



Internal images: Human anti-idiotypic Fab antibodies mimic the IgE epitopes of grass pollen allergen Phl p 5a

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Abstract

Background: The role of anti-idiotypic antibodies in allergic disease is still poorly understood. According to Jerne, anti-idiotypic antibodies to IgE should represent internal images of an allergen. Our aim was to ultimately prove whether this hypothesis holds true in allergy. Here, we describe the selection of anti-idiotypic antibodies against Phl p 5a-specific IgE directly from the B-cell repertoire of a grass pollen allergic individual.

Methods: Taking *Phleum pratense* grass pollen allergen Phl p 5a as a model, we selected anti-idiotypic antibodies against allergen-specific IgE directly from the B-cell repertoire of an allergic individual. We screened a combinatorial phage display library of human monovalent antibody heavy and light chain fragments (Fabs) with anti-Phl p 5a-IgE to identify and characterize Fabs with anti-idiotypic specificity.

Results: Five different Fab clones with anti-idiotypic specificity for anti-Phl p 5a-IgE were identified. Their hypervariable regions revealed partial sequence homology with solvent accessible antigenic sites of Phl p 5a, which have been identified by our previous mimotope approach. Phagemid DNA derived from the phage clones was used to produce two soluble recombinant anti-idiotypic Fab clones in *E. coli*. As a proof of molecular mimicry, both Fabs induced anti-Phl p 5a-specific antibodies in immunized BALB/c mice. Molecular modeling of the heavy and light chain hypervariable loops of the anti-idiotypic Fabs illustrated structural similarity with dominant IgE epitopes of Phl p 5a.

Conclusion: In this straightforward phage technology approach, antibodies with anti-idiotypic specificities could be isolated from a human allergic's repertoire. As predicted by the immune network hypothesis, their hypervariable domains mimic IgE epitopes like internal images and, more importantly, induce allergen-specific immune responses in the absence of the allergen.

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1. Introduction

In his immune network theory, Jerne (1974) proposed that the immune system responds to a given antigen besides the production of antibodies (Ab1) with the appearance of anti-idiotypic antibodies (anti-Ids) presenting “internal images” of

the original antigen (Ab2). Moreover, upon immunizations with anti-Ids, anti-anti-Ids may be generated (Ab3), again directed towards the initial antigen and possibly potentiating protective immune responses (Nisonoff and Lamoyi, 1981; Roitt et al., 1981). Since then, several models of immune networks have been proposed, and it has been shown experimentally that immunologic memory can be maintained by idiotypic network interaction (Weisbuch et al., 1990).

Also in the field of allergy, the relevance of anti-Ids to allergen-specific human IgE has been demonstrated early by in vitro downregulation of specific IgE using “anti-IgE Id” purified from human sera (Malley and Dresser, 1982, 1983). Moreover, the induction of natural anti-Ids was observed in patients

Abbreviations: anti-Id, anti-idiotypic antibody; IgE, immunoglobulin E; Phl p 5a, *Phleum pratense* allergen 5a; WT, wild-type

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URL: <http://www.allergology.at>.

successfully treated with allergen immunotherapy (Tamir and Pick, 1988) and may explain the inhibition of allergen–IgE interaction after grass pollen immunotherapy (Simon, 2004; Wachholz et al., 2003). It can be speculated that anti-Ids directed against IgE B-cell receptor could regulate allergen-specific B-lymphocytes as the degree of crosslinking may be decisive for their activation (Schöll et al., 2005) or, on the other hand, apoptosis (Mouhamad et al., 2004). The involvement of anti-Ids in basophil mediator release has not been clarified so far, probably due to methodological difficulties when using purified antibodies (Weyer et al., 1995; Wheeler et al., 1990). To circumvent this chimeric monoclonals Ab1 and Ab2 were created recently from murine templates, representing surrogates for investigating the idiotype–anti-idiotype interaction between Der p 1-specific IgE and its Ab2 on a molecular basis (Furtado et al., 2000, 2002).

In this study we aimed to generate anti-Ids by selecting them directly from a human antibody repertoire with allergen-specific IgE. The library used was derived from a grass pollen sensitized individual (Vogel et al., 1994). The timothy grass allergen Phl p 5 was considered a good model as it is an important and highly crossreactive major allergen. It binds IgE with outstanding affinity, being likely a reason for the great clinical significance of this allergen (Hantusch et al., 2005). In a previous study this journal we reported the localization of the IgE epitopes of isoallergen Phl p 5a by means of mimotopes (epitope-mimics) selected from a phage display library (Hantusch et al., 2004). In this study, we investigated whether anti-Ids could truly mimic these IgE epitopes like internal images.

