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Delineating the specificity of an IgE-encoding transcriptome

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Abstract

Background: Although much is known about the reactivity of polyclonal populations of antibodies targeting the wide array of allergens produced by timothy (*Phleum pratense*) and other grass species, little is known about the finer details at the level of individual antibody specificities.

Objective: We sought to investigate the IgE repertoire as it occurs in a grass pollen allergic patient.

Methods: For this purpose, a human IgE library, constructed from peripheral blood B-cells of a timothy allergic individual, was used. The library was screened, using phage display, against a panel of six different timothy allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, and Phl p 11).

Results: Highly diverse antibody fragments with respect to gene usage were identified. The binders were highly specific for their respective target antigen, except for clones selected on Phl p 6 which also recognized Phl p 5, most likely reflecting the high sequence homology between these allergens. Interestingly, by using this approach we were able to determine the specificity of over 25% of all IgE-producing transcripts in this allergic individual.

Conclusion: The human IgE repertoire is produced by a limited number of highly related B-cell clones and as such restricted in its recognition of a limited number of antigens.

Clinical Implication: Human allergen-specific antibodies can, by defining the specificity of IgE responses, aid in the development of allergy vaccines or even by themselves be used in passive immunotherapy.

Capsule summary: Findings from this study demonstrate that just a few allergens account for a high percentage of the complex IgE repertoire in a grass-pollen allergic patient, a factor of importance for the development of allergy therapies.

Key words: allergy, antibody library, antibody repertoire, diversity, grass pollen allergen, IgE, phage display, specificity

Abbreviations

BSA: bovine serum albumin

CDR: complementarity determining region(s)

HRP: horse-radish peroxidase

PBS: phosphate buffer saline

scFv: single chain antibody fragment(s)

V_H: variable region of the antibody heavy chain

V_L: variable region of the antibody light chain

INTRODUCTION

Grass pollen allergy has been estimated to affect up to 20% of the population in the industrialized countries,[1, 2] consequently affecting the quality of life of millions of people. The disease mechanism is associated with increased serum-levels of specific IgE that are mainly directed towards a limited number of proteins that are released from the pollen grain upon hydration. Binding of these allergens to basophil- and mast cell-bound IgE leads to Fc-receptor cross-linking, degranulation and the release of inflammatory mediators which in turn causes the symptoms associated with grass pollen allergy, ranging from rhinitis and conjunctivitis to asthma. As the antibody-allergen interaction is a key event in the allergic reaction, a deeper understanding of it is a prerequisite for understanding the mechanisms behind allergy and such knowledge will also aid in the development of new allergy treatments.

Although much is known about the make-up of genes encoding antibodies in general and also to some extent of genes encoding IgE, much less is known about sequences of antibodies targeting specific allergens. In a previous investigation,[3] we set out to study the IgE repertoire as it occurs in grass-pollen allergic patients. Sequencing the heavy chain of random transcripts of this repertoire, we found these antibody populations to highly resemble immunoglobulin populations of other isotypes with respect to genetic diversity and mutational patterns, a finding supported[4-6] as well as contradicted[7-10] by other studies. However, in contrast to IgG populations,[11] a high number of clonally related sequences were identified among the analyzed clones, suggesting that despite its rather diverse genetic make-up the peripheral IgE population is populated by a limited set of related clones, or so called clonotypes. This latter finding confirms the results of previous studies.[4, 12, 13] Taken together, this lead us to hypothesize that the relatively small IgE population of allergic individuals is limited in its recognition of just a few allergens at a given time. In order to define the

specificities of the identified clonotypes, a combinatorial phage display library was constructed from the IgE repertoire of the patients mentioned above and selections were initially performed on the major grass pollen allergen Phl p 5.[3] Highly specific antibody fragments, binding to several different epitopes on Phl p 5, were identified. Interestingly, one of the selected binders was highly similar to a set of sequences found among the randomly picked IgE-encoding transcripts. Numerous studies have explored the reactivity of polyclonal IgE populations to many different allergens, including those of grass pollen, but when it comes to the reactivity pattern of individual IgE-molecules data is limited.[14-18] In contrast to our investigation,[3] however, none of the previous studies have described the antibodies within the larger context of the whole IgE repertoire. Our combined approach of both studying the overall IgE repertoire and the IgE repertoire targeting specific allergens therefore put us in a unique position, allowing us to investigate the specificity profile of this population at a much higher resolution and further providing us with new insights on how allergen-specific responses arise.

