

Immunogenetic analysis of the heavy chain variable regions of IgE from patients allergic to peanuts

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Peanuts are one of the most allergenic of the foods, and hypersensitivity responses to peanut allergens can be fatal. Although the nature of the antigenic components of peanuts is being defined at the molecular level, there is little information on the induced IgE antibodies, which are central to the allergic reaction. Recognition sites of IgE antibody molecules arise from the variable regions of heavy and light chains (V_H and V_L). By using nested polymerase chain reactions with specific primers for the available repertoire of V_H genes, together with primers in the constant ϵ region, we have amplified V_H sequences of IgE from blood lymphocytes of two patients with peanut allergy. After cloning and sequencing the products, we found a predominance of V_H1 family use in both patients, which was not found in control IgM-specific primers. The IgE V_H sequences were highly somatically mutated, but in only six of 17 cases was there clear evidence for clustering of amino acids indicative of antigen selection. Previous results from patients with allergy to house dust mites have indicated predominance of V_H5 use and little evidence for antigen selection. Although results from two patients allergic to peanuts must be regarded as preliminary, they do suggest that the IgE response to peanuts may have a different V_H bias, with a similar mutational pattern. (*J Allergy Clin Immunol* 1998;101:391-6.)

Key words: IgE, allergy, peanut, variable region genes, V_H , B cells

Peanut is one of the most common foods causing acute, severe, and fatal allergic reactions, both in adults and children.¹⁻³ It appears to be increasing in prevalence and is characterized by life-long persistence.⁴ Investigation of the nature of the allergic components of peanuts has thus far revealed two major allergens, Ara h 1 and Ara h 2.^{5,6} Recently, a peanut expression library has been used to identify sequences recognized by serum IgE from patients, and Ara h 1 clones have been isolated.⁷ This has allowed production of recombinant

Abbreviations used

C ϵ :	Constant region of epsilon heavy chain
CDR:	Complementarity determining region
cDNA:	Complementary deoxyribonucleic acid
FWR:	Framework region
HDM:	House dust mite
PCR:	Polymerase chain reaction
RNA:	Ribonucleic acid
V_H :	Heavy chain variable region
V_L :	Light chain variable region

peanut protein, which will help to define allergenic sequences.⁸

In contrast to the refined molecular analysis of allergen, study of IgE in patients with peanut allergy has been confined to whole serum reactivities. It has generally proved very difficult to isolate and clone IgE-producing B cells from patients with allergies, making studies of individual antibodies difficult.⁹ The sites in the immunoglobulin molecule involved in recognition of antigen lie in the variable regions of the heavy and light chains of immunoglobulin, V_H and V_L . With the use of genetic technology, it is now proving possible to investigate the structures of V_H regions of IgE by sequence analysis.^{10,11} The V_H region of an antibody molecule of a normal B lymphocyte arises by genetic recombination of a single V_H gene from the available repertoire to a combined D_H - J_H gene to create a V_H - D_H - J_H transcriptional unit. Choice of V_H is from the approximately 51 potentially functional unrearranged V_H genes, grouped into seven families, V_H1 - V_H7 .¹² The recombination process generates heterogeneity of sequence important for providing recognition sites for the myriad of external antigens.^{13,14} Somatic hypermutation then adds more available sequences that can be selected by antigen, leading to higher-affinity antibodies.^{15,16} Conventional antigens bind to the complementarity determining regions (CDRs); therefore amino acid changes arising from mutation tend to be concentrated in these sites.¹⁷ However, some antigens bind to framework regions (FWRs) of V_H , and, because this leads to stimulation of a wider range of B cells, these are known as "superantigens." Because the FWRs are characteristic of the V_H gene segments and are unaffected by the recombination process, an influence of superantigens can be deduced

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TABLE I. Profile of V_H family use in IgE and IgM sequences derived from patients with B cells

Ig sequences (n*)	V _H 1 %	V _H 2 %	V _H 3 %	V _H 4 %	V _H 5 %	V _H 6 %	V _H 7 %
Patient 1 IgE (12)	66.6	0	8.3	16.6	8.3	0	0
Patient 2 IgE (5)	60.0	0	40.0	0	0	0	0
IgE sequences (17) (Mean)	63.7	0	17.6	11.7	5.8	0	0
IgM sequences (14)† (Mean)	14.2	7.1	28.5	35.7	7.1	7.1	0
Expected use‡ (Mean)	21.5	5.8	43.1	21.5	3.9	1.9	1.9

*Number analyzed.

