Anti-idiotypic Fab Fragments Image a Conserved N-terminal Epitope Patch of Grass Pollen Allergen Phl p 1

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Abstract:

Background and Aims: Naturally occurring anti-idiotypic antibodies structurally mimic the original antibody epitope. Anti-idiotypes, therefore, are interesting tools for the portrayal of conformational B-cell epitopes of allergens. In this study we used this strategy particularly for major timothy grass pollen (*Phleum pratense)* allergen Phl p 1.

Methods and Results: We used a combinatorial phage display library constructed from the peripheral IgG repertoire of a grass pollen allergic patient which was supposed to contain anti-idiotypic Fab specificities. Using purified anti-Phl p 1 IgG for biopanning, several Fab displaying phage clones could be isolated. 100 amplified colonies were screened for their binding capacity to anti-Phl p 1-specific antibodies, finally resulting in four distinct Fab clones according to sequence analysis. Interestingly, heavy chains of all clones derived from the same germ line sequence and showed high homology in their CDRs. Projecting their sequence information on the surface of the natural allergen Phl p 1 (PDB ID: 1N10) indicated matches on the N-terminal domain of the homo-dimeric allergen, including the bridging region between the two monomers. The resulting epitope patches were formed by spatially distant sections of the primary allergen sequence.

Conclusion: In this study we report that anti-idiotypic specificities towards anti-Phl p 1 IgG, selected from a Fab library of a grass pollen allergic patient, mimic a conformational epitope patch being distinct from a previously reported IgE epitope area.

Keywords: Grass pollen, allergy, B-cell epitope, Phl p 1, mimotope.

INTRODUCTION

In the early 1970ies N.K. Jerne proposed in his network hypothesis that, additionally to antibodies (Ab1) against an antigen, also anti-idiotypic antibodies (Ab2) against the paratope of Ab1 are induced [1]. Later it was also proven that these antibodies are part of the normal immune response [2]. The antigen binding sites of anti-idiotypic antibodies portrays the antigen epitope recognized by Ab1, and additionally may even mimic functional characteristics of the original antigen like enzymatic activity [3]. Furthermore, they seem to play an important anti-inflammatory role in the therapy of autoimmune diseases with intravenous immunoglobulins (IVIg) [4]. Moreover, they may modulate the results of passive anti-cancer immunotherapy based on monoclonal antibodies [5].

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In allergy, anti-idiotypic antibodies may adapt opposing functions by enhancing or inhibiting the IgE-mediated response [6]. Anti-idiotypic specificities may bind to B-cell receptors and counter-regulate B-lymphocyte function [7], or to FcεRI-bound IgE and thereby enhance [8] or inhibit the effector phase of type I allergy, and, interestingly, anti-idiotypic specificities may be directed against the T-cell receptors of allergen-specific T-lymphocytes [9].

Phleum pratense (P. pratense), also called timothy grass, belongs to the Poaceae family and is one of the most prevalent hypersensitizers in Europe. 14.7% of the population included in the first European Community Respiratory Health Survey (ECRHS-I) were found to have positive skin prick test (SPT) to this pollen species [10]. Epidemiologically, *P. pratense* major allergen Phl p 1 is of special importance, as 85% of timothy grass pollen allergic patients show IgE reactivity to this protein [11].

Although there are 13 allergenic proteins in *P. pratense* [12], major allergens Phl p 1 and Phl p 5 are of special im-

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portance as they are responsible for a majority of IgE reactivities in grass pollen allergic patients [13]. Phl p 1 is a glycoprotein of around 30 kDa and is, like all members of group I grass pollen allergens, a β-expansin. It is composed of two stable domains containing anti-parallel β-sheets [14]. This class of protein has cell wall loosening activity and is mainly found in mature pollen. Its main function lies in the separation of pollen grains and in germination [15]. The expansins show a high level of clinical cross reactivity among class I allergens from Poaceae [16,17]. This cross reactivity is based on high structural homology (~90% for Poaceae) especially in their N-terminal domains [18]. Phl p 1 induces IL-6 and IL-8 expression in epithelial cells (without acting on the mucosal barrier by degrading tight junctions) which may be an explanation for its sensitizing capacity [19].