In this study, we have continued on the path of determining the specificity profile of the IgE-encoding repertoire of a grass-pollen allergic patient. We show that 6 timothy pollen allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, and Phl p 11) account for over 25% of the IgE producing transcripts of this donor, further supporting the notion of IgE repertoires having limited recognition ranges.

METHODS

IgE-scFv library

The local ethical committee gave approval for the procedures in this study. The construction of the combinatorial single chain antibody fragment (scFv) library used has previously been described.[3] Briefly, peripheral blood lymphocytes had been isolated from a grass pollen allergic donor, A3, during season of antigen exposure (June) as part of a study investigating immunological principles in allergy.[19] Except for showing reactivity in clinical skin-prick testing to timothy extract, the patient also had clear symptoms of rhinitis and had high levels of timothy extract specific IgE (55 kUA/l) at the time of sampling. Additionally, skin prick testing on A3 revealed sensitivity to cat and birch.[19] The heavy (V_H) and light chain (V_L) encoding genes were obtained by a semi-nested PCR approach, the V_H genes having an origin in the IgE repertoire, while the V_L genes were derived from B cells producing different antibody isotypes. The V_H and V_L encoding genes were assembled using overlap-extension PCR and subsequently incorporated into a modified form of the pFab5c.His phagemide.[20] The ligation product was transformed into electrocompetent Top10F' (Invitrogen, Carlsbad, CA, USA), giving a library size of approximately 3×10^5 clones. The library was infected by VCSM13 helper phage (Stratagene, La Jolla, CA, USA) and phage harvested by standard polyethylene glycol/NaCl precipitation.

Phage display selections of antibody fragments specific for timothy allergens

To identify timothy-specific IgE, phage selections were performed on 7 different allergens from timothy grass (*Phleum pratense*); Phl p 1, Phl p 2, Phl p 4, Phl p 5 (isoform a), Phl p 6, Phl p 7 and Phl p 11. All antigens were purchased from Biomay AG (Vienna, Austria) except for Phl p 4 and Phl p 11, which were kindly provided by Dr. Jonas Lidholm (Phadia AB, Uppsala, Sweden). Recombinant versions of the allergens were used, except in

the case of Phl p 4 where the natural form was utilized. Selections were performed in

microtiter plates (Costar, Cambridge, MA, USA) by first coating the plates with allergens

diluted to 5 μg/ml in phosphate buffer saline (PBS). Unbound antigen was washed away and

blocking solution (1% (w/v) bovine serum albumin (BSA), 0.05% Tween 20 (v/v) in PBS)

added before the phage library, described above, was applied. After washing, unbound phages

were eluted by the addition of trypsin, which cleaves at a site located between the displayed

scFv and protein III. After two and/or three rounds of selections, phage stocks were produced

from individual randomly picked clones and analyzed for binding specificity. The clones were

named with a prefix indicating the antigen selected on (p1 for Phl p 1, p2 for Phl p 2, etc.)

followed by a clone number.

ELISA analysis

The phage-displayed scFv were analyzed for binding specificity by phage ELISA. The

allergens, BSA and extracts of *Phleum pratense* and *Lolium perenne* (both extracts were

kindly provided by P. A. Würtzen, ALK-Abelló, Hørsholm, Denmark) were coated in

microtiter plates at a concentration of 5 μg/ml. Skim milk was used as blocking agent (1%

(w/v) milk, 0.05% Tween 20 (v/v) in PBS) and bound phage were detected by a horse-radish

peroxidase (HRP) conjugated anti-M13 monoclonal antibody (GE Healthcare, Uppsala,

Sweden) using *o*-phenylenediamine as chromogen. Absorbance was measured at 490 nm. The

clones were considered positive if the absorbance for their respective target antigens was at

least 6 times higher than that obtained for control antigens, like BSA.

The reactivity of donor A3's IgE to the different allergens used during the selections as

well as to recombinant Phl p 12 (a kind gift from Dr. Jonas Lidholm, Phadia AB) was verified

by ELISA analysis performed on serum aliquots from this donor, collected during season of

grass pollen exposure (June). Bound IgE was detected with the HRP-labeled goat-anti-human

IgE (KPL Inc., Gaithersburg, Maryland, USA). Coating, blocking and detection were

performed as described above.