2. Methods

2.1. IgE antibodies

Human polyclonal IgE specific for Phl p 5a and Cyp c 1 (carp parvalbumin) were purified from sera of grass pollen or fish allergic patients by affinity chromatography on recombinant allergens (Biomay, Vienna, Austria) as described previously (Hantusch et al., 2005). Human myeloma IgE PS (a kind gift from Drs. T. Ishizaka and K. Ishizaka, La Jolla, CA) and human monoclonal IgE SUS11 (Zurcher et al., 1995) were used for control. IgE amounts were determined with the UniCAP System (Pharmacia, Uppsala, Sweden).

2.2. Panning of human immune Fab library with anti-Phl p 5a-IgE

The combinatorial Fab-library “LB” was used for screening with Phl p 5a-specific IgE. In this library, IgG heavy and light chain PCR products derived from mRNA of PBMCs of a grass pollen allergic donor have been inserted into the *XhoI/SpeI*- and *SacI/XbaI*-sites of phagemid vector pComb3 (Barbas et al., 1991; Vogel et al., 1994). Three rounds of biopanning were performed essentially as described before (Leitner et al., 1998). Briefly, anti-Phl p 5a-IgE was coated at a concentration of 0.05 µg/ml on microtiter plates (Maxisorp, Nunc, Roskilde, Denmark). After blocking and washing, wells were incubated with 20 µl library LB (1.08×10^{10} cfu) diluted in 280 µl PBS,

2.5% BSA for 2 h at room temperature. Wells were washed 10 times with PBS, 0.1% Tween-20, and 10 times with PBS. Bound phages were eluted with acidic buffer (0.1 M glycine, pH 2.2) or competitively, using 10 µg/ml recombinant Phl p 5a in PBS, 2.5% BSA. Eluted phages were used for infection of logarithmic *E. coli* XL-1Blue under coinfection with 10^{12} pfu of helper phage VCSM13 (Stratagene, La Jolla, CA) and were precipitated from supernatants with 2.5% polyethylene glycol 8000, 0.4 M NaCl.

2.3. Characterization of IgE-reactive Fab phage clones

After the third panning round, 100 colonies of each elution method were randomly chosen and grown in 250 µl SB-medium in microtiter plates overnight. Phage containing supernatants were used directly for specificity ELISA on plates coated with Phl p 5a-IgE (0.005 µg/ml in NaHCO₃-buffer, pH 9.6). After washing and blocking phage supernatants were added in duplicates to wells at 4 °C overnight. Bound phages were detected with HRP-labeled mouse anti-M13 mAb (Amersham Pharmacia, Uppsala, Sweden). Washing and developing was done as described before (Myers et al., 2000). Phages giving OD values above the mean + 3S.D. of the background control were considered to be specifically reactive with capturing IgE. Specificity ELISAs of reactive phage clones compared to WT phage were essentially performed as described above, adjusting phage titers to 2×10^{11} cfu/ml and testing on Phl p 5a-specific IgE or control IgEs PS- and SUS11 in serial dilutions.

2.4. Fab sequence analysis and alignments with Phl p 5a

Phagemid double-stranded DNA was purified from bacterial cells (Biorad, Hercules, CA) and subjected to sequencing on an automated LI-COR 4000L DNA sequencer (LI-COR, Lincoln, NE), applying the Sanger dideoxy nucleotide method (SequiTherm ExcelII DNA Sequencing Kit-LC, Epicentre, Madison, WI). Primers Hfwd: 5'-ggattgttattactcgctgccc-3' and CHγ1: 5'-cgctgtgccccagaggt-3' were used for the heavy chains, and Lfwd: 5'-gtggaattgtgagcggataac-3', Cλ: 5'-gagacacaccagtgtggc-3' and Cκ: 5'-cacaacagaggcagttcc-3' (MWG-biotech, Ebersberg, Germany) for the light chains. Comparison of derived amino acid sequences with germline sequences was performed with the IgBLAST database of NCBI. Sequences for the heavy and light chains were deposited in GenBank. Sequence-comparisons of the CDR regions with Phl p 5a (GI: 1171011) were done by the alignment algorithm of Vector NTI Suite (InforMax Inc., Frederick, MD), which uses a modified Clustal W algorithm to create multiple sequence alignments (Thompson et al., 1994).