Selection of mimotopes from phage display libraries is a powerful tool for identification of antigen epitopes. The mimotopes obtained by biopanning are actually structural mimics of B-cell epitopes [20-25]. As they do not necessarily possess primary sequence homology to the allergen, they lack the corresponding allergen-specific T-cell epitopes [26,27]. Nevertheless, mimotopes are able to induce immune responses, which can be modulated by the choice of adjuvants and application route [28]. Instead of using random peptide phage libraries, one may also apply combinatorial phage libraries displaying antibody fragments. Vogel et al. [29] have constructed such a library from the peripheral IgG repertoire of a grass pollen allergic patient. In a previous approach, we succeeded to extract anti-idiotypic Fab-mimotopes from this library, which enabled us to study the IgE epitopes of major grass pollen allergen Phl p 5 [30]. To enhance our understanding of allergenic B-cell epitopes, here we aimed to study the equally important grass pollen allergen Phl p 1 using the same approach.

MATERIALS AND METHODS

Purification of Phl p 1-specific Antibodies

Phl p 1-specific polyclonal antibodies were purified from 4 grass pollen allergic patients' sera by affinity chromatography. After coupling recombinant Phl p 1 (rPhl p 1, Biomay, Vienna, Austria) to CNBr-activated sepharose 4B (GE Healthcare, Uppsala, Sweden) and intensive washing with phosphor buffered saline (PBS), the serum pool was diluted 1:10 in PBS, loaded onto the column, followed by a washing step with PBS. Bound antibodies were eluted by pH shift (0.1 M glycine/150 mM NaCl/50 mM $MgCl_2$; pH 2.2), and increasing salt concentration (3 M KSCN; pH 7). Antibody eluates were immediately adjusted to pH 7.0 and concentrated by centrifugation at 3000 g using a centrifugal filter with a cut-off of 100 kDa (Amicon Ultra- 15, Millipore, Carrigtwohill, Ireland). To remove concomitantly eluted IgE antibodies, a HITrap™ Protein G HP column (GE Healthcare) was used. The purity of the eluted antibodies was tested in immunoblot on *P. pratense* pollen extract. The pollen extract was separated by electrophoresis according to Laemmli [31] using 12% separation and 8% stacking gel. Thereafter protein was electroblotted onto nitrocellulose membrane (Protran®, Whatman Inc, Florham Park, NJ, USA). Membranes were blocked using Tris-buffered saline (TBS) containing 0.1% Tween-20 and 5% dried milk powder (DMP) over night (ON) at 4°C. Purified antibody was diluted 1:30, as a control the serum pool was diluted 1:50 for IgE binding, and 1: 500 for IgG detection using the same blocking buffer but with a reduced DMP concentration of 0.5% and incubated ON at 4°C. For detection of bound antibodies goat anti-human IgG labeled with horse radish peroxidase (HRP) (Thermo Scientific Rockford Illinois, USA) or HRP-labeled goat anti human-IgE (KPL Gaithersburg, MD, USA) were used. The reaction was detected using Supersignal West Pico Chemoluminiscenct Substrate (Thermo Scientific Rockford Illinois,USA).

Phage display-Fab-library

For biopanning we used a combinatorial phage display Fab-library, displaying the repertoire of IgG heavy and light chains from a grass pollen allergic patient not having undergone SIT [29]. This library was constructed by inserting Fab-cDNA from PBMCs of this allergic donor into the XhoI/SpeI and SacI/XBaI sites of the pComb3 vector allowing the fusion of the Fd chain to the C-terminal domain of the phage coat protein III (library LB) [29,32]. For propagation of phages, the tetracycline resistant XL-1 strain of *Escherichia coli* and the kanamycin resistant lysogenic M13 helper phage VCSM 13 (Stratagene, La Jolla, CA) were used. The latter, a wild type phage lacking inserts was also used as a negative control for assessing specificity.

