Supplementary information for

The human IgE repertoire

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Supplementary Table 1. Examples of studies of human IgE repertoires.

Condition of subjects	Lymphocyte source; Number of donors / PCR products analysed	IgE-encoding repertoire – main finding	Reference
Atopy	PBMC§; 2/104	Mutated sequences; Oligoclonal population	1
Atopic dermatitis	PBMC; 3/19	Diverse repertoire (only IGHV5 analyzed in detail); oligoclonal repertoire	2
Atopic dermatitis	PBMC; 2/29	Diverse	3
Peanut allergy	PBMC: 2/17	Overrepresentation by IGHV1; mutated	4
Allergic asthma	Bronchial biopsy: 1/30	Overrepresentation of IGHV5	5
Atopic subjects (dermatitis, rhinitis, conjunctivitis, asthma)	PBMC; 10/ *	Diverse IGHV repertoire	6
Atopic dermatitis	PBMC; 1/51	Overrepresentation of IGHV6-1. These antibodies were multireactive.	7
Seasonal rhinitis	PBMC; 3/62	Diverse, oligoclonal antigen-driven IgE	8
Infants with allergic disease	PBMC; 3/50 (36 independent)	Diverse repertoire; similar clones found in different individuals	9
Allergic rhinitis	Nasal biopsy: 11/62	Overrepresentation of IGHV5 and of mutations in FR	10
Allergic rhinitis	PBMC; 7/50	Diverse with respect to VH subgroup usage	
Allergic rhinitis	PBMC; 2/72	Diverse, mutated	11
Atopic dermatitis; multiple allergies	PBMC; 1/76	Diverse but overrepresentation of IGHV4; mutated but little evidence of antigenic selection	12
Non-allergic	PBMC; 2/60	Diverse but with fewer mutations in comparison to IgE of allergic subjects or to IgG	
Atopic eczema (IgE > 10 IU/ml)	PBMC; 14/†	Diverse with respect to VH subgroup usage. Mutated sequences. Oligoclonal repertoires.	13

Allergic asthma (children)	PBMC; 13/1366	Diverse with respect to VH subgroup usage; oligoclonal population; highly mutated; evidence of antigenic selection	14
Atopic dermatitis (children)	PBMC; 4/129	Diverse with respect to VH subgroup usage; less oligoclonal and less mutated as compared to sequences from patients with allergic asthma; little evidence of antigenic selection	
Chronic rhinosinusitis with nasal polyps	Nasal tissue; 4/217	Diverse with respect to VH subgroup usage; mutated but with little evidence of antigenic selection	15
Allergic subjects monosensitized to Bet v 1 analyzed over a period of four years of allergen exposure	PBMC; 3/152	Diverse with respect to subgroup usage; mostly somatically mutated sequences but no evidence of increase of somatic mutations over time of allergen exposure. Similar IgE variable regions found over four years of allergen exposure in single individuals and in genetically non-related individuals.	16
Chronic rhinosinusitis	Sinus mucosa; 4/50	Diverse, mutated	17
Non-allergic fungal eosinophilic rhinosinusitis	Sinus mucosa; 4/36	Diverse, mutated	
Parasitized subjects, no history of allergic disease	PBMC; 14/118	Diverse with respect to VH subgroup usage; mutated but with reduced evidence of antigenic selection	18

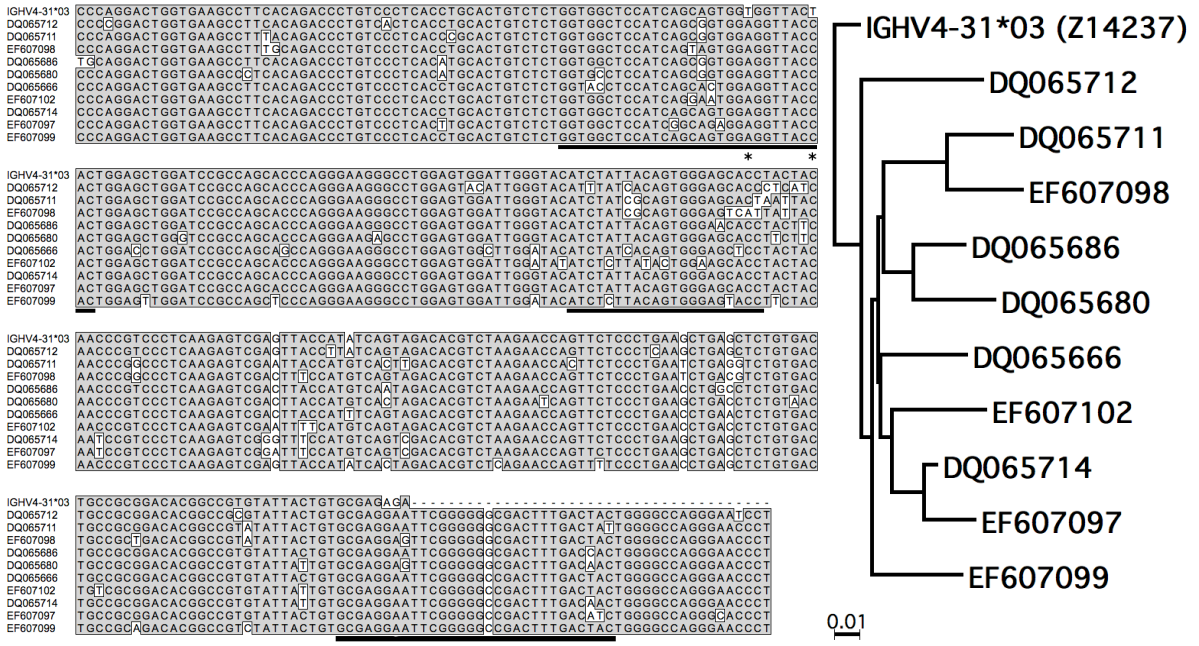
§ PBMC: peripheral blood mononuclear cells.
* Southern blot based analysis – individual clones not analysed by sequencing.
† Major results reported from quantitative PCR-based assay. Only partial protein sequences available.

