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Direct versus sequential immunoglobulin switch in allergy and antiviral responses

E. Svirshchevskaya^{a*}, G. Fattakhova^a, S. Khlgatian^b, D. Chudakov^a, E. Kashirina^a,
D. Ryazantsev^a, O. Kotsareva^a, S. Zavriev^a

^a*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, 16/10, Miklukho-Maklaya St., GSP-7, 117997, Moscow, Russian Federation*

^b*Mechnikov's Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Maliy Kazenny pereulok, 5A, 105064 Moscow, Russian Federation*

e.mails: esvir@mx.ibch.ru (E.S.); fattakhova@yahoo.com (G.F.); laball@yandex.ru (S.Kh.); boris-chudakov@yandex.ru (B.Ch.); helen-kas@mail.ru (E.K.); d.yu.ryazantsev@gmail.com (D.R.); olga.kotsareva@gmail.com (O.K.); skz123@mail.ru (S.Z.)

**corresponding author*

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.

Key words: *allergy; house dust mites; alternaria alternata, direct class switch recombination; Epstein-Barr virus; antiviral response; sequential class switch recombination*

Abbreviations: HDM – house dust mites, Der f – *Dermatophagoides farinae*; Der f 2 – major allergen from *D. farinae*; Alt a 1 – major allergen from *Alternaria alternata* fungi; pEBV – polypeptide from EBNA-1 protein; CSR – class switch recombinations; ILC2 – innate lymphoid type 2 cells.

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-Limon P *et al.* demonstrated that IL-4, IL-5, IL-13, and IgE were produced after parasite 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 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* or 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). 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'-TTAGGATCCATGCAGTTCACCACCATC-3', reverse: 5'-CGACTGCAGTTAATCACGGATTTTACCATGG-3'); DNA fragment encoding a part of EBNA-1 protein of human herpesvirus 4 (pEBV, sequence is shown in supplemental Figure S1) was assembled from oligonucleotides and amplified by PCR (forward primer: 5'-ATAGGATCCCCGCGTAGTCCGAGCA-3', reverse: 5'-TTGAAGCTTCTGGCCTTC TTCGCTTC-3'). Alt a 1 and pEBV were cloned into the pQE30 expression vector (Qiagen, USA) using restriction endonucleases BamHI/SalI and BamHI/HindIII, 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 (Figure 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 1:1,000 dilution (Sorbent, Moscow, Russian Federation) for 1 hour and streptavidin-peroxidase (Biolegend, USA) at 1:1,500 dilution for 1 more hr. Direct anti-human IgG-HRP conjugate (SantaCruz, USA) was used at 1:2,000 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% H₂SO₄ 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.

2.4. Statistical analysis

Average and standard deviation were calculated by Excel software. Correlations of IgE antibody responses between groups of patients were assessed by parametric Pearson's χ^2 test and Student's t-test. A result was considered statistically significant when the *p* value was less than 0.05 in two tailed analysis.

3. RESULTS

3.1. Comparison of allergic response to HDM and Alt a

Among children primarily sensitized to *D. farinae* (HDM patients) or Alt a, only those with high IgE response (class>2.5) as determined by RIDA®AllergyScreen system were selected. Among these, Der f 2 specific IgE was found in 83% of HDM patients and Alt a 1 specific ones – in 73% of Alt a positive sera (Table). As the aim of this work was to estimate relations between IgE and other Ig specific to allergens, only patients with IgE to Der f 2 or Alt a 1 were selected for the correlation analysis. Additionally healthy controls of the same age were included in the study (Table). Most patients demonstrated multiple sensitizations even at their first visit to allergy department (Table). Cross recognition of *D. farinae* and *D. pteronyssinus* in RIDA test was 91%. Cross recognition of Alt a and other fungi such as *Aspergillus fumigatus*, *Cladosporium herbarum* or *Penicillium notatum* was 56% with *A. fumigatus* and *C. notatum* predominant. Average RIDA class of hyperreactivity did not differ between HDM and Alt a patients (Table).

RIDA results for both HDM and Alt a sera did not correlate with total IgE (Figure 1, A and B). Der f 2 specific IgE also did not correlate with *D. farinae* RIDA scores (Figure 1, C) while it did significantly correlate for Alt a sera (Figure 1, D). Moreover, RIDA Alt a linear range was not sufficient to discriminate the differences in IgE in half of samples (Figure 1 D, the highest RIDA class is 6). Total IgE did not correlate with Der f 2 or Alt a 1 specific IgE (Figure 1, E and F).