2.5. Purification of soluble Fab fragments from *E. coli* periplasm

pComb3 phagemid DNA was digested with *SpeI* and *NheI* to remove the phage gene III sequence (Barbas et al., 1991). The DNA was religated and transformed into competent XL-1 *E. coli* cells. Positive transformants were grown in SB containing

50 µg/ml carbenicillin until OD₅₀₀ = 0.2. IPTG was added to 1 mM final concentration and induction continued at 37 °C for 3 h. Bacterial pellets were resuspended with Tris–buffer (20 mM Tris, pH 8.0, 1 mM EDTA), 0.1 µg/ml lysozyme and were French-pressed, applying 1000–1500 psi. The lysates were centrifuged, supernatants were diluted 1:10 with PBS and transferred directly onto an anti-Fab antibody coupled (Accurate Chemical, Westbury, NY) CNBr-Sepharose affinity column (Amersham-Pharmacia, Uppsala, Sweden). Bound Fabs were eluted with 3 M KSCN, pH 7 and tested in Western blotting using a HRP-conjugated goat anti-human F(ab)₂ antibody (Accurate Chemical, Westbury, NY, USA).

2.6. Mouse immunizations

Mouse studies were performed according to European Community Rules of Animal Care (Waldegrave, 1986) with permission 66009/117-Pr/4/00 of the Austrian Ministry of Science. Three-month-old BALB/c mice (Charles River, Sulzfeld, Germany) (*n* = 5 per group) were immunized intraperitoneally with rPhl p 5a (1 µg/100 µl 0.9% NaCl per dose) and with affinity-purified Fab fragments C29 and E39 (0.2 µg/100 µl per dose) adsorbed to 50 µl Al(OH)₃ on days 0, 14 and 28. Blood samples were taken from the tail vein on days 7, 21 and 35. Antibody titers against Phl p 5a, Bet v 1 (Biomay) and Der p 1 (a kind gift by Prof. Jean-Marie Saint-Remy, University of Leuven, Belgium) were determined in ELISA (Schöll et al., 2002). Statistical significance of the results was calculated by Student's *t*-test. Differences of *p* < 0.05 were considered significant.

2.7. Molecular modeling of variable Fab heavy and light chains

The Swiss-PdbViewer program suite (Guex and Peitsch, 1997) and TINKER molecular modeling package (<http://www.dasher.wustl.edu/tinker/>) were used for comparative modeling of C29 and E39 Fab heterodimers. Homologous molecules deposited in the Brookhaven Protein Data Bank (Bernstein et al., 1977) were aligned with C29 and E39 and submitted to ProModII for model building. The models were structurally aligned with the CAα backbone of a Fab heterodimer model origin to obtain an approximate contact

interface and examined for unfavorable van der Waals overlaps and side chain intersections, which were removed by minimal rotation around side chain torsion angles. Resulting models were converted to XYZ format using the TINKER implemented OPLSAA force field parameter set. An energy minimization was performed to a RMS of 10 using the MINIMIZE function of TINKER. Then, a short rigid body molecular dynamics was applied to the models to relax the interface side chains, followed by a further energy minimization to a RMS gradient of 1. All calculations were done in vacuo. The quality of the models was evaluated manually and with a variety of computational techniques in the WHAT.CHECK validation package (Hooft et al., 1996). The C-terminal half of Phl p 5a was modeled as described previously (Hantusch et al., 2004) based on the crystallographic structure of Phl p 5b (Rajashankar et al., 2002).

3. Results

3.1. Selection of human Fab Abs against Phl p 5a-specific IgE

Affinity-purified human polyclonal Phl p 5a-specific IgE was used to select ligands from a combinatorial M13 phage library displaying Fab fragments at the N-terminus of surface protein pIII (Leitner et al., 1998). After three rounds of panning, a 300-fold enrichment of Phl p 5a-IgE specific phage titers was observed when ligands were eluted by low pH, while with the competitive method an even 8000-fold enrichment was observed (Table 1). Biopannings with parvalbumin-specific IgE did not achieve phage selections from this library obviously due to its bias towards grass pollen specificity (data not shown).

3.2. Phage clones display anti-idiotypic Fabs

Ten highly reactive phage clones from both elution methods of the third selection round were analyzed further in terms of specificity and binding strength towards IgE in capturing ELISA. Five of them (A23, B36, C29, D32, E39) reacted strongly with Phl p 5a-IgE and less or not with the control IgEs. Three of the clones derived from the acidic (A23, B36, E39), and two from

Table 1
Enrichment of Fab-clones from the LB-library in three panning rounds

Elution method	Round	Phage input ^a (cfu/ml)	Phage output ^b (cfu/ml)	Ratio (output/input)	Phage enrichment ^c
Acidic	1	1.0 × 10 ¹¹	4.0 × 10 ⁵	4.0 × 10 ⁻⁶	
	2	1.5 × 10 ¹⁰	2.0 × 10 ⁵	1.3 × 10 ⁻⁵	3
	3	5.0 × 10 ⁹	7.0 × 10 ⁶	1.4 × 10 ⁻³	108 324
Competitive	1	1.0 × 10 ¹¹	3.0 × 10 ⁴	3.0 × 10 ⁻⁷	
	2	5.0 × 10 ⁹	1.0 × 10 ⁵	2.0 × 10 ⁻⁵	67
	3	2.0 × 10 ⁹	5.0 × 10 ⁶	2.5 × 10 ⁻³	125 8375

^a Phage input means the titer of the phage amplificate applied in the respective panning round.