Genetic analysis

The allergen-specific clones were subjected to DNA sequencing (MWG Biotech,

Ebersberg, Germany). The genetic origin of their V_H, excluding the bases that were part of the

primer definition when constructing the library,[3] were analyzed by the bioinformatics tools

IMGT/V-Quest[21] and IMGT/Junction[22] (http://imgt.cines.fr/). The V_H sequences of the

selected clones were also compared to previously described Phl p 5-specific scFv and to

randomly sequenced IgE VH sequences, all with an origin in the A3 donor.[3] Clones were

considered clonally related if having an origin in the same IGHV gene and having a highly

homologous CDRH3, having identical length (between C104 and W118) and sharing at least

80% similarity on nucleotide level. Throughout this article, the IMGT nomenclature is used

for complementarity determining region (CDR) definitions and residue numbering.[23]

RESULTS

IgE-reactivity profile to different timothy allergens

The combinatorial IgE library used in this study had been derived from a donor, A3,

which previously had been tested positive in skin prick testing to timothy extract.[19] In

addition, serum from this donor was tested in an ELISA for the presence of IgE antibodies

specific to the individual timothy allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7,

Phl p 11 and Phl p 12) used in this study (Figure 1). Reactive antibodies were found in all

cases except for Phl p 7 and Phl p 12. The lack of reactivity to these two allergens is not

surprising since only around 10-20% of grass-pollen allergic individuals have an IgE response

against them.[1, 2]

Selection of scFv specific for different timothy allergens

The IgE library was subjected to phage selections on 7 different timothy allergens (Phl

p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7 and Phl p 11). In most cases, high output/input

ratios could initially be observed, underscoring the high frequency of specific binders in the

library. Following two or three rounds of selections, randomly picked clones were analyzed

for specificity using phage-ELISA (Figure 2). Consistent with the IgE serum data we were

able to isolate binders to all of the antigens except for Phl p 7, demonstrating that the selected

binders most likely had a counterpart *in vivo*. The isolated binders were highly specific to

their respective target allergen, except those selected on Phl p 6, which also bound Phl p 5, an

allergen with extensive sequence homology to Phl p 6.[24, 25]

Most of the isolated scFv also bound the natural form of the antigen as shown by the

observed reactivity, although of various strengths, with pollen extracts of timothy and/or rye

grass (Figure 2). In most cases these species produce similar allergens, which explain why

both extracts can be recognized. An exception is Phl p 6, which have been identified in

timothy but not in rye grass.[26] The obtained signal to rye grass of the Phl p 6-selected clones

is therefore likely due to the above mentioned cross-reactivity resulting in binding to the

group 5 allergens of this extract. The low signal that was observed for the Phl p 11-specific

scFv does not necessarily mean that these binders do not recognize the natural antigen, but

may reflect a low concentration of this allergen in the extracts. Furthermore, a potential

glycosylation site found in this allergen might possible explain the observed differences.[27]

Clonal relationship of the heavy chains of the IgE-encoding repertoire

Our previous analysis of the IgE transcriptome of two donors[3] indicated that few

clonotypes populate the IgE-encoding repertoire, i.e. multiple related sequences were

identified when sequencing these transcriptomes. For example, among the 63 randomly

sequenced V_H transcripts of donor A3, only 33 different clonotypes were identified (Table I,

clonotype 1-33), suggesting that many of the clones had an origin in the same precursor clone.

The allergen-specific clones isolated in this study could similarly be organized into

clonotypes based on heavy chain sequence homology (sharing the same IGHV gene and

having highly similar CDRH3 sequences). Altogether, 12 different clonotypes, with an origin

in all major IGHV subgroups (1, 3, 4 and 5), were identified (Table I). Except in the case of

Phl p 2, at least two clonotypes for each allergen target were found, utilizing diverse sets of

genes, demonstrating that the IgE response against these allergens are not clonally restricted.

When comparing sequences of the allergen-specific scFv with the randomly picked IgE-

encoding transcripts, obtained from the donor (A3) who contributed the sequences of the

library, it was shown that 5 of the 12 clonotypes were found in both sets of sequences (Table

I, clonotype 5, 18 and 24-26). Two Phl p 5-specific clones have additionally, in two separate

studies[3] (J. Persson *et al*., manuscript in preparation), been identified among the random

transcripts (Table I, clonotype 16 and 29) using the same library, bringing the total number of

clonotypes with a defined allergen specificity to 14 (Table II). 7 of the clonotypes, including

the three Phl p 6-selected clonotypes, could not be found among the previously described

clonotypes (Table I, clonotype 34-40), suggesting that they were minor components of the

IgE-encoding transcriptome.