Biopanning

For the selection of Phl p 1-specific Fab-mimotopes, three rounds of biopanning were performed as follows:

On microtiter plates (Maxisorb, Nunc, Roskilde, Denmark) Phl p 1-specific IgGs were coated in 100 mM $NaHCO_3$ (pH=8.6) in duplicates ON at 4°C (5 ng/well for the 1st and 2nd round; 2.5 ng/well for the 3rd round). Plates were blocked using PBS/5% BSA (bovine serum albumin) for 2 hours at room temperature (RT). After washing, phage library or phage amplificates from previous rounds of biopanning were diluted 1:10 in PBS/2.5% BSA and incubated 2 hours at RT. Unbound phages were removed by 10 washing steps using PBS with or without 0.1% Tween-20 respectively. Bound phages were eluted using either a pH drop or a competitive method: for pH elution 150 µl/well of 0.1 M glycine buffer (pH 2.2) were incubated on the corresponding wells for 2 minutes, subsequently the eluate was transferred to a new tube and the pH was immediately adjusted to 7.0. Competitive elution was performed by incubating the respective wells with 150 µl/well of 10 µg/ml rPhl p 1 in PBS/2.5% BSA for 15 minutes. Residual phages were removed using 50 µl/well 0.1 M glycine buffer (pH 2.2) for 2 minutes, adding this elution to the pH elution followed by pH adjustment to 7.0. The elutions were then amplified overnight, using tetracycline resistant *E. coli* XL-1Blue and 10^{12} cfu/ml of helper phage in SB-culture medium. Amplified phages were precipitated using 2% polyethylene glycol (PEG) in 4 M saline solution.

To monitor the success of the biopanning procedure, phage titer of eluates was determined by colony forming units (cfu), the specificity of amplificates for the selection antibody was defined by ELISA.

Colony Screening ELISA

To select phage clones possessing binding activity tot Phl p 1-specific antibodies, colony screening was performed.

50 clones from the second round of both elution methods were randomly picked and cultivated in 270 µl of SB-medium on ELISA plates. Bacteria were spun down by centrifugation and 100 µl/well phage supernatants were transferred as duplicates on microtiter plates, already coated with selection antibody (5 ng/ml) and incubated ON at 4°C. After washing, bound phages were detected using an HRP labeled anti-M13 antibody (Amersham, Pharmacia, Uppsala, Sweden).

Phage clones giving more than 3 standard deviations of absorption compared with background control (VCSM13) were considered reactive and chosen for further analysis in a specificity ELISA.

Specificity ELISA

To confirm the binding specificity of selected phages, specificity ELISA was performed. Human myeloma IgG (World Health Organization references, kindly provided by Dr. Skvaril, Bern, Switzerland) was used as irrelevant antibody. For this purpose, clones selected during colony screening and wild type phage (wt) as a control were cultivated in 10 ml SB medium ON. Phages were precipitated using 2% PEG in 4M saline solution and were resuspended in PBS/2.5% BSA. Phage concentration was adjusted to $2x10^{11}$ phages/ml and then used in a 1:10 dilution in ELISA. The phages were transferred in duplicates onto microtiter plates coated with selection or irrelevant antibody (5ng/ml) and ELISA was performed as described for colony screening.

Patient Sera on Selected Phage Clones

To test human antibody binding activity to the selected phage clones, sera of 10 randomly selected grass pollen allergic patients obtained from the Floridsdorf Allergy Center (FAZ, Vienna, Austria) containing at least 800 ng/ml of Phl p 1-specific IgE were used. Collection of patient sera was approved by the ethics committee of the Medical University of Vienna and performed according to ethical guidelines. Phl p 1-specific IgG of sera was adjusted to 40 ng/ml for a modified ELISA procedure. In short, microtiter plates were coated with 100 µl/well of 10^7 cfu/ml of either one of the selected phage clones or with control wt M13 phages lacking an insert. The 10 grass pollen sera were tested individually on coated phages and bound IgG detected using HRP coupled goat-anti-human IgG antibody. Statistical analysis was performed using PASW Statistics 18 using non-parametric testing (Friedmann and Wilcoxon test) for linked samples.