^b Phage output denominates the titer of the phage eluate of the respective panning round.

^c The phage enrichment is calculated as the multiplier of the output/input ratio from one round to the next.

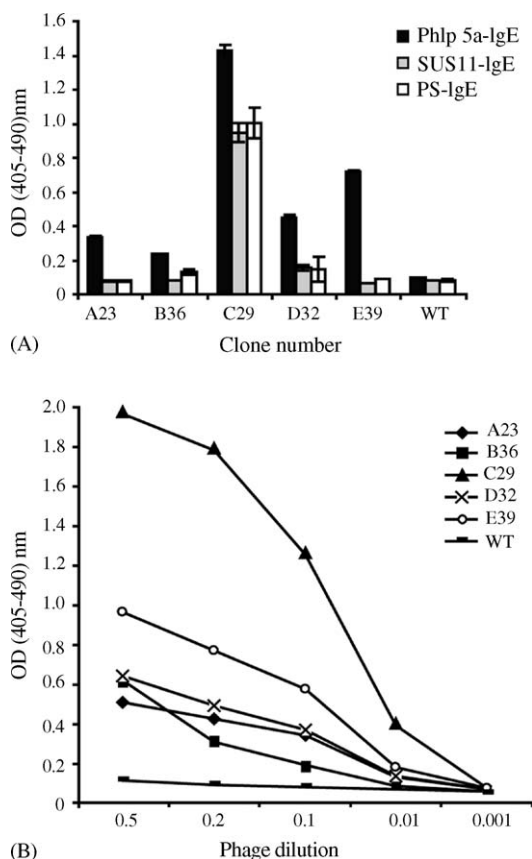


Fig. 1. Fab clones bind specifically to Phl p 5a-specific IgE, but not control IgE. (A) Individual phage clones (2×10^{11} cfu/ml) were added to wells coated with Phl p 5a-specific IgE or with the control isotypes SUS11- and PS-IgE. Bound phages were detected with a HRP-labeled mouse monoclonal anti-M13 Ab. (B) Dose-dependent titration of phage clones. ELISA plates coated with Phl p 5a-specific IgE were incubated with serial dilutions of the five phage clones starting at an equipotent titer of 2×10^{11} cfu/ml of all clones.

the competitive elution method (C29, D32). Among them, C29 and E39 showed the highest binding capacity, and clone E39 the highest specificity for Phl p 5a-IgE in comparison with WT phage (Fig. 1).

3.3. Sequence analysis of anti-idiotypic Fabs

Phagemid DNA of the five Phl p 5a-IgE specific phage clones was sequenced and the Fab amino acids and nearest germline sequences were determined (Table 2). The Fab sequences have been deposited in GenBank under accession numbers AY950789 until AY950798. For the heavy chain genes, usage of the large VH3 and VH4 gene families was observed. Four clones used the VH4-39 gene, with mutations accumulating in FR1, CDR2 and FR3. The fifth clone (C29) exhibited a VH3-53 sequence with few mutations in FR1, CDR2 and FR3. Concerning the light chain genes, four clones (A23, C29, D32, E39) displayed λ -chains, whereas one clone (B36) possessed a κ -chain. Three clones (A23, D32, E39) had a V λ 1 gene, but derived from different germplines, namely 1–19 α and 1–17 λ . Again, clone C29 diverged from the other four clones by usage of a V λ 3 gene.

3.4. Alignments of Fab CDRs with the primary sequence of Phl p 5a

Fab CDR sequences were compared separately with the linear sequence of Phl p 5a by using a modified Clustal W algorithm. CDR residues aligned to, or were in close vicinity of the IgE epitopes of Phl p 5a previously identified by our mimotope approach (Fig. 2) (Hantusch et al., 2004). High score alignments could mainly be detected in the C-terminal half of Phl p 5a, preferentially with the IgE epitope 3 and with the alanine-rich C-terminus of the molecule containing IgE epitope 4. Matches were preferentially found within the CDR2 and CDR1 or CDR3 regions of the heavy and light chains, respectively. No similarities were observed with CDR2 of light chains. VH-CDR3 of Fab C29 revealed 43% identity and 21% similarity with aa 201–214 of Phl p 5a. VL-CDR3 of Fab E39 showed 33% identity versus 44% similarity with Phl p 5a aa 193–201. In the same region, VH-CDR2 of Fab A23 matched with 38% identity and 38% similarity. An additional alignment spot with the CDRs of the heavy and light chains of B36 was detected in a loop region of Phl p 5a comprising aa 222–235.