Overall, it was shown that the repertoire targeting just 5 allergens (Phl p 1, Phl p 2, Phl

p 4, Phl p 5 and Phl p 11) alone covered 21-27%, 7 of 33 clonotypes or 17 of 63 members, of

the IgE V_H-encoding transcriptome as represented by randomly sequenced transcripts. This

implies that just a few allergens elicit the IgE repertoire encoded by circulating B cells at a

given point in time.

The light chain of selected allergen-specific antibodies

The combination of combinatorial library and display technologies like phage display is

commonly exploited to investigate specific antibody repertoires. The combinatorial principles

of library construction, i.e. the random assortment of light chains with heavy chains in

proteins encoded for by the library, however, adds diversity to the population that does not

have a counterpart in the repertoire as found *in vivo*. In addition, the library used in this study

utilized light chains from antibodies of all isotypes, not just restricted to the IgE population,

thereby increasing the diversity even further. Having this in mind, one would assume the

selected scFv to be highly diverse in the choice of light chains. However, this was generally

not the case. All but one of the allergen-specific V_H clonotypes that were selected in multiple

copies, were highly restricted in the choice of light chain partner, i.e. the selected binders used

very similar light chain sequences (Table II). Altogether, these results strongly suggest that an

allergen-specific binder derived from the V_H of these clones in general could only be formed

upon combination with a very restricted set of V_L.

DISCUSSION

In a previous study,[3] we defined the IgE repertoire as it occurs in a grass pollen

sensitized individual. Among a limited set of randomly sequenced IgE V_H transcripts highly

related sequences were identified. These and similar results obtained in other studies[4, 12, 13]

indicate that the peripherally located IgE-producing B-cell population is populated by a

relatively restricted set of clones. The 63 clones that were analyzed could based on sequence

homology, i.e. sharing the same IGHV and having highly similar CDRH3 loops, be clustered

into 33 different groups or clonotypes (Table I). Interestingly, among the Phl p 5-specific

scFv that were isolated from the combinatorial phage library constructed from the IgE

repertoire of the same donor, a scFv belonging to one of these 33 clonotypes was retrieved

(Table I, clonotype 29). By fusing the light chain of this Phl p 5-specific antibody fragment to

the V_H of two other members of clonotype 29, antibodies with retained allergen specificity

were created, thus suggesting that clones belonging to the same clonotype most likely have

the same specificity.[3] This is consistent with the fact that the heavy chain, especially CDRH3,

play a key role in antigen recognition.[28] The differences observed between members of the

same V_H clonotype likely describe different evolutionary pathways obtained as a result of the

affinity maturation process to the antigen in question.

Encouraged by these results, we have now embarked on a journey with the aim of trying

to obtain a more complete picture of the specificity of the IgE repertoire. In the current study

we have, using the same library, identified antibody fragments with specificity to additional

five timothy allergens (Phl p1, Phl p 2, Phl p 4, Phl p 6, Phl p 11). As in the case of the Phl p

5-specific antibody fragments, many of the newly selected binders belonged to clonotypes

previously identified among the IgE V_H transcripts.[3] Although the specificities have not been

proven for the randomly picked V_H transcripts, it may be assumed, based on the above

reasoning, that they represent the same specificity as their phage-selected homologues.

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Altogether, by using this procedure it was shown that the repertoire targeting this restricted

set of allergens alone covered around 25% of the IgE-encoding transcriptome. Previously,

absorbent studies on polyclonal populations have similarly concluded a limitation in the

number of recognized allergens by IgE repertoires.[29, 30] However, the allergen-specific

antibody fragments as identified and characterized here give an important insight into the way

the immune system recognizes the allergen at a level of resolution reaching beyond that

achievable by assessing polyclonal antibody repertoires as found in the serum. The current

study provides a picture of the immune response during a single time-point during allergen

peak season and it is therefore unclear whether the identified population is a snapshot in time

of an evolving response or if they represent a constant final set of clones. Niederberger *et al.*[31]

recently suggested that allergen exposure leads to the activations of an established pool of IgE

memory cells rather than an activation of immature B-cells, indicating that the IgE producing

population is rather fixed over time. However, the existence of an IgE memory B cell

compartment has been questioned[32] and further investigations regarding IgE plasma cell

homeostasis is needed in order to shed further light on the subject.