Sequencing

Phage DNA was extracted from bacteria using the PureLink™ HiPure Plasmid DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instruction. Sequence information was obtained by sequencing according to the Sanger didesoxy nucleotide method by Microsynth (Microsynth, Balgach, Switzerland). The following primers were used for heavy chain: forward: 5´-cctctgaggagcttcaagccaac-3´ and reverse: 5´-taccaccgtcaccttgttcaa-3´. For the light chain: 5´:-gtggaattgtgagcggataac-3´was used as a forward primer and: 5´-gagacacaccagtgtggc-3´ or 5´-cacaacagaggcagttcc-3´ were used as reverse primers for lambda- and kappa light chains respectively.

From this information amino acid sequence of Fabs was deduced and CDR sequences identified by IgBLAST comparison with GenBank sequences (http://www.ncbi.nlm.nih.gov/igblast/).

To assess potential for T-cell crossreactivity, mimotope sequences were compared to the amino sequence of Phl p 1 using Vector NTI Advance 11.5.0 (Invitrogen, Carlsbad, CA, USA).

3D-Matching

Structural epitope matching of Fab-clones with Phl p 1 allergen was performed as described before [24]. Position of amino acids in the crystal structure of Phl p 1 (PDB ID: 1N10 Fedorov, A.A., deposited 2002) and the CDR sequences of the clones were compared, similarity scores computed and further assessed for solvent exposure.

In a second step, the matchings of the three CDRs of one chain were evaluated for proximity to each other on the allergen surface. Results were visualized using UCSF Chimera version 1.4 (http://www.cgl.ucsf.edu/chimera).

RESULTS

Antibody Purity and Biopanning

Phl p 1-specific IgG antibodies affinity purified from patients´ sera were quality controlled by immunoblot (Fig. **1**).

Table 1. Total Phage and Specific Phage Titres During Biopanning Procedure

Elution Method		1st Round	2nd Round	3rd Round
low pH	total phages (cfu/ml))	$8x10^5$	$2x10^6$	$2x10^7$
	specific titre (OD 405-490)	0.439	0.678	0.318
allergen competition (comp)	total phages (cfu/ml)	$8x10^5$	$1x10^7$	$1.1x10^7$
	specific titre (OD 405-490)	0.276	0.618	0.225

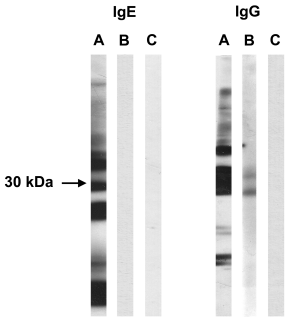

Fig. (1). The pool of grass pollen allergic patient's sera used for purification shows multiple IgG (1:500 dilution) and IgE (1:50 dilution) reactivities to blotted aqueous *Phleum pratense* extract (lanes A). Lanes B: Purified selection antibody (1:50 dilution) showed binding to a double band around 30 kDa when testing with anti-human IgG, while no binding could be detected when testing for IgE. Lanes C: negative buffer controls.