The absence of correlation between RIDA or allergen specific IgE and total IgE possibly means that Der f 2 and Alt a 1 specific IgE responses represent only a minor fraction of serum IgE pool which is in accord with multiple sensitizations found for both HDM and Alt a patients. The absence of correlation between *D. farinae* RIDA and Der f 2 specific IgE is likely a result of other allergen binding in the commercial extract. At the same time a significant correlation of Alt

a 1 specific IgE and Alt a RIDA score manifests either that Alt a 1 is a major protein in the extract or it is the major allergen recognized by allergic sera.

3.2. Allergen specific Ig distribution

Total IgE levels were elevated only in HDM sera (Figure 2, A). Rather unexpectedly, total IgE in Alt a sera and in control ones were comparable (Figure 2, A). Of note 41% of HDM sera cross recognized Alt a, and 26% of Alt a sera cross recognized *D.farinae* (Table). Still the difference in total IgE was highly significant ($p=0.0003$).

Allergen specific IgE levels were significantly higher both in HDM and Alt a groups in comparison with controls (Figure 2, B) and allergen specific IgE level was higher ($p<0.05$) in Alt a patients in comparison with HDM ones (Figure 2, B). Specific IgG4 was significantly increased in Alt a 1 group in comparison with Der f 2 and control sera (Figure 2, E). No differences were found in allergen specific IgA1, IgA2 and in Der f 2 specific IgG4 in comparison with controls (Figure 2, C-E).

Estimation of IgG titers resulted in extremely high values (titers from $2 \cdot 10^4$ to 10^6) in all groups. It was hypothesized that a significant portion of these Ig originates from a natural pool of Ig antibodies. To address this problem, 28 sera (7 from HDM, 7 from Alt a and 14 from control sera) were run against Der f 2 and two irrelevant proteins - sperm whale myoglobin (SWM) and KLH at 10 fold dilution scale starting from 1/50, and IgG titers were determined. It appeared that the average natural IgG titers were around 5000 (Figure S2). There were no differences in Der f 2, SWM, or KLH specific IgG titers between groups. Basing on these results antigen specific IgG titers were divided by 10^3 as the least estimation of natural IgG pool.

The results on Der f 2 and Alt a 1 specific IgG distribution in HDM, Alt a and control groups are shown in Figure 2, F. Sera were titrated 1/3 starting from 1000 dilution. Almost all the sera were negative if to consider 5000 titer as a cut-off level of natural IgG pool. Still some small however significant difference was found between HDM and control groups. Der f 2 specific IgG titers were higher in control in comparison with HDM sera. The highest ratio between low and high responders was around 30. Of note, at 1/1000 serum dilution significant errors can occur due to technical problems. Consequently, care should be taken in the interpretation of allergen specific IgG results.

3.3. Analysis of IgG specific to pEBV

Analysis of anti-EBV IgG frequency in HDM, Alt a and control groups demonstrated that the ratio of high to low responders was around 1000 which is much higher than for allergen specific IgG titers. Sera showing anti-EBV IgG titers more than 10000 were considered as positive ones. Among HDM, Alt a, and control patients 44, 25, and 38 %% accordingly were found positive with no statistical difference between groups (Figure 3, A-B). Low level of IgE production was occasionally found with no differences between groups (data not shown).

3.4. Correlation analysis of Ig specific to pEBV and allergens

No significant correlation between anti-pEBV IgG and other Ig classes were found possibly because of overwhelming dominance of major IgG1 and IgG2 production (data not shown). However anti-pEBV IgG4, IgA1, and IgA2 titers correlated significantly both in allergic (Figure 3, C and E) and control (Figure 3, D and F) sera. Of interest to note different slopes of data approximation in HDM and Alt a groups (Figure 3, C and E) with more IgG4 and IgA1 production by Alt a patients and more IgA2 production by HDM patients.

Among Igs, specific to allergens, Alt a 1 specific IgA2 and IgE titers correlated significantly (Figure 4, A). No correlation was found between all other data pools both for HDM and Alt a sera showing either independent IgE, A1, A2, G4 production or the absence of others than IgE antibodies specific to the allergens. However analysis of Der f 2 specific IgA2 and IgE distribution demonstrated a split of data in three different groups: low IgE/IgA2, low IgA2/high IgE, and high IgA2/high IgE (Figure 4, C) in comparable sample numbers per group. This could manifest a sequential switch from IgE to IgA2 production as IgE titers were 10 times higher than IgA2 ones.