3.5. Recombinant production of anti-idiotypic Fabs C29 and E39

Because of their high-score homologies with an epitopic region of Phl p 5a and because they resembled the two Fabs most distantly related concerning their VH and VL genes, clones C29 and E39 were chosen for further analysis. The purification of Fab fragments from phagemid libraries using the pComb3 vector, which allows the export of recombinant fragments to the periplasm has been described in several reports (Barbas et al., 1991; Siegel and Silberstein, 1994). The integrity of Fab fragments in the periplasmic and affinity purified fractions were determined by SDS-PAGE and Western blot (Fig. 3). The production yields of Fabs varied between 15 and 20 μ g Fab/l of culture.

3.6. Fabs C29 and E39 induce Phl p 5a-specific Ab responses

Fabs C29 and E39 were tested for their ability to induce anti-Phl p 5a antibodies (Ab3). BALB/c mice immunized with Fabs C29 and E39 formed significant levels of IgG that bound specifically to Phl p 5a, but not to the unrelated control allergens Bet v 1 and Der p 1 (Fig. 4). Compared to immunizations with rPhl p 5a, the response was relatively weaker, but equally specific. As expected from the initial experiments, highest Ab titers were achieved with clone E39, which obviously had the best specificity and mimicry potential.

3.7. Fab clones C29 and E39 image IgE epitopes of Phl p 5a

To visualize the anti-idiotypic interface responsible for the observed biological mimicry, we applied computational molecular modeling of anti-Ids C29 and E39. These structural models

Table 2

Deduced amino acid sequences of V_H and V_L genes of phage clones selected with Phl p 5a-specific IgE from the LB Fab library

Heavy Chains							
Clone	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
VH-39	QLQLQESGPGLVKPKSETLSLTCTVSGGSIS	SSSYWG	WIRQPPGKLEWIG	SIYSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSVTAADTAVYYCAR	QADNWFD	WGQGLTVTVSS
A23	-VK-L-----K-----L	-G-----	-----	N--N-N--S--	-----N-----M-----	-----	-----
B36	-VK-L-----K-----D---	-TG-----	-----	-V--N-N--Q--	-L-M-A-----L--S-Q-----	STYWGSQDFDN	-----A-----
D32	-VK-L---QR-----S--A--R	--D--A	-----M--	---D---H-DSW-RN	-L---L---Q-HM--T-T-----	-----	-----
E39	-VK-L---R-----K---K-F--A--R	--D--A	-----	---D---H-DS--RN	-L---L---Q-HI--T-T-----	-----	-----
VH-53	EVQLVESGGGLIQPGGSLRL-SCAASGFTVS	SNYMS	WVRQAPGKLEWVS	VIYSGSTYYADSVKG	RFTISRDNSKNTLYQMNSLRAEDTAVYYCAR	DGGTGRWESWFD	WGQGLTVTVSS
C29	Q--L--V-----L-----LS--	---N	-----	AL---N-----D	-----SF---P-----P-----	-----	-----

Light Chains

Clone	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
V1-19 λ	VLTQPPSVSAAPGQKVTISC	SGSSSNIGNNYVS	WYQQLPGTAPKLLIY	DNNKRPS	GIPDRFSGSGSGTSATLGITGLQTGEADYYC	GTWDSLSLAG	FGGGLTKLTVL
A23	-V-----	-----S---	-----E-----	-D-----	-----G-----A-----	-S--G--N-WV	-----V---
O2 x	QMIQSPSSLSASVGDRTITC	RASQSISSYL	WYQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGIDFTLTITSLQPEDFATYYC	QQSYSSRP	FGGGLTKVEIK
B36	V-T-----	-----	-----	-----	-----T-----	-----LT	-----L-R-
V3-2 λ	QTVVTQEPSTLVSPGGTIVLTC	ASSTGAVTSGYYPN	WFQKPGQAPRALIY	STSNKHS	WTPARFSGSLGGKAALTLGSGVQPEDEAEYYC	LLYYGGAQ	FGGGLTKLTVL
C29	..-L-----V-----	-----T--	-----	-A-T---	-----D-----T---	-----WV	-----
V1-17 λ	QSVLTQPPSVASGTPGQRTVISC	SGSSSNIGSNVY	WYQQLPGTAPKLLIY	SNNQRPS	GVPRFSGSGSGTSASLAIISGLRSEDEADYYC	AAWDDSLSGP	FGGGLTKLTVL
D32/E39	..-V-----	-----	-----	R-----	-----	-----WV	-----