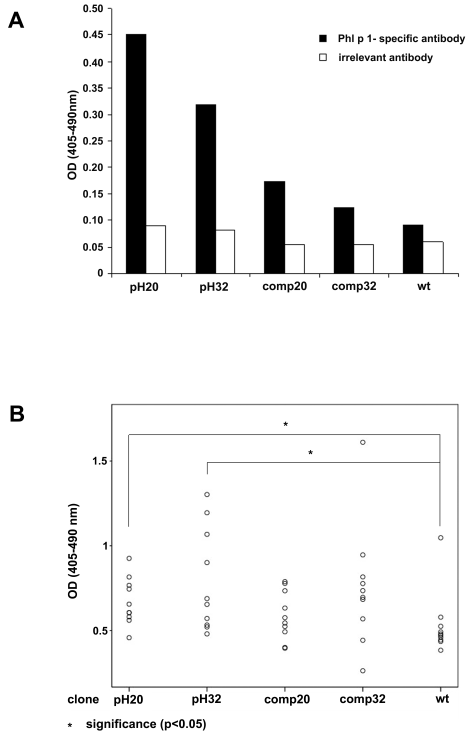

Fig. (2). 50 single phage clones from the second rounds of biopanning from both elution methods were randomly picked and tested for binding activity to Phl p 1-specific antibody by colony screening. Panel A: The clones with the highest binding activity, were further tested in ELISA for their binding to the purified anti-Phl p 1 antibody (dark bars) or isotype control (light bars). Depicted are the four best clones, two from pH elution (pH20; pH32) and two from competitive elution (comp20; comp32). For comparison also wild type phage (wt) lacking inserts was included.
Panel B: Scatter plot analysis of IgG binding activity of 10 grass pollen allergic patient's sera to immobilized specific phage clones, or wt control (10^7 phages/ml) in ELISA. Binding activity to the specific clones largely varied between individual patients. (significances indicated by brackets marked with *).

IgG from pooled crude sera recognized multiple protein bands in blotted grass pollen extract. Purified antibodies contained IgG, but no IgE against bands around 30 kDa, representing natural Phl p 1. The purified Phl p 1-specific IgG antibodies were used for selection of anti-idiotypic specificities from the combinatorial phage display Fab-library displaying Fab-fragments on coat protein pIII [29]. During the three rounds of biopanning using low pH- or competitive elution 25 or 13.75 fold increases of total phage titres were achieved (Table **1**). Specific titers of amplified phage eluents to selection antibody in ELISA increased from the first to the second, but decreased again in the third round of both biopanning procedures. Therefore, 50 clones of each elution method were picked from the second rounds and amplified for screening. Four clones were selected based on binding specificity to the purified allergen-specific antibody (Fig. **2A**), with two of them being derived from low pH (clones pH20 and pH32) and two from competitive elution (clones comp20, comp32), respectively. Among them, phages originating from the pH-shift elution showed the highest binding specificity in comparison to wild type phage, and when testing with an irrelevant control antibody.

As a further proof of specificity the clones were subjected to ELISA testing using sera of 10 randomly selected grass pollen allergic patients. The extent of IgG reactivity varied substantially among the individual patients. Sera containing anti-phage specificities caused a high background. In case of the clones derived from the low pH-elution method, binding was significant as compared to the binding to wt phage at (clone ph20: $p=0.049$; clone ph32: $p=0.037$). This was not the case for clones derived from competitive elution.

Fab-mimotope Sequences

The DNA of the four selected clones was sequenced and the most closely related germ line sequence determined (Table **2**).

Light chain sequences of all four clones, and heavy chain sequence of all except for clone comp20 could be identified. All heavy chain sequences obtained derived from germ line sequence VH4 and shared the same sequences for CDR1 and 2 only differing in CDR3. Clone pH32 and comp32 shared light chain sequences originating from Vλ7 gene, clone pH20 from Vλ3, only in clone comp20 a kappa light chain was found deriving from gene Vκ3.

Computational Matching of Fab-mimotopes to the 3D-structure of Phl p 1

To pinpoint the Phl p 1 epitope anti-idiotypic Fabs were matched on the allergen structure as described previously [24] using the pdb file representing dimeric Phl p 1 (PDB ID: 1N10). The conformational match is illustrated in Fig. **3A**: The CDR1 and CDR2 sequences shared by the heavy chains of clone pH20, pH32 and comp32 were matching at the N-terminus of the protein. The heavy chain sequence of clone comp20 was unfortunately not decipherable. In the case of CDR2 the match identified a region where two chains of each half of the dimeric protein overlap. Thus, the mimicked epitopes are made up by CDR1 and CDR3 matching to one half, and an interdigitating match by CDR2 to the other half of the dimer. The CDR3 sequences of the clones with highest specificity, pH20 and pH32, also matched similar regions of the protein, altogether forming a rather condensed epitope patch on the Phl p 1 surface.