The isolated binders were highly specific for their respective target antigen except those

obtained from the Phl p 6 selections, which showed high cross-reactivity with Phl p 5. These

allergens are highly homologous, having regions of over 90% sequence identity.[24, 25] Identical

or over-lapping epitopes between the two allergens are likely to be the reason for the observed

cross-reactivity. These findings demonstrate the close immunological similarity between Phl

p 5 and Phl p 6 and support, at a clonal level, the notion that epitopes found on Phl p 6 have a

counterpart in Phl p 5.[1, 2]

Since combinatorial libraries randomly recombine heavy and light chains one might ask

if the antibodies selected from such libraries constitute a valid representation of the *in vivo*

immune response. Importantly, as demonstrated by the inability of identifying Phl p 7-

specific scFv, an allergen for which the donor had no measurable serum IgE, the

combinatorial process as such did not create novel specificities that had no counterpart *in

vivo*. Quite contrary, the sequences of many of the allergen-specific scFv of the current study

suggest that the pairing could have developed in B-cells. A predominance of certain V_H-V_L

pairs, particularly involving different but highly related clones, suggests that the observed

combinations are associated with antigen binding *in vivo*. This was very apparent for

sequences belonging to clonotype 26 encoding Phl p 2 specificity. This common sequence

was only selected in combination with light chain sequences originating from the two highly

related germline genes IGKV1-5 and IGKV1-12 (Table II). Furthermore, all of the selected

light chains had undergone a rearrangement creating highly similar CDRL3 of the same

length, hence indicating that combinations very similar to the original pairing were formed in

these clones.

   Although phage display has been reported to bias the selection of promiscuous heavy

chains[33] and certain allergen-specific IgE V_H have been characterized as promiscuous,[16, 18, 34]

only one of the identified clonotypes in the current study was isolated with a number of

unrelated light chains (Table II, clonotype 35). This clonotype was found to dominate the Phl

p 5 response and it was also most commonly found among clones selected on Phl p 6.

Interestingly though, whereas the Phl p 5-specific paratope could be established from this

particular V_H sequence using a range of light chains with an origin in both λ and κ, only one

particular V_L created a Phl p 6-reactive binding site (Table II). Despite very similar (Figure

3), and sometimes even identical V_H, these clones had quite different binding behaviors. The

Phl p 5-selected clones did not cross-react with Phl p 6, whereas, as noted above, the opposite

was true; all the isolated Phl p 6-specific clones also recognized Phl p 5. These findings

demonstrate that the ability of this particular V_H to form a specific binding site for the smaller

allergen was much more limited and depended much more on the light chain than was its

ability to create a Phl p 5-specific binder. However, our investigations neither define which type of light chain that was found in combination with this particular V_H in the B-cell, nor define precisely which of these two allergens (or their homologues in other grass species) that initially activated its naïve B-cell precursor.

In conclusion, this investigation together with our previous study[3] has allowed us to connect the overall makeup of the IgE-encoding repertoire with the parts of the repertoire targeting a specific allergen. The results demonstrate that not only is the number of IgE-producing peripheral B-cells during active disease limited, the number of antigens recognized by the IgE repertoire is also highly restricted. These studies have allowed us to pinpoint, at a molecular level, the nature of antibodies with a role in allergy. This type of molecular characterization is a prerequisite for understanding the interplay between the allergen and the antibody response. The strategy further gives the opportunity to understand antibody repertoire development and restrictions as they occur in an individual. We foresee that this approach to characterize specific antibody repertoires will have an impact on allergen vaccine construction and standardization of allergen-specific IgE assays. Furthermore, the isolated antibodies may themselves, following transfer into an appropriate format (e.g. IgG1 or IgG4) be used for passive immunization.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. Serum IgE reactivity of a grass pollen allergic patient, who was utilized for the

construction of the combinatorial IgE library used in this study, to various grass pollen

allergens; recombinant and natural (as found in timothy extract, Phl p E).