FR, framework region; CDR, complementary determining region. The most upper sequences in bold are given for comparison and represent the deduced amino acid sequence of the closest published germline gene sequence. Dashes indicate identities with germline gene sequence. Heavy chains: the first three amino acids (QVQ) are determined by the vector sequence of pComb3. Sequence data are available from GenBank under accession numbers AY950789, AY950791, AY950793, AY950795, AY950797 for the heavy chains, and AY950790, AY950792, AY950794, AY950796, AY950798 for the light chains, respectively.

allowed a direct comparison of the anti-Ids C29 and E39 with the Phl p 5a-IgE epitopes (Fig. 5). Regions of homology between anti-Id and allergen are given in yellow, blue and red. The linear alignments of C29 with Phl p 5a (Fig. 2) had indicated that VH-CDR3 exhibited a high homology with IgE epitope 3 of the allergen, and VL-CDR1 with IgE epitope 4. The models in Fig. 5A show that these two regions are located in the binding groove of C29 (Fig. 5A). For E39, VH-CDR2 and VL-CDR3, which had aligned with IgE epitope 3 of Phl p 5a, form one

closed patch located in the central part of the binding groove (Fig. 5B).

4. Discussion

Only recently, murine antibodies with Der p 1 specificity were translated into chimeric variants, representing excellent tools for analyzing the molecular interface between Ab1 and Ab 2 at a molecular level (Furtado et al., 2002). However, the mimicry



Fig. 2. Homology between CDRs of Fabs and Phl p 5a. A linear homology alignment of isolated CDRs of several Fab clones with the amino acid sequence of Phl p 5a, using Vector NTI Suite, is shown. Previously identified IgE epitopes of Phl p 5a are underlined. Fab clone numbers refer to Table 2 and are indicated at the left margin in the order of their appearance. The respective CDRs of the Fabs are listed at the right margin. Residues present in the CDRs that are identical with amino acids of Phl p 5a are indicated in bold, physicochemically related amino acids are drawn in bold-italics. GenBank accession number of Phl p 5a—GI: 1171011.

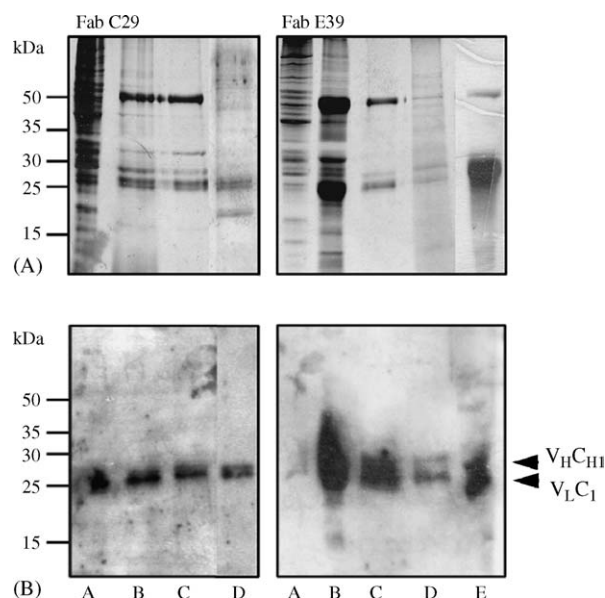


Fig. 3. Analysis of affinity purified recombinant Fab fragments C29 and E39. (A) SDS-PAGE analysis of Fab purified from *E. coli* periplasm by affinity chromatography. Proteins were analyzed on a 12% gel under reducing conditions and silver stained. (B) Examination of the integrity of produced Fab fragments on Western blot using an anti-human (Fab)₂-specific antibody. Lane A, French press lysates of *E. coli*; lane B, column load before Fab-elution; lane C, column load after elution of Fab for control of elution efficiency; lane D, purified Fab protein; lane E, control IgG for size comparisons of Fab fragments.

potential of a natural human anti-IgE Id with the original antigen has so far not been analyzed. In this straightforward approach, we applied phage libraries displaying Fab antibody fragments from the B-cell repertoire of a grass pollen allergic patient (Vogel et al., 2000) for retrieving anti-Id specificities against allergen-specific IgE.