†From a cDNA pool of four patients allergic to peanuts.

‡Calculated from the available repertoire.

by asymmetry in use of certain genes in responses to antigen.¹⁸ This has been well described for the antibodies against staphylococcal protein A.¹⁹

Genetic studies of V_H from IgE in blood lymphocytes of patients with atopic dermatitis and allergic asthma have also indicated a possible asymmetry in gene use, with evidence for increased involvement of the V_H5 family.^{10,11} A further feature of the V_H5 sequences is a high degree of somatic mutation but a pattern of mutations that did not indicate a role for antigen selection.^{10,11} These findings led to the suggestion that the house dust mite (HDM) allergen may be acting as a B-cell superantigen.¹⁷ This study describes a similar study of V_H use by IgE in patients with allergy to peanuts and indicates possible bias to a different V_H family.

METHODS

Patients and cell preparation

Approval for this study was obtained from the local Southampton University Hospital Ethical Committee. Two patients with a clinical history of hypersensitivity to peanuts were studied. Both were female and had positive reactions to skin prick tests against peanut extract. Patient 1 (age 23 years) had a total serum IgE of 330 kU/L and peanut-specific IgE of RAST class 4. However, she had multiple allergies, with additional skin prick test reactions against grass pollen, milk, egg, and other nuts. Patient 2 (age 15 years) had a total serum IgE of 220 kU/L and peanut-specific IgE of RAST class 6. This patient appeared to have the single allergy, with no further detectable specific IgE reactivities. Both patients reacted positively to a challenge of peanut rubbed onto the lower lip, with patient 2 showing lip swelling and patient 1 showing pharyngeal pruritus and ear itch. Peripheral blood mononuclear cells were isolated from 50 ml blood samples by Lymphoprep (Nycomed, Birmingham, England) density gradient centrifugation.

Preparation of cDNA, amplification, and sequence analysis of V_H genes in IgE (V_H-Cε)

To bias the genetic analysis toward functional genes, ribonucleic acid (RNA) was used as source material.²⁰ Total RNA was isolated from the peripheral blood mononuclear cells by using RNazol B (Cinna Biotex, Houston, Tex.), and complementary deoxyribonucleic acid (cDNA) was prepared with an oligo-dT primer and the AMV reverse transcriptase system (Promega, Madison, Wis.). For analysis of V_H genes in combination with the constant region of epsilon heavy chain (Cε), a nested polymerase chain reaction (PCR) was applied.¹¹ The primary PCR amplification was carried out with a mixture of 5'-

oligonucleotide primers, specific for the V_H leader sequences of families V_H1-6,²¹ together with a 3'-primer specific for the first exon of the ε-constant region.¹⁰ This was followed by a second nested PCR, with a mixture of inner oligonucleotide 5'-primers based in the FWR 1 of the V_H families,²² together with an internal 3'-Cε primer.¹⁰ For testing the V_H primer mixes for possible bias, the same nested approach was used with a pool of cDNA from four patients with peanut hypersensitivity, including patients 1 and 2. For this, the outer and inner 3'-Cε primers were replaced by primers based in the Cμ constant region Cμ₃ (outer): 5'-GGG TTG CCG AAG AAG CCG-3'; Cμ₃₀₀ (inner): 5'-AGG CAC GTT GTC GAC TTT GTT GCC GTT GGG GTG CTG GAC TTT GCA-3'. Conditions for the PCR reactions were as described.¹¹

All PCR products were gel-purified, ligated into pGEM-T vector, and used to transform JM109-competent bacteria (Promega). Clones were sequenced as described,²³ and sequences were analyzed by searching European Molecular Biology Library and current databases (V Base sequence directory: I.M. Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, U.K.), with MacVector 4.5.3 software.