Table 2. CDR Sequences of Selected Fab-clones

Clone	Chain		Sequence	Matched Amino Acids on Phl p 1
pH20	heavy	CDR1 CDR2 CDR3	-GYYWS- -EINHSGSTNYNPSLKS- -VPRGDDYYYYGMDV-	26-22 16-1 39-30; 58; 81-79
	light	CDR1 CDR2 CDR3	-GGNNIGSKNVH- -RDSNRPS- -QVWDSSTVV-	27-30; 58; 81-86 83-89 180-188
pH32	heavy	CDR1 CDR2 CDR3	-GYYWS- -EINHSGSTNYNPSLKS- -EGPRAYCGGDCDDAFDI-	26-22 16-1 53-58; 81-91
	light	CDR1 CDR2 CDR3	-ASSTGAVTSGYYPN- -STSNKHS- -LLYYGGAWV-	103-90 116-110 17-25
comp20	heavy	CDR1 CDR2 CDR3	not deliverable	
	light	CDR1 CDR2 CDR3	-RASQSVSSYLA- -DASNRAT- -QQRSNWPD-	84-94 118-112 22-15
comp32	heavy	CDR1 CDR2 CDR3	-GYYWS- -EINHSGSTNYNPSLKS- -GHKRTSHAFDI-	26-22 16-1 14-24
	light	CDR1 CDR2 CDR3	-GSSTGAVTSGHYPY- -DTSNKHS- -LLSYSGARV-	27-40 118-112; 112-110 89-97

Although CDR sequences of the light chains were much more diverse and showed little amino acid similarities, they similarly matched the N-terminal domain of the allergen. Furthermore, even though the light chain CDR2 sequences showed no amino acid homology, three of the clones (pH32, comp20, comp32) matched to an almost identical position within the Phl p 1 sequence and structure (Table **2**, Fig. **3**). Turning back to the primary structure, the location of the matching amino acid on the sequence indicates the discontinuous nature of the epitope (Table **2**, Fig. **3C**).

DISCUSSION

Type-I hypersensitivity reactions consist of an immediate phase elicited by cross-linking of receptor bound IgE antibodies to specific B-cell epitopes on the allergen, and a late phase reaction induced by allergen-specific T-cells [33]. Therefore, information about both T- and B-cell epitopes are of great interest for elucidating the intricate details of the allergic reaction. As T-cell epitopes are by their nature sequential, approaches screening overlapping synthetic allergen peptides with isolated T-cell clones have produced good results for characterizing T-cell epitopes on grass pollen allergen Phl p 1. The main T-cell reactive regions being located at the N-terminal domain, between amino acid 70-84, 97-114, and 127-141 [34]. A pragmatic adaptation of this method using longer stretches of the allergen sequence for screening was developed for characterization of Phl p 1 B-cell epitopes [35]. However, also sequentially widely separated amino acids may be spatially close in the folded protein B-cell epitopes. Moreover, also postranslational modifications of the protein contribute to the correct folding and thus to formation of antibody epitopes [14]. Integrity of allergen structure has been shown to be of crucial importance for IgE binding as a mosaic protein containing the whole Phl p 1 sequence but re-assembled of four large fragments lost its IgE reactivity [36].

An alternative approach for studying B-cell epitopes is to generate mimotopes, peptides mimicking the structure of these epitopes but not necessarily their sequence [28]. Therefore, mimotope vaccines usually lack T-cell epitopes [26,27] and thus may be advantageous when inflammation caused by activation of allergen-specific T-cells has to be avoided [37]. Mimotopes can be readily selected from phage display libraries presenting peptides, Fab-fragments or hairpin libraries by screening them with antigen specific antibodies (reviewed by [6]).

Our group has previously concentrated on grass pollen allergen Phl p 5, generating peptide [38], DNA [39] and anti-idiotypic mimotopes [30] which have been proven to be suitable for epitope studies, and for immunotherapy of grass pollen induced asthma in BALB/c mice [27]. In the present work we aimed to expand this strategy to the second important *Phleum pratense* allergen Phl p 1.

