIDA	Cross sensitive[b]
HDM	27 (1–9)	6.2 ± 2.2	3.8 ± 1.1	3.8 ± 1.5	11/27 (41%)
Alt a	26 (1–9)	6 ± 2.2	3.4 ± 1.4	4.3 ± 1.6	8/35 (26%)
Controls	21 (1 − 12)	7.1 ± 3.5	0	0	0

[a] RIDA scores for Der f - *Dermatophagoides farinae* (Der f) or *Alternaria alternata* (Alt a).
[b] Number/percentage of patients sensitive to both HDM and Alt a.