Analysis of mutational patterns in V_H genes

The number of expected replacement mutations in the CDR was calculated according to the method of Chang and Casali.²⁴ The binomial distribution model was used to calculate whether the excess or scarcity of replacement mutations in CDRs or FWRs resulted by chance.²⁴

RESULTS

V_H sequence profiles obtained from IgE

The profile of V_H family use found in IgE from the two patients is shown in Table I. For patient 1, a total of 12 distinct clones was identified, with several of these being obtained as repeated sequences, as indicated in Fig. 1, A. For patient 2, only five distinct clones were identified, with many repeated sequences (Fig. 1, B). These same sequences were obtained from patient 2 through four separate PCRs, indicating that the available V_H-Cε transcripts were limited. Each of the 17 individual V_H-Cε sequences was potentially functional and was derived from a single B cell. Sequences have been compared with the closest germ line V_H gene in the database. As seen in Table I, the majority of sequences from both patients were derived from the V_H1 family (eight of 12 and three of five for patients 1 and 2, respectively). This is also shown in Fig. 1, A, with the germ line genes 12M28 and DP88, both from the V_H1

A		
V ₁ -2	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGYTFT GYYMH	WVRQAPGQGLEWMG WINPSSGGTNYAQKPGG RVITMRDTSISTAYMELSLRSEDITAVYYCAR
CO 9.4 (2)	-----v---aC-----L-	--r---H-----L---N---TcKF-k---r---It-----K---l-----T DGGPESP
CO 4.1 (2)	-----Q---P-----	-----CIP-----PR-----e---T---v---aS NRDWYCSGGSCYSDDAFDWDGQGTMTVTVSS JH5b
V ₁ -46	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGYTFT SYVMH	WVRQAPGQGLEWMG IINPSGGSTSYAQKPGG RVITMRDTSISTAYMELSLRSEDITAVYYCAR
CO 15.4 (3)	E-----k-----L---T-ED---N-FI-	--r---H-----L---N---TcKF-k---r---It-----K---l-----T DGGPESP
CO 4.4 (2)	---R-----T-----G---	-----G-----K---T-----Q- CGELSC
CO 9.1 (2)	-----R---T-----	-----G---KV---R---L---L---vY-----r QGSCSGGTCTY EFDS WQGTMTVTVSS JH4b
V ₁ -18	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGYTFT SYGIS	WVRQAPGQGLEWMG WISAYNGKNTYNAQKPGG RVITMRDTSISTAYMELSLRSEDITAVYYCAR
CO 17.4 (1)	QV-----R-----Ts--A-t s---	-----g---l-s-S---Y-D-----e---rY--K----- DNYQLLWE YWQGTMTVTVSS JH4a
12M28	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGGTFS SYAIS	WVRQAPGQGLEWMG GIIPILGIANYAQKPGG RVITITADKSTSTAYMELSLRSEDITAVYYCAR
CO 8.1 (2)	-----R-----W-----S- -DG--	-----g---A-----gRI---P-----Ttsta-----L----- YTLGUVSH YNYHYMDWQGTMTVTVSS JH6b
DP88	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGGTFS SYAIS	WVRQAPGQGLEWMG GIIPITGTANYAQKPGG RVITITADKSTSTAYMELSLRSEDITAVYYCAR
CO 11.1 (2)	E---C-----g-----E---ST NS--T	-----e---R-----gRT--a---t-t-Ia---TIV---l---T----- DNYGELAVR WLDWQGTMTVTVSS JH5b
V ₃ -11	<u>QVQLVDSG</u> GLLVQPGSSVRLSLSAASGPTFS DYVMS	WVRQAPGQGLEWVS YISSSGSTIYADSVKG RPTISRDNAKNSLYLQMNSLRSEDITAVYYCAR
CO 6.1 (2)	-----D-----Q-----g-R -y-N	---P-----G---ANN---T-S---l---T-----aI--- ARD GMDWQGTMTVTVSS JH6a
V ₄ -74	<u>QVQLVDSG</u> PLVQPKPSLTSLTCTVSGGSIS SGDYVMS	WVRQAPGQGLEWIG YIYSGSTIYNPSLKS RVITISVDTSKNQPSLKSSVTAADTAVYYCAR
CO 23.4 (1)	E-----d---s -Y---pj---G---y---gs---L---r-M-v---nh-W-N---tP-S-v--- SGAPGESL	DYWGQGTMTVTVSS JH4a
V _{1V} -4/4.35	<u>QVQLVDSG</u> PLVQPKPSLTSLTCTVSGGSIS SYVMS	WVRQAPGQGLEWIG RIYPSGSTIYNPSLKS RVITISVDTSKNQPSLKSSVTAADTAVYYCAR
CO 10.4 (1)	-----A-----g-T -Y--	-----L---PD-----s-M-----N-----I----- DESLVPATSSR AFDWQGTMTVTVSS JH3a
V ₅ -VHCV	<u>EVQLVDSG</u> AEVKKPGESLKISKSGSYST SYVMG	WVRQMPGQGLEWMG ILYPGSDITRYSPSPQG QVITISADKSISTAYLQWSSLKASDTAVYYCAR
CO 7.1 (1)	-----E-----P-----A---N-D N--M-	-----L- V-----G-----q- -----st----- PGGTLSPY DHDWQGTMTVTVSS JH4b
B		
V ₁ -69	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGGTFS SYAIS	WVRQAPGQGLEWMG GIIPITGTANYAQKPGG RVITITADESTSTAYMELSLRSEDITAVYYCAR
KH 17.4 (6)	E-----g-----sD-----	-----M---T---f---N-----V----- DRYDSSGYALVYS YGMDWQGTMTVTVSS JH6
V ₁ -8	<u>QVQLVDSGA</u> EVKKPGASVKVSKASGYTFT SYDIN	WVRQATGQGLEWMG WNPNSGNIGYAQKPGG RVITMTNTSISTAYMELSLRSEDITAVYYCAR
KH 1.4 (3)	E-----k-----G---S	-----A-----g-N-E---V-----g-----G---v-F--- GQLMTYRNYDRL YFDYWGQGTMTVTVSS JH4t
KH 7.4 (4)	E-----S-----	-----T-----e-----F--- GRGSSYYESSG YXWQGTMTVTVSS JH4t
V ₃ -74	<u>EVQLVDSG</u> GLLVQPGSSVRLSLSAASGPTFS SYVMH	WVRQAPGQGLWVS RINSIGSSTSYADSVKG RPTISRDNAKNSLYLQMNSLRSEDITAVYYCAR
KH 3.4 (5)	-----C---t- sy---	---E-E-----NaPT-TRFSE-----H----- ELVDYGVS FDCWQGTMTVTVSS JH4t
KH 15.4 (8)	-----p-----N---	-----n---TH-RD-----r---T-----n-----v----- VNIGGGATVINDA FDIWQGTMTVTVSS JH3t