Although screening of the phage library was performed with polyclonal IgE, only five clones with anti-Id specificity were selected, possibly indicating a dominant idiotope on Phl p 5a-IgE (Ab1). A sequence analysis of the V_H and V_L genes of our clones revealed favorable usage of the VH4-39 gene in four out of five clones, with differences between the clones through several mutations mainly located in CDR2 and FR3. Interestingly, for anti-Ids no preferential usage of immunoglobulin gene families has been described before (Cheng et al., 1988).

As the antigen-mimicking sites have been localized in the hypervariable regions of anti-Ids in other studies (Laune et al., 2000; Pride et al., 1992; Spendlove et al., 2000), a homology alignment of our Fab CDRs with the Phl p 5a sequence was done. Significant similarities of several CDRs with previously identified IgE epitopes 3 and 4 of Phl p 5a (Hantusch et al., 2004) were seen. They may thus provide the motifs for mimicry (Pride et al., 1992; Williams et al., 1988), although antigenic mimicry can also occur independent from sequence homologies (Braden et al., 1996).

According to Jerne, and as a proof of principle the identified anti-Ids should also immunologically mimic the original allergen Phl p 5a. The anti-idiotypic Fabs with the highest (C29) and most specific (E39) binding capacity towards Phl p 5a-IgE were chosen for mouse immunizations, and did indeed both elicit Phl

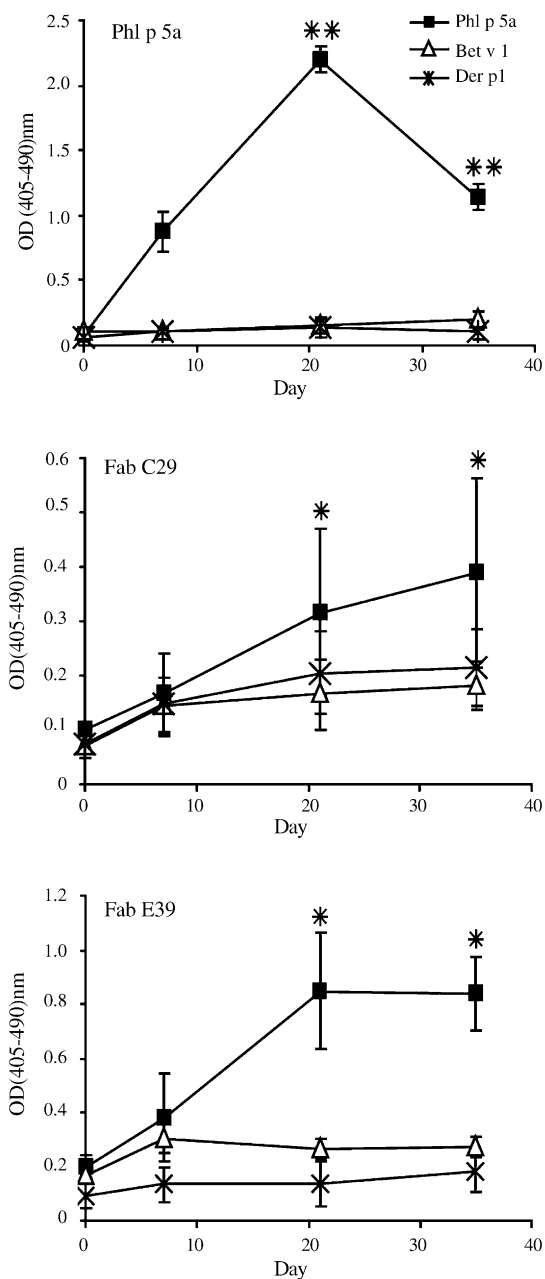


Fig. 4. Fab immunization leads to production of Phl p 5a-specific IgG antibodies in mice. IgG-reactivity of BALB/c mice immune sera elicited by Phl p 5a, Fabs C29 and E39 were tested against Phl p 5a and control antigens Bet v 1 and Der p 1. The X-axis shows the time-schedule of blood taking. Results are expressed on the Y-axis as OD (405–490) nm of mean IgG reactivities and standard deviations of mouse groups ($n = 5$). Mouse sera were tested individually at a 1:100 dilution on Phl p 5a and on control antigens Bet v 1 and Der p 1. ** p -Value < 0.01 of Phl p 5a and * p -value < 0.05 of Fab C29 and Fab E39 immune sera from days 21 and 35 compared to the respective signals obtained on the control antigens.

p 5a-specific antibody responses (Ab3). C29 induced, however, a relatively weaker antibody response, maybe due to the relatively lower density of homology regions across its Fab binding groove. Importantly, these experiments immunologically proved the principle of molecular mimicry of a human IgE antibody with an extrinsic allergen, and demonstrated that Fab E39 was the best anti-idiotypic candidate.