Figure 2. Binding specificity of representative examples of selected scFv as determined by

ELISA on different recombinant timothy allergens and on the natural extracts Phl p E

(timothy) and Lol E (rye grass). The clones have preferred recognition of the substrate used in

the selection, except for the clones selected on Phl p 6 (here exemplified by clone p6-1) which

show cross-reactivity to Phl p 5.

Figure 3. Sequences of scFv selected on Phl p 6 (clone p6-1) and Phl p 5 (clones p5-9 and

p5-13), having essentially the same V_H (clonotype 35) but differing in the light chain. The

CDR of both chains are high-lighted, identical residues are shown with dots and gaps are

indicated by dashes.

Table I. Genetic origin of IgE V_H from donor A3, as found among randomly picked transcripts. Previously, based on sequence homology, the 63 randomly sequenced IgE V_H transcripts of donor A3 were grouped into 33 different clonotypes (1-33). As indicated in the specificity column, 7 of these clonotypes were identified among the grass pollen specific scFv. New clonotypes, not found among the random transcripts, were also identified among the grass pollen specific antibody fragments (clonotype 34-40).[a]

Clonotype	Number of identified members	Heavy chain gene origin	Specificity
1	2	IGHV1-2	
2	1	IGHV1-2	
3	1	IGHV1-2	
4	4	IGHV1-18	
5	1	IGHV1-18	Phl p 1
6	1	IGHV1-58	
7	2	IGHV1-69	
8	1	IGHV1-69	
9	2	IGHV3-7	
10	1	IGHV3-11	
11	1	IGHV3-21	
12	2	IGHV3-21	
13	2	IGHV3-23	
14	6	IGHV3-23	
15	1	IGHV3-23	
16	1	IGHV3-23	Phl p 5
17	1	IGHV3-23	
18	2	IGHV3-30	Phl p 11
19	3	IGHV3-30	
20	1	IGHV3-30	
21	1	IGHV3-30	
22	1	IGHV3-30	
23	1	IGHV3-43	
24	2	IGHV3-43	Phl p 4
25	1	IGHV3-53	Phl p 1
26	6	IGHV4-31	Phl p 2
27	2	IGHV4-31	
28	2	IGHV4-39	
29	4	IGHV5-a	Phl p 5
30	1	IGHV5-a	
31	1	IGHV5-51	
32	4	IGHV7-4	
33	1	IGHV7-4-1	
34	-	IGHV1-18	Phl p 5
35	-	IGHV3-11	Phl p 5/6[b]
36	-	IGHV3-11	Phl p 5/6[c]
37	-	IGHV3-21	Phl p 5/6[c]
38	-	IGHV3-23	Phl p 5
39	-	IGHV3-30	Phl p 11
40	-	IGHV4-39	Phl p 4

[a] An extended version of this table (Table E1) including IGHJ and IGHD gene origin, CDRH3 lengths and GenBank accession numbers is available in the Online Repository at www.jacionline.org.

[b] All members of this clonotype recognize Phl p 5 and some also recognize Phl p 6.

[c] Members of this clonotype recognize both Phl p 5 and Phl p 6.

Table II. Genetic origin of genes encoding the isolated grass pollen specific scFv.[a]

Clonotype	Specificity	Heavy chain gene origin	Light chain gene origin
5	Phl p 1	IGHV1-18	IGLV3-25
25	Phl p 1	IGHV3-53	IGKV2-28
26	Phl p 2	IGHV4-31	IGKV1-5
			IGKV1-12
24	Phl p 4	IGHV3-43	IGLV1-44
			IGLV1-47
40	Phl p 4	IGHV4-39	IGKV1-6
16	Phl p 5	IGHV3-23	IGLV3-1
29	Phl p 5	IGHV5-a	IGLV1-44
34	Phl p 5	IGHV1-18	IGLV1-44
38	Phl p 5	IGHV3-23	IGLV3-25
35	Phl p 5	IGHV3-11	IGKV1-39
	Phl p 5		IGLV1-44
	Phl p 5		IGLV2-8
	Phl p 5		IGLV2-11
	Phl p 5		IGLV2-14
	Phl p 5/6[b]		IGLV2-14
36	Phl p 5/6[b]	IGHV3-11	IGLV3-1
37	Phl p 5/6[b]	IGHV3-21	IGLV2-14
18	Phl p 11	IGHV3-30	IGLV2-14
39	Phl p 11	IGHV3-30	IGKV4-1