FIG. 1. Deduced amino acid sequences of the V_H-C_ε sequences derived from patient 1 (**A**) and patient 2 (**B**). Comparisons have been made with the closest germ line genes. *Upper case*: replacement amino acids; *lower case*: silent mutations. Replacement amino acids in J_H are underlined. Numbers in brackets indicate number of repeated sequences. Accession numbers (EMBL Nucleotide Sequence Database): Patient 1 9.4 Z92888; Patient 1 15.4 Z92889; Patient 1 4.4 Z92890; Patient 1 17.4 Z92891; Patient 1 23.4 Z92892; Patient 1 10.4 Z92893; Patient 2 17.4 Z92894; Patient 2 1.4 Z92895; Patient 2 7.4 Z92896; Patient 2 3.4 Z92897; and Patient 2 15.4 Z92898.

family. Remaining sequences were from the V_H4 (two of 12 for patient 1), V_H3 (one of 12 for patient 1 and two of five for patient 2), and V_H5 (one of 12 for patient 1) families. No sequences were identified from the smaller V_H families, V_H2, V_H6, and V_H7. Dominance of the V_H1 would be even greater if repeated sequences, which might indicate expanded clones, were taken into consideration.

Although numbers are small, the profile differs from that expected on the basis of random usage of V_H genes from the available repertoire, which is dominated by the V_H3 family (Table I). In fact, the apparent excess of V_H1 use (64% of individual clones as compared with 22% expected) reaches statistical significance for both patients (patient 1: $p < 0.01$; patient 2: $p < 0.05$ by χ^2 analysis). The V_H nested primers have been checked for bias previously,¹¹ but we have recently repeated this check procedure by using pooled cDNA from eight patients with asthma (Snow RE, unpublished data). For

this, the 5'-V_H primers were used with 3'-mixed J_H primers to sample all immunoglobulin isotopes. V_H1-derived clones made up 25%; V_H3 = 45%, V_H4 = 20%, and V_H5 = 5%, figures in line with those expected from the known repertoire (Table I). We also checked their operation by using cDNA from four patients with peanut hypersensitivity, by amplification from IgM sequences. These would be expected to be derived from the wide range of IgM-positive B cells in blood. Results obtained (Table I) showed only 14% of clones derived from V_H1, confirming no preference for V_H1 by using the V_H primer mixes for IgM from these patients.