Allergic responses to nonreplicative innocuous allergens such as HDM and opportunistic allergens such as fungi differed in major IgG subclass response. A significant positive correlation was found between Alt a 1 specific IgG titers and age of patients (Figure 4, B) which was not found in Der f 2 specific IgG in HDM patients (Figure S3, A). The same increase in Alt a 1 specific IgG with age was found in control and HDM sera (data not shown). The increase in allergen specific IgG in Alt a patients with age did not affect Alt a 1 specific IgE and IgG4 formation (Figure S3, B, C).

The titers of total IgE tended ($p=0.1$) to decrease with age in both HDM and Alt a groups (Figure S3, D). Of note affinity of IgE specific to Alt a 1 was significantly higher than of Der f 2 specific one as determined by the slope of titration curves (Figure 4, D). Higher affinity was due to some intrinsic property of Alt a 1, as allergen degradation by trypsin completely abrogated IgE binding (data not shown) showing that IgE specific to Alt a 1 was conformational.

Comparison of average allergen and EBV specific Ig titers is shown in Figure 4, E and F. Allergic response to Der f 2 was characterized by a significant increase in Der f 2 specific IgE production and a decreased IgG level in comparison with control sera, while only increased production of IgE to Alt a 1 was found in the sera of patients with fungal allergy.

Analysis of antiviral response demonstrated no significant difference between groups however slightly higher levels ($p>0.05$) of IgG, IgG4, and IgA1 to EBV were found in Alt a sera (Figure 4, F).

4. DISCUSSION

Understanding the mechanism of allergy formation is an important issue which predetermines the development of an efficient anti-allergic treatment as well as allergy prevention. In this paper we have studied Ig distribution in children with primarily established diagnosis of allergy to innocuous nonreplicative allergen HDM and opportunistic fungus *A. alternata*. Sera from HDM patients demonstrated significantly increased titers of Der f 2 specific IgE but not IgG, IgG4, IgA1, and IgA2. No correlations were found between any Ig classes in HDM sera. These findings correspond to a direct mu to epsilon CSR and support the results obtained by Niederberger *et al.* [21].

Immune response to Alt a significantly differed from anti-HDM one and was characterized by an increased Alt a 1 specific IgE and IgG4 production, a correlation between IgE and IgA2 levels, and age-dependent increase in Alt a 1 specific IgG which did not affect allergen specific IgE formation. These results can be explained as a result of two independent immune responses one of which - allergic - is initiated outside of central immune organs in mucosal tissue, and the other one - central - is formed in the lymph nodes where germinating conidia are delivered by antigen-presenting cells. Our results show that these two types of responses do not correlate and do not affect each other.

Allergic immune response to Alt a demonstrated a correlation between IgE and IgA2 production showing these two events are linked. The titers of IgE and IgA2 antibodies and $\epsilon/\alpha 2$ gene location in the germline show that most likely CSR occurs via a direct mu-epsilon and later – a sequential mu-epsilon-alpha 2 switches. At the same time central immune response to Alt a is characterized by IgG production which increases with age. Persistence of Alt a 1 specific IgG did not affect specific IgE and IgG4 production supporting the hypothesis of two independent immune responses against opportunistic fungi.

Many groups demonstrated IgG formation alongside with IgE to allergens [35-39]. It can be speculated that repeated contacts with the allergens lead to CSR to other Igs independently of mu to epsilon switch. Resch Y *et al.* showed significantly higher IgE titers in children with allergic asthma but fewer asthmatic children showed IgG reactivity than nonasthmatic children [22]. Jarvis D *et al.* showed that cat or HDM specific IgG or IgG4 were not associated with a decreased risk of IgE sensitization or respiratory symptoms [23] which also biases independent CSR. Smith *et al.* showed higher IgG production in children with high HDM exposure, however Ig responses were divergent in twin patients [40] showing the role of other than environmental exposure and genetic susceptibility factors. The interesting results were obtained by Orivuori *et al.* who studied Ig distribution at age 1 and 6 years in children with β -lactoglobulin and gliadin allergy [41]. Contrary to respiratory allergy, in food allergy IgA and IgG specific to β -lactoglobulin and gliadin at age 1 were predictive to IgE formation at age 6 [41]. The authors concluded that aberrant barrier functions could lead to Ig and allergy formation.