[a] An extended version of this table (Table E2) including GenBank accession numbers is
available in the Online Repository at www.jacionline.org.
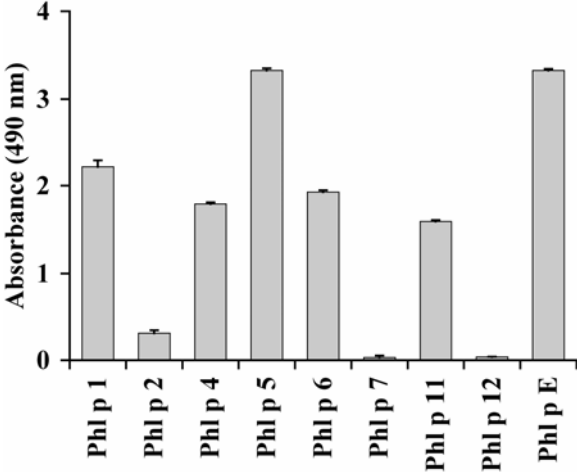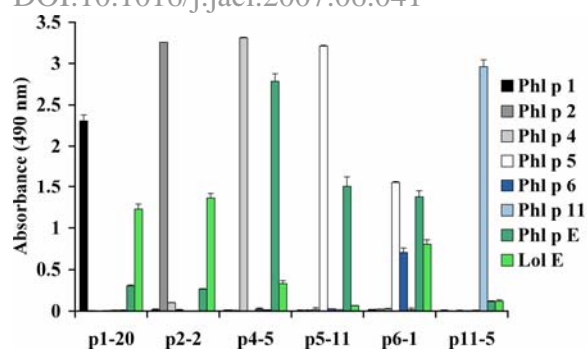[b] Clone recognizes both Phl p 5 and Phl p 6.

Figure 1

Figure 2

Heavy chain

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                                          CDR1                              CDR2
p6-1   E V Q L V Q S G G G L V K P G G S L R L S C A A S G F I F S D H Y M A W V R Q T P G K G L E W I S Y I S M T S S Y T N Y A
p5-9   . . . . . E . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
p5-13  Q . . . Q . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

                                                            CDR3
p6-1   D S V K G R F T I S R D N A K N S L S L E M N S L R V E D T A V Y Y C A R D R L R N W N D D A F D I W G P G T M V T V S
p5-9   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
p5-13  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
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Light chain

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                                            CDR1                            CDR2
p6-1   S Y E L T Q P A S - V S G S P G Q S I T I S C T G T P S D I G T Y P Y V S W Y Q Q H P D R A P K L L I Y G V T N R P S G V
p5-9   Q S A . . . . . . - . . . . . . . . . . . . . . . . S . . V . S . N L . . . . . . . G K . . . . M . . E G S K . . . . .
p5-13  D I Q M . . S P . S L . A . I . D R V . . T . R A S Q T I S S Y - - - L N . . . . K . G K . . E . . . H A A S T L Q . R .