V_H-C_ε-deduced amino acid sequences

Nucleotide sequences of the 17 clones have been submitted to the EMBL database. Deduced amino acid sequences (Fig. 1) demonstrate that the CDR3 sequences, which represent the "clonal signature" of the B cell, are clearly distinct in both length and composition

TABLE II. Analysis of replacement and silent mutations in framework regions and complementarity determining regions of the observed V_H-IgE sequences

IgE sequence	% Mutation from germ line	FWR			<i>p</i> (FWR)	CDR		Expected R	<i>p</i> (CDR)
		Observed		Expected R		Observed			
		R	S			R	S		
Patient 1									
9.4	6.2%	4	6	9.7	0.0044*	5	2	3.4	0.1317
4.1	4.8%	8	3	7.4	0.2087	2	0	2.6	0.2693
15.4	9.6%	7	6	14.7	0.0015*	10	3	5.2	0.0149*
4.4	2.6%	5	1	4.0	0.2304	1	0	1.4	0.3678
9.1	3.7%	3	2	5.6	0.6264	5	0	2.0	0.0229*
17.4	6.6%	5	6	10.1	0.0108*	4	3	3.6	0.2142
8.1	7.4%	5	6	11.3	0.0033*	7	2	3.8	0.0442*
11.1	9.2%	8	7	14.2	0.0080*	6	4	4.8	0.1528
6.1	6.3%	5	4	9.5	0.0182*	7	1	3.4	0.0259*
23.4	9.1%	9	9	13.7	0.0327*	2	5	5.2	0.0630
10.4	5.2%	5	4	7.8	0.0728	4	1	2.6	0.1565
7.1	7.4%	11	4	11.6	0.1171	4	1	3.8	0.2171
Patient 2									
17.4	2.9%	1	2	4.5	0.0136*	3	2	1.5	0.2014
1.4	5.1%	4	4	7.9	0.0252*	5	1	2.9	0.0897
7.4	1.5%	2	1	2.3	0.3628	1	0	0.8	0.4111
3.4	7.7%	4	2	11.7	0.0006*	11	4	4.2	0.0008*
15.4	4.8%	1	4	7.2	0.0004*	6	2	2.6	0.0228*

R, Replacement mutations; S, silent mutations; *p* (FWR/CDR), the probability that an excess or scarcity of replacement mutations in the FWRs or CDRs resulted by chance.

*Denotes significant probability values.

in each case. Analysis of D-segment genes revealed a complex pattern, with use of single and double D_H genes in normal and reverse orientation, and evidence for N-region addition (not shown). Analysis of J_H genes indicated that the majority were derived from J_H4, which is commonly used by normal B cells.²⁵

The amino acid sequences have been compared with those deduced from the closest germ line gene, and inspection shows that there are many differences from germ line sequence, apparently the result of somatic mutational events (Fig. 1). Thirteen of seventeen sequences also had mutations in the J_H regions. The percentage of mutation in the nucleotide sequences is shown in Table II.

Analysis of the distribution of somatic mutations

Clustering of replacement amino acids in CDR1 and CDR2 is considered to be indicative of conventional antigen selection and is evident in some of the V_H sequences in Fig. 1 (for example, KH 3.4) on visual inspection. There is also a tendency for there to be more replacement mutations in the CDRs than expected on a random basis (Table II). However, significant clustering is revealed objectively by applying a codon-by-codon analysis of nucleotide substitutions and examining the statistical probability of its being due to chance.²⁴ This has been carried out for all the V_H-C_ε sequences (Table II) and has indicated significant clustering in CDRs of only 6 of 17 sequences, four from V_H1 and two from V_H3. Conversely, preservation of sequence, with fewer

than expected replacement mutations, in the FWRs is also revealed by this analysis and is present in 11 of 17 sequences (Table II). These results are consistent with only a limited influence of conventional antigen selection on the identified sequences of the IgE binding sites.