Random direct CSR means that different allergen specific Ig classes can be formed. The data on allergen specific IgA are limited. Miranda *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]. Our results demonstrated that in HDM group significant difference was found only in Der f 2 specific IgE but not in other Ig classes. At the same time Guhl *et al.* found an increased IgA level in Birch pollen allergic patients [42], while den Hartog *et al.* showed a protective role of IgA2 in HDM allergy [43]. These and other findings show that other than IgE allergen specific classes and subclasses of Ig are produced in a discordant manner. High-throughput DNA sequencing of immunoglobulin heavy chain rearrangement in B-cells obtained from adult allergic and non-allergic subjects demonstrated the existence of comparable multiple variants [44].

To address the question whether mu to epsilon switching in allergy occurs in the germinal centers or extrafollicular loci in lymph nodes [45] or outside the lymph nodes in nasal or bronchial mucosa we studied EBV specific Ig distribution in the same patients. It is well known that B-cells latently infected with EBV [46] bring the viruses to the lymph nodes where memory adaptive immune response is reactivated [47]. Thus, EBV specific Ig distribution can reflect the typical adaptive lymph node germinal center mediated humoral immune response. EBV infection was selected because it starts early in childhood via virus transmission through their parents' infected saliva. Our results demonstrated that among HDM, Alt a, and control patients 44, 25, and 38 %% of children were infected with EBV accordingly. Earlier seroprevalence of EBV in children was found to be from 36 to 68 %% [28-29] which is in agreement with our results. Analysis of Ig distribution in antiviral response demonstrated that dominant IgG response did not correlate with any of the minor IgG4, IgA1, IgA2 classes. However IgG4, IgA1, IgA2 correlated significantly both in allergic and control groups. Dominance of non-allergic IgG is specific to adaptive Th1 type immunity as was shown by multiple research groups [46-47]. The results of EBV study demonstrate that humoral immune response formed in the germinal centers of lymph nodes is characterized by interdependent CSR and concordant production of different Ig classes and subclasses.

Taken collectively it can be concluded that allergic response is initiated locally outside of lymph nodes as it was shown earlier [19-20] and can be augmented by ILC2. Other than mu to epsilon or mu to gamma 4 CSR can be stimulated by innate lymphoid cells of type 1 [48].

One more parameter reflecting a direct mu to epsilon CSR is the number of somatic hypermutations in IgE and IgG genes which is much lower in IgE [49-50]. This is shown not only for a middle form of allergy such as HDM but also for IgE able to induce anaphylactic reactions to bee or wasp venom or peanut allergens [49]. Determination of allergen specific IgE to IgG4/IgG ratio by more sensitive quantitative methods such as real time immuno-PCR can show the stage of allergy progression. Immuno-PCR has 100-1000 [51] times higher compared to ELISA sensitivity and can be used for the analysis of small samples (1-2 ul) of serum.

5. Conclusions

The results presented in this work demonstrate a high probability of a direct mu to epsilon class switch recombination both in HDM and *A. alternaria* allergy onset which occurs most likely outside of lymph nodes in nasal or bronchial mucosa. Primary allergic response is characterized by IgE production in the absence of other Igs and can be triggered by alarmins and type 2 innate lymphoid cells. Allergens such as opportunistic fungi which are able to activate antigen presenting cells sufficiently to deliver antigens to lymph nodes are likely to induce two independent immune responses. Allergic response in this case is formed outside of lymph nodes in nasal or bronchial mucosa and is characterized by predominant IgE production mediated by a direct mu-epsilon CSR. The other one is formed in the lymph nodes and is characterized by the formation of IgG subclasses other than IgG4, titers of which increase with age. This dual mechanism can explain why simultaneous allergen specific IgE and IgG production can occur in allergic patients. Moreover, our results show that these two types of immune responses to some allergens are independent and increase in IgG does not lead to the decrease of IgE. Results of allergen specific immunotherapy demonstrate that in spite of clinical improvements allergen specific IgE decreases very slowly [52]. Contrary to allergic response, antiviral humoral immunity is characterized by high titers of antiviral major IgG subclasses and a coordinated IgG4, IgA1, and IgA2 formation characteristic to adaptive immune response initiated in the germinal centers of lymph nodes.

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Table. 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

^aRIDA scores for Der f - Dermatophagoides farinae (Der f) or Alternaria alternata (Alt a).

^bNumber/percentage of patients sensitive to both HDM and Alt a.