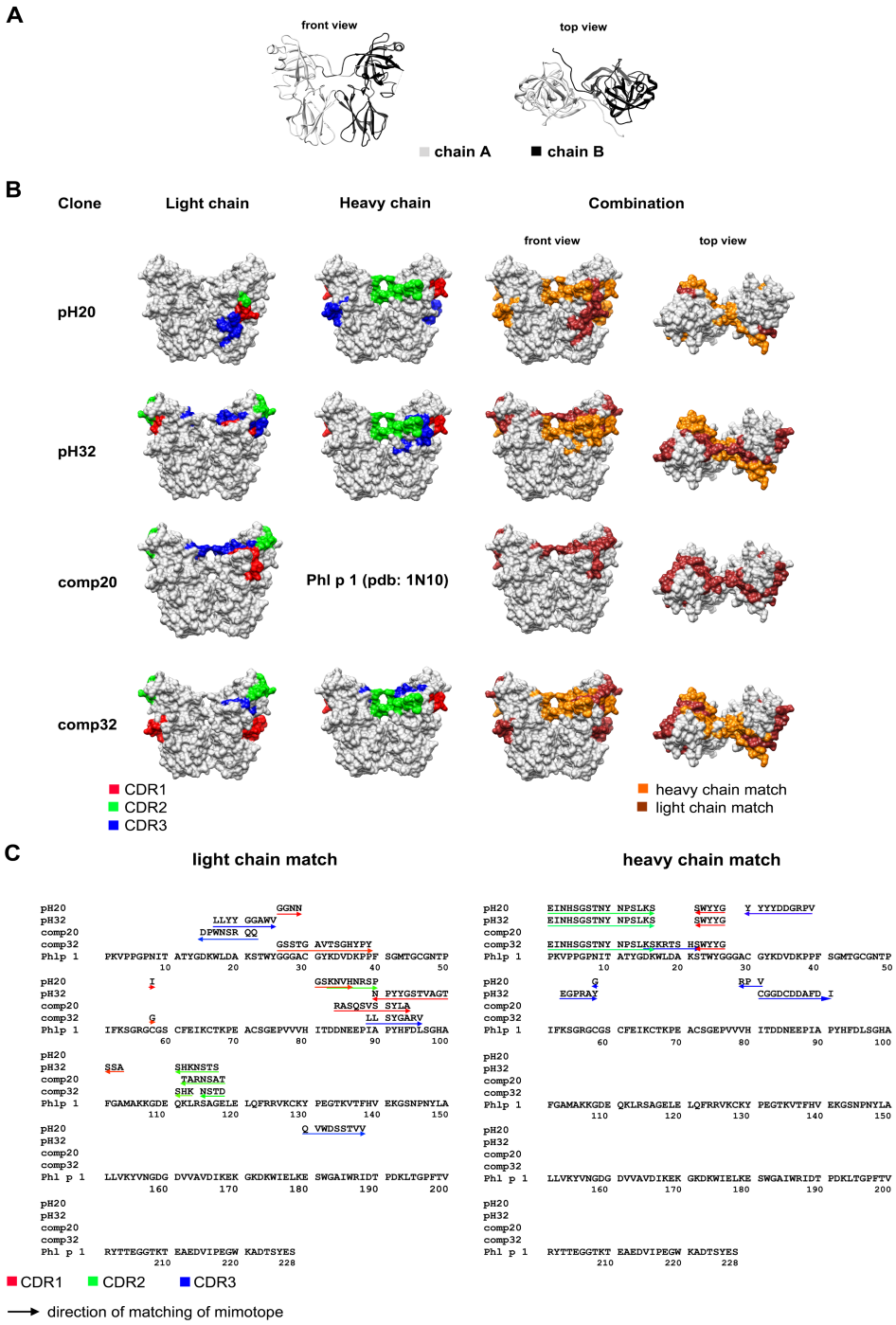

Fig. (3). Panel A shows the ribbon model of the dimeric Phl p 1 molecule (PDB ID: 1N10) illustrating the N-termini of the two monomers (chain A: light gray; chain B: black) interdigitating with the respective other chain.