Supplementary Table 2. Basic outline of library construction and initial steps in the isolation of human allergen-specific antibody fragments using phage display technology.

1. Isolation of samples (peripheral blood or tissue) containing B cells from allergic donors.
2. Isolation of mRNA (often as total RNA)
3. cDNA synthesis for instance using oligo-dT or, for the amplification of genes encoding the H chain V domains of IgE, primers specific for genes encoding constant domains of IgE [11].
4. PCR-amplification of antibody encoding genes
    a. Amplification of genes encoding the H chain V domains using primers that limit the amplification to those encoding IgE.
    b. Amplification of genes (or creation of synthetic genes) encoding L chain V domains.
5. Cloning of antibody V domain-encoding genes in a vector suitable for phage display technology.
6. Transfection of library into F-pilus carrying *E. coli*.
7. Production of phage stock displaying antibody fragment on filamentous phage.
8. Selection of phages displaying allergen-specific antibody fragments using panning.
9. Infection of selected phages into F-pilus carrying *E. coli*.
10. Repeated selection (steps 7-9), if necessary.
11. Primary screening of allergen-specific binders (either displayed on phage or as soluble proteins.
12. Downstream analysis of productivity, reactivity and specificity as required.

Supplementary Table 3. Examples of factors that may affect the outcome of selection allergen-specific antibody fragments from combinatorial antibody libraries

	Problem	**Effect**
1.	PCR primer design may affect the ability to incorporate certain antibody-encoding genes in a library.	Certain V genes may not be efficiently amplified, skewing the library. Optimised primers and reaction conditions [19] should be employed.
2.	Sequence features will affect display potential and thus potential for selection for instance through toxicity to *E. coli*.	Some fragments based on certain V genes will not be properly displayed or selected, a fact that may introduce artificial skewing of selected repertoire or complete failure of the selection process. Selection of appropriate bacterial strains, stringent control of recombinant protein production, co-expression of chaperone and steps that minimize advantages of fast-growing clones etc. may improve selection outcome [20-23].
3.	Display format (e.g. single chain fragment variable (scFv) or Fab) may affect selection efficiency.	ScFv is often displayed more efficiently than Fab but dimerization and linker effects may affect scFv behaviour.
4.	The degree of dependence of a H chain V domain on the L chain sequence for establishment of a specific binder will affect selection.	Dependence of an H chain sequence on a certain L chain sequence for antigen binding will reduce the likelihood of selecting that binder.
5.	Library size will determine the likelihood of recreating a specific binder through random combinations of H and L chain V domains.	Large libraries will be more likely than small libraries to recreate rare binders but they may also create new specificities that were not present in the patient.
6.	Selection conditions (factors such as but not limited to mode of antigen immobilization, antigen concentration, washing procedure and nature of antigen (glycosylation, folding, isoform composition etc.)) will greatly effect selection outcome.	Selection procedure will identify only a fraction of binders available in the library. Different sets of conditions may collect different sets of binders.



Supplementary Figure 1. Alignment of clonally related genes encoding the H chain V domain of IgE that have been derived from a library made from transcripts derived from a single allergic individual. All sequences have an origin in the IGHV4-31 germline gene and carry very similar CDRH3 sequence (with a maximum of 3 nucleotide differences from a consensus CDRH3 sequence). CDR are underlined. Note two mutations (indicated by stars) in CDRH1 that are conserved among all members of this set of clones. One of them is silent (GGT⇒GGA) while the other (TAC⇒CAC) results in a Y⇒H substitution). Nucleotides that aligned to primers during PCR amplification are not shown. Sequences were obtained either after sequencing of random PCR products (names starting with DQ) [11] or after selection for specificity for group 2 grass pollen allergen Phl p 2 by phage display technology [24] (names starting with EF). A phylogenetic tree, made using the Neighbour-Joining method, describing the potential relationship of the sequences is shown.

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