Figure legends

Figure 1. Correlation between RIDA and ELISA results. Correlation between HDM or *A. alternaria* RIDA classes, total IgE levels (A-B), allergen specific IgE (C-D), and total IgE and allergen specific IgE (E-F) in HDM (A, C, E) and Alt a (B, D, F) sera; Pearson's coefficients r and probability p are shown for each correlation.

Figure 2. Serum distribution of allergen specific Ig. Titers of total IgE (A), allergen specific IgE (B), IgA1 (C), IgA2 (D), IgG4 (E), and total IgG (F) in sera from HDM (black circles), Alt a (grey circles), and healthy patients (Der f 2 and Alt a 1 recognition is shown in the same colors). Significant difference ($p < 0.05$) is shown with bars.

Figure 3. Characteristic of immune response to EBV. Distribution of high and low IgG responders in HDM (stripped bars), Alt a (grey bars) (A), and control (B) patients. Correlations between EBV specific IgG4 and IgA2 (C and D) and between IgA1 and IgA2 (E and F) in HDM (black circles), Alt a (grey circles) (C, E), and control (D, F) patients. Approximations are shown in lines. Pearson's coefficients r and probability p are shown for each correlation.

Figure 4. Comparison of allergic responses to HDM and Alt a. A-B. Correlation between allergen specific IgE and IgA2 in Alt a (A) and HDM (B) groups. HDM data split in three types shown in circles corresponding to transition of B-cells from IgE to IgA2 production (arrows). C. Dependence of Alt a1 (grey circles) or Der f 2 (black circles) specific IgG on age of patients. D. Different affinity of Alt a 1 and Der f 2 specific IgE is shown by approximation (solid lines) of average titration curves (dotted lines). E-F. Comparison of Ig distributions in allergy (E) and anti-EBV response (F). Significant ($p < 0.001$) increase in allergen specific IgE in comparison with appropriate control is shown with asterisks, a significant increase in Der f 2 specific IgG in control group is shown with two asterisks.