Panel B is depicting the computational matching of the four selected mimotope clones onto the 3D-structural model of Phl p 1 molecule. Locations of the matchings of the three CDR regions are indicated with the following colors: CDR1 red; CDR2 green; CDR3 blue. On the right side under the subtitle "Combination" the superposition of heavy (orange) and light chain (brown) epitope location is shown in front and top view on the molecule.

Panel C shows the 3D matched amino acids superimposed on the linear sequence of the mature allergen (UniProt ID: P43213 excluding signal peptide) revealing the discontinuous nature of the matched epitopes. Mimotope sequences are indicated directly above the matched amino acids of the allergen, using the same color scheme as in the single chain matchings of panel A, the orientation of the underscoring arrows indicate N to C-terminal direction of mimotope sequence.

By screening of a combinatorial phage display Fab-library deriving from the repertoire of a chronic grass pollen allergic patient with polyclonal anti-Phl p 1 IgG, we identified four clones. According to Jerne´s network hypothesis,

these clones should resemble anti-idiotypic antibodies (Ab2) and also internal images of the allergen [1]. The Fab clones were also recognized by polyclonal anti-Phl p 1 IgG from other patients´ sera. As binding of the clones to Fc̣ domain of IgG could be excluded by negative isotype control experiments, we classified them as anti-idiotypic specificities. Heavy chains of all clones derived from VH4 germ line sequence which was previously found to be preferred by atopic patients [40]. The heavy chain sequences were almost identical, differing only in CDR3, indicating a limited repertoire of the anti-idiotypes. Also four out of five from the same combinatorial library previously identified anti-idiotypic Fabs for Phl p 5 allergen, originated from the same germ line sequence [30].

Matching of the anti-idiotypic Fabs identified an epitope patch being assembled by several independent stretches of the allergen sequence, thus representing a discontinuous, conformational B-cell epitope. Although the deduced amino acid sequences of the light chains showed little similarity with each other, their matches on Phl p 1 apparently clustered between amino acid 80-116 of the mature allergen. All identified clones matched on the N-terminal half of the protein, where all allergenic Pooidea β-expansins share a high level of cross reactivity [16] due to sequence and structural homology [18]. Therefore, the IgG epitope patch defined here may also be relevant for other class-I allergens. In contrast, other approaches screening monoclonal human antibody on recombinant Phl p 1 fragments matched major IgE epitopes to the C-terminal domain [35,41]. As already previously shown IgE and IgG reactive epitopes of patients may differ [42].

Further, the epitope characterized in our study overlaps with previously defined cross reacting T-cell epitopes [34] which suggests that the anti-idiotypic Fab fragments might contain T-cell stimulatory activity, although homology search indicated only 8-26% aa homology between mimotopes and matched protein sequence (data not shown). This would be in contrast to our findings with mimotopes from random peptide libraries which were devoid of T-cell stimulatory capacities [26,27]. It is even conceivable that they directly activate T-cells as it has been repeatedly demonstrated that B- and T-cell receptors may share the same idiotypes [6,9]. These aspects should be addressed in future functional studies. Based on our molecular modelling we conclude that, in accordance with Jerne's network hypothesis, the isolated anti-idiotypes represent internal images of a conserved IgG epitope on grass pollen allergen Phl p 1.

CONCLUSION

We could define a conformational epitope patch on the N-terminal end of the grass pollen allergen Phl p 1, located at the interface region of the homo-dimeric protein. The mimotopes obtained from the phage display Fab-library show little sequence homology to the matched regions on the allergen. As the location of previously identified T-cell epitopes is included, possible cross reactivity with allergen-specific T-cells have to be assessed before considering them as candidates for development of new safer immunisation strategies.

ACKNOWLEDGEMENTS

This study was financed by grant SFB F01808-B13 of the Austrian Science Funds. Jan Fuhrmann and Dirk Neumann are financially supported by European Social Fund and the German Federal Ministry of Economics and Technology due to a resolution by the German