DISCUSSION

Immunogenetic analysis of IgE-variable regions is providing some intriguing data that may distinguish IgE from other immunoglobulin isotypes. Evidence is accumulating that appears to indicate that the IgE response may be partly driven by "superantigens," which bind to FWRs of certain V_H genes.^{11, 18} Selective binding gives rise to detectable bias in the use of those genes by IgE. In asthma associated with hypersensitivity to HDM, a predominant use of V_H5 genes by IgE has been noted.^{10, 11} Our initial observations have been confirmed recently in a study of seven patients with mild HDM-associated asthma, in which 20% of the V_H-C_ε sequences obtained were derived from the V_H5 family, as compared with an expected frequency of 4%.²⁶ This suggests that HDM allergen may bind preferentially to naive B cells expressing IgM encoded by V_H5 genes and that in allergy-prone individuals, these B cells then switch to IgE production.^{11, 26} In this sense, the allergen is acting as a B-cell superantigen and could be mimicking or replacing a useful broad stimulation of B cells by parasite antigens. Clinical effects of hypersensitivity to food allergens differ from those to HDM, possibly because of differences in the level and timing of expo-

sure to the inducing allergen. Food allergens such as peanuts are ingested intermittently and possibly at high doses, whereas exposure to HDM is usually at a constant low level. There is also a tendency for a slow reduction in sensitivity to HDMs in early adult life that appears not to occur for peanuts.⁴ Structural studies of IgE have now suggested that a superantigen may be involved in driving B cells in both individuals allergic to HDMs and those allergic to peanuts but that the response is different between the two types of allergen. Perhaps that is to be expected because the nature of the two allergens is quite different,⁵ and it might be expected therefore that they would bind to different framework region sequences in V_H. It will now be interesting to compare the V_H family use of the IgE response to peanuts with that in other food allergies. It should be noted that it has been very difficult to amplify V_H-C ϵ sequences from normal individuals¹⁰ (Janezic et al., unpublished findings), indicating that the identified sequences in patients are associated with allergic disease.

Although our two patients differ in having either multiple allergies (patient 1) or a single allergy (patient 2), the pattern in the V_H-C ϵ sequences was similar. Many more cases must be analyzed before drawing firm conclusions about frequency of V_H gene use, but it is quite remarkable that both have shown a bias toward the V_H1 family (64%) and low involvement of V_H5. Predominant transcripts from V_H1 have also been found in a further three of three patients allergic to peanuts (unpublished observations). This can be contrasted with findings in the seven patients with HDM-associated asthma in which V_H1 use showed no increase over that expected from the available repertoire, varying from 0% to 25% of sequences with a mean of 12%.¹¹ In a study of patients with atopic dermatitis, which is often associated with food allergies, transcripts from the V_H5 family were apparently predominant, but no information on V_H1 transcripts was available because the primers used were only for the V_H3-V_H6 families.¹⁰

Comparison of the V_H-C ϵ sequences obtained from the patients allergic to peanuts with the closest germ line genes has revealed extensive somatic mutation, which indicates that the B cells have traversed the germinal center where the somatic mutation mechanism is activated.¹⁶ Selection of mutated sequences by antigen also occurs at this site, with conventional antigen binding leading to a focus of replacement amino acids in the contact points in the CDRs.¹⁷ However, this pattern was not seen in most sequences, possibly supporting the idea that the influence of allergens is mainly on V_H gene family choice, with binding occurring outside the CDRs.^{11,18} It appears that HDM allergen may select V_H5, whereas, although results are preliminary, peanut allergen may select V_H1-encoded sequences.

The application of PCR to whole blood lymphocytes can reveal only crude V_H gene bias, with no information on the specificity of the IgE. Currently we are developing the approach of cell separation and single-cell PCR, which will provide both V_H and V_L sequences from an

IgE-expressing B cell. We are able to express these sequences in phage²⁷ and can then test the specificity of the IgE. Clearly, this approach will allow testing of the hypothesis that IgE binds allergens through FWR sequences, which has been suggested by our findings.

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