                                              CDR3
p6-1   S N R F S G S K S G N T A S L T I S E L Q A E D E A D Y Y C S S Y T L T S I V V F G G G T K L T V L G
p5-9   . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . S . . . . . S I N T . . . . . . . . . . .
p5-13  P S . . . . . G . . T D F T . . . . S . . P . . F . T . . . Q Q S Y - S T P L T . . . . . . V E I K R
```

Figure 3

Online Repository

Delineating the specificity of an IgE-encoding transcriptome

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Table E1. Genetic origin of IgE V_H from donor A3, as found among randomly picked transcripts. Previously, based on sequence homology, the 63 randomly sequenced IgE V_H transcripts of donor A3 were grouped into 33 different clonotypes (1-33). As indicated in the specificity column, 7 of these clonotypes were identified among the grass pollen specific scFv. New clonotypes, not found among the random transcripts, were also identified among the grass pollen specific antibody fragments (clonotype 34-40).

| | | Heavy chain gene origin | | | | | |
Clonotype	Number of identified members	IGHV	IGHJ	IGHD	CDRH3 length	GenBank accession nos. of random V_H transcripts (DQ065XXX)	Specificity
1	2	1-2	4	1-26	13	683, 691	
2	1	1-2	4	4-23	13	697	
3	1	1-2	6	3-10	20	690	
4	4	1-18	4	3-22	14	675, 679, 688, 695	
5	1	1-18	4	1-26	7	722	Phl p 1
6	1	1-58	6	3-3	19	681	
7	2	1-69	4	2-8	12	677, 701	
8	1	1-69	4	6-19	12	705	
9	2	3-7	6	3-3	18	716, 724	
10	1	3-11	6	3-9	24	715	
11	1	3-21	4	2-2	9	704	
12	2	3-21	4	3-3	14	668, 694	
13	2	3-23	4	3-22	18	713, 723	
14	6	3-23	6	3-16	15	672, 678, 685, 693, 721, 728	
15	1	3-23	4	1-26	12	684	
16	1	3-23	4	2-15	16	707	Phl p 5
17	1	3-23	1	6-19	15	708	
18	2	3-30	4	3-22	18	718, 725	Phl p 11
19	3	3-30	4	3-22	16	674, 689, 709	
20	1	3-30	6	2-15	26	669	
21	1	3-30	2	3-9	17	696	
22	1	3-30	4	3-22	17	692	
23	1	3-43	4	3-9	20	726	
24	2	3-43	3	6-19	12	670, 719	Phl p 4
25	1	3-53	4	3-16	11	720	Phl p 1
26	6	4-31	4	3-10	10	666, 680, 686, 711, 712, 714	Phl p 2
27	2	4-31	4	6-19	14	700, 706	
28	2	4-39	4	3-10	10	676, 717	
29	4	5-a	4	2-15	20	702, 703, 710, 727	Phl p 5
30	1	5-a	3	6-19	14	698	
31	1	5-51	4	6-13	12	673	
32	4	7-4	4	3-22	15	667, 671, 682, 699,	
33	1	7-4-1	4	3-3	17	687	
34	-	1-18	6	3-22	23	-	Phl p 5
35	-	3-11	3	1-1	15	-	Phl p 5/6[a]
36	-	3-11	4	3-22	9	-	Phl p 5/6[b]
37	-	3-21	4	6-13	13	-	Phl p 5/6[b]
38	-	3-23	6	3-3	21	-	Phl p 5
39	-	3-30	4	3-22	16	-	Phl p 11
40	-	4-39	4	4-4	24	-	Phl p 4

[a] All members of this clonotype recognize Phl p 5 and some also recognize Phl p 6.
[b] Members of this clonotype recognize both Phl p 5 and Phl p 6.

Table E2. Genetic origin of genes encoding the isolated grass pollen specific scFv.

Clonotype	Specificity	Heavy chain gene origin	Light chain gene origin	GenBank accession nos.
5	Phl p 1	IGHV1-18	IGLV3-25	EF607095-6
25	Phl p 1	IGHV3-53	IGKV2-28	EF607094
26	Phl p 2	IGHV4-31	IGKV1-5	EF607100
			IGKV1-12	EF607097-9, EF607101-2
24	Phl p 4	IGHV3-43	IGLV1-44	EF607108-9
			IGLV1-47	EF607103-7
40	Phl p 4	IGHV4-39	IGKV1-6	EF607110
16	Phl p 5	IGHV3-23	IGLV3-1	EF601887[b]
29	Phl p 5	IGHV5-a	IGLV1-44	DQ065730[c]
34	Phl p 5	IGHV1-18	IGLV1-44	EF607117
38	Phl p 5	IGHV3-23	IGLV3-25	EF607113
35	Phl p 5	IGHV3-11	IGKV1-39	EF607116, DQ065729[c]
	Phl p 5		IGLV1-44	EF607118
	Phl p 5		IGLV2-8	EF607115
	Phl p 5		IGLV2-11	EF607119
	Phl p 5		IGLV2-14	EF607112, EF607114, DQ065733[c]
	Phl p 5/6[a]		IGLV2-14	EF607120
36	Phl p 5/6[a]	IGHV3-11	IGLV3-1	EF607122
37	Phl p 5/6[a]	IGHV3-21	IGLV2-14	EF607121, EF607123-4
18	Phl p 11	IGHV3-30	IGLV2-14	EF607125-6, EF607128
39	Phl p 11	IGHV3-30	IGKV4-1	EF607127

[a] Clone recognizes both Phl p 5 and Phl p 6.

[b] Clone isolated from the same library but in a separate study (J. Persson *et al.*, manuscript in preparation).

[c] Clone isolated from the same library but in a separate study.[E1]

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