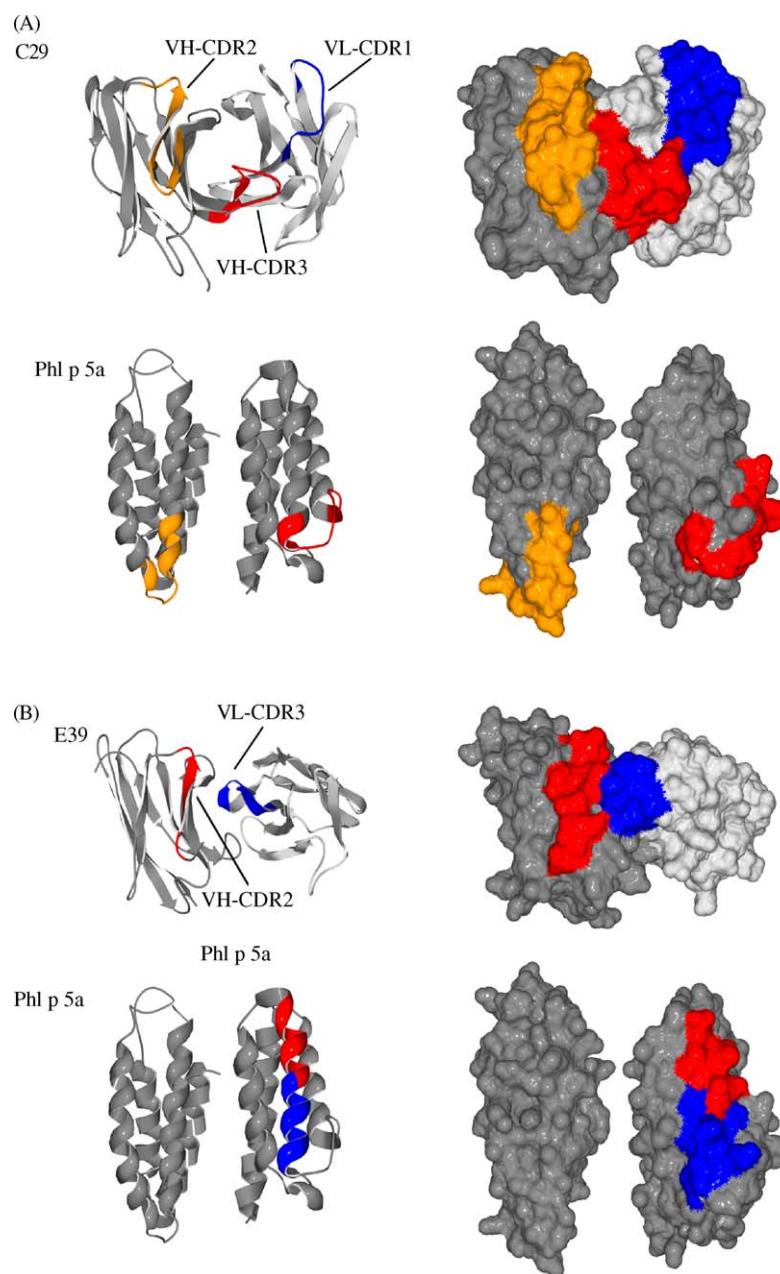


Fig. 5. Molecular models of Fabs C29 (A) and E39 (B), each shown together with models of Phl p 5a AR repeats 1 and 2. Ribbon models are shown on the left, and the Conolly surfaces on the right. The backbone structures of the Fabs are represented in dark and light grey for the heavy and light chains, respectively. Amino acids of CDRs exhibiting similarities with Phl p 5a are indicated in red and orange on the heavy chains versus blue on the light chains. The corresponding homologous sites on Phl p 5a are indicated in the same colors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Several conclusions can be drawn from the existence of anti-Ids in the repertoire of an allergic individual. Firstly, the anti-idiotypic Fabs generated in this work are true internal images of the IgE epitopes of Phl p 5a, just as predicted by Jerne's network theory. Second, their presence could maintain B-cell memory during the off-season of grass pollen dissemination. And third, the anti-Ids against allergen-specific IgE could serve as tools for epitope-specific immunotherapy of type I allergy, because the risk of crosslinking IgE on effector cells in a sensitized individual would be greatly reduced with the monovalent anti-idiotypic Fab fragments (Ganglberger et al., 2001; Schöll et al., 2005).

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