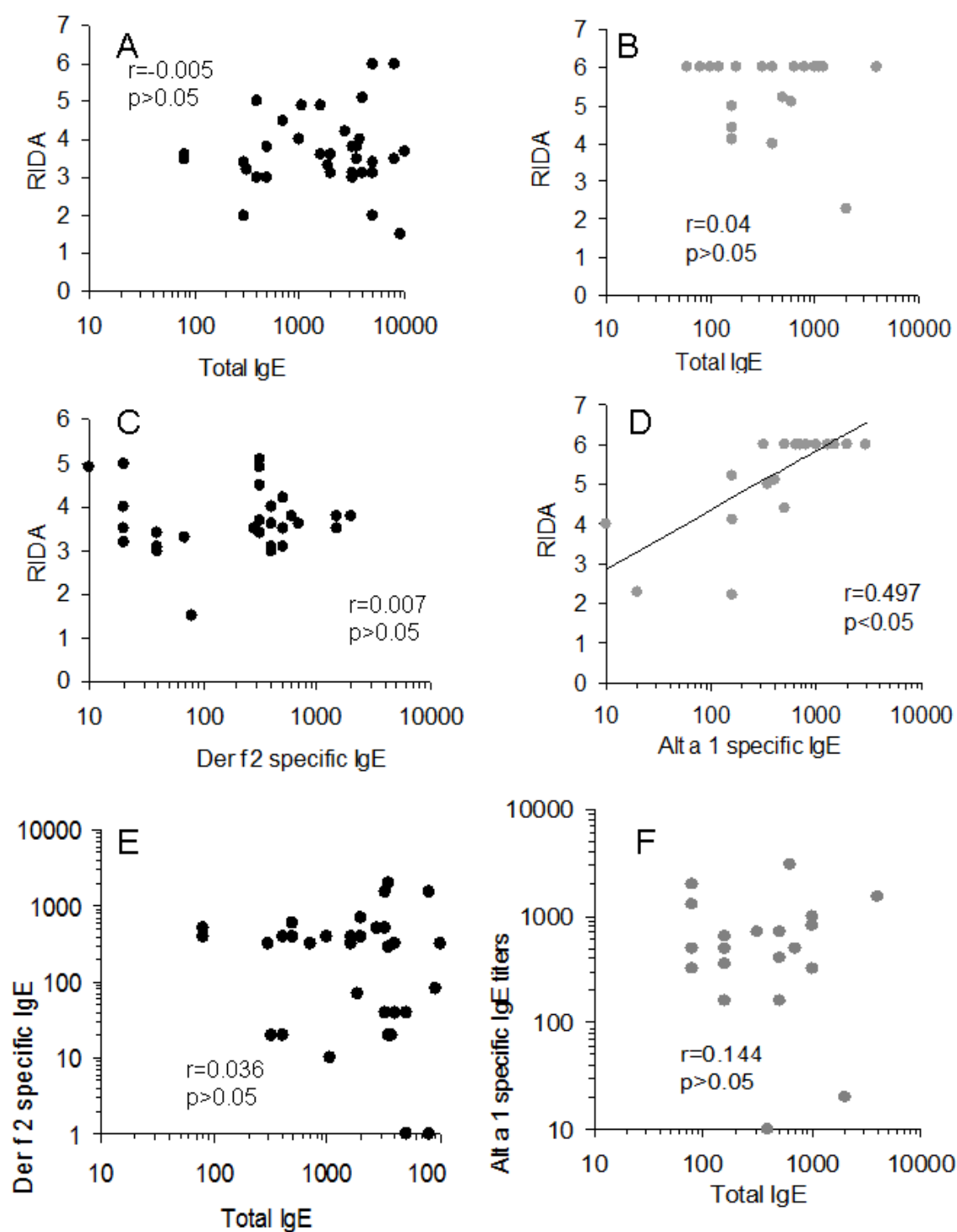


Figure 1

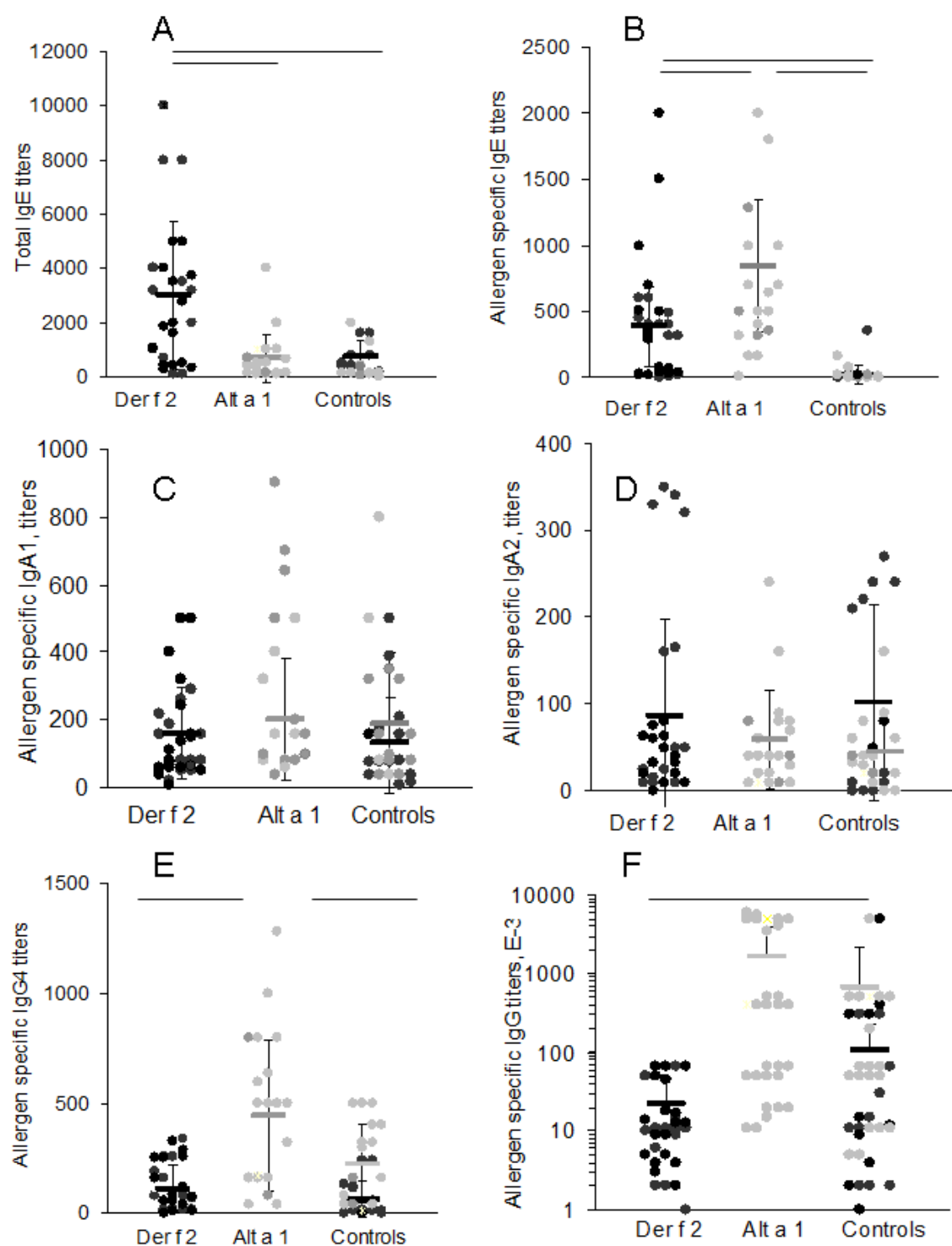


Figure 2

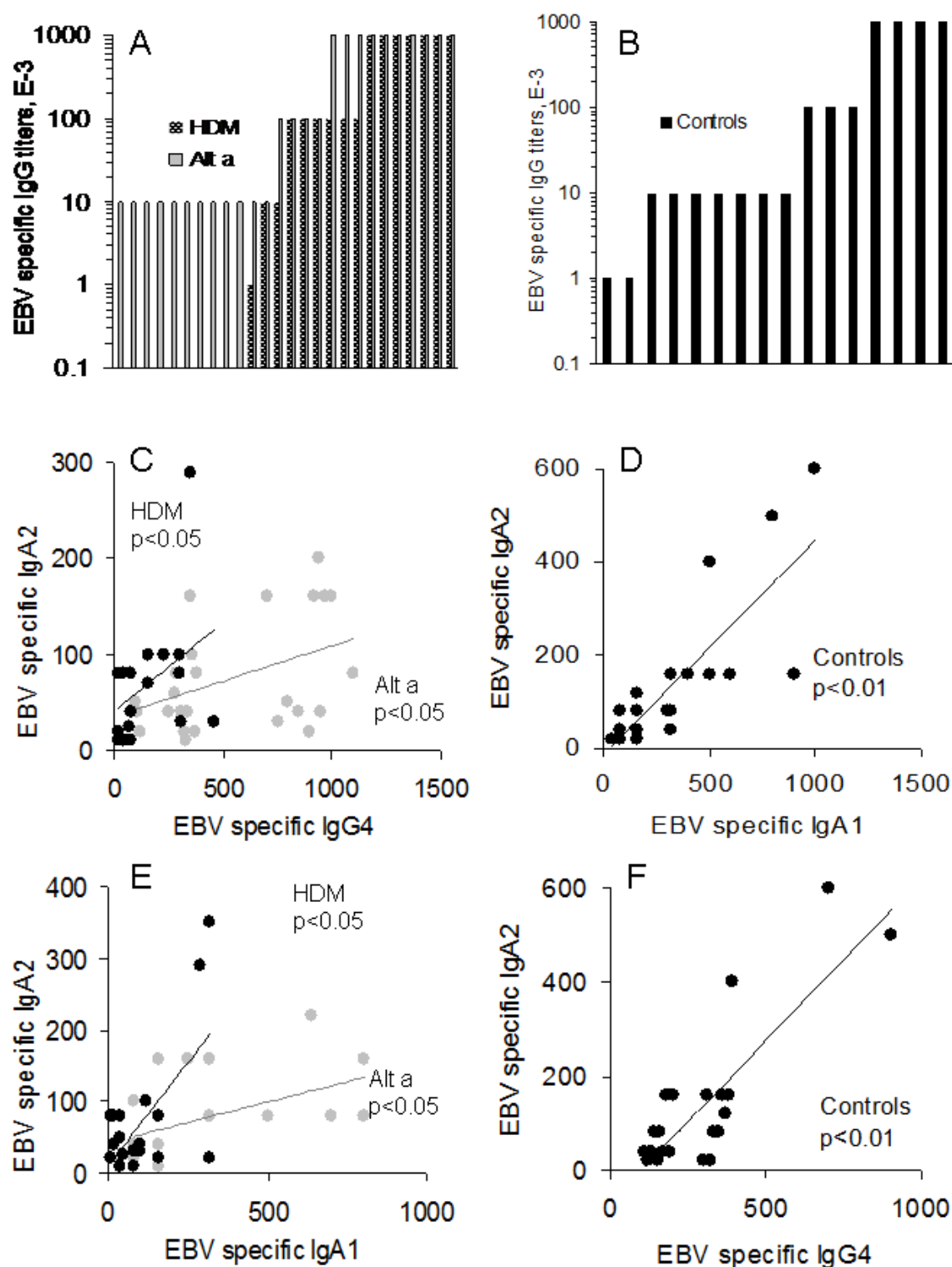


Figure 3

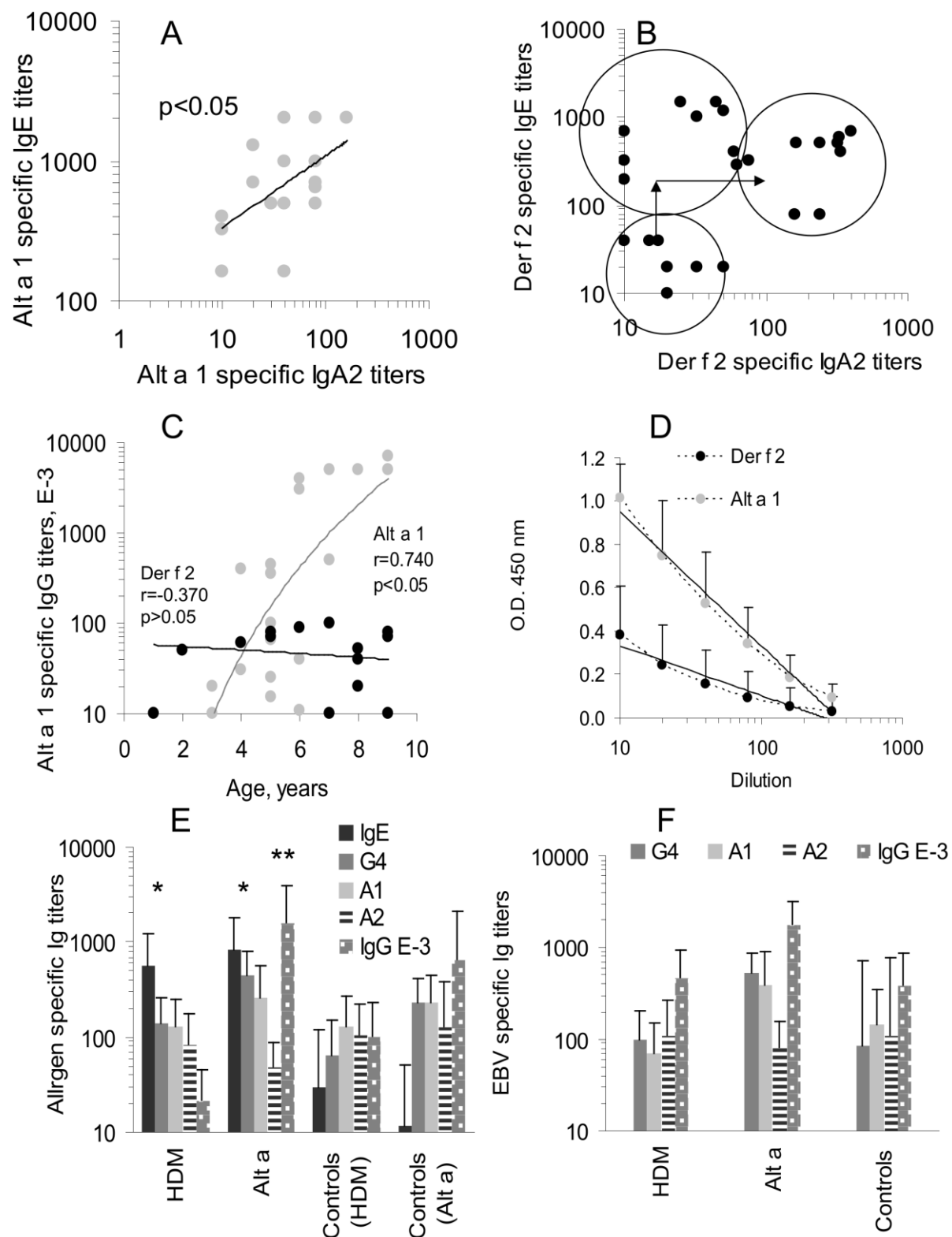


Figure 4

Highlights

- The onset of HDM or *A. alternata* allergy is manifested almost exclusively by the increase in specific IgE
- These results are consistent with a direct μ to epsilon CSR in allergy
- Antiviral response is characterized by IgG production with minor input of IgA and IgG4
- Antiviral response is characterized by dominant direct μ to gamma switch
- Sequential switches to IgA1, IgG4 and IgA2 do occur but represent rare events even in anti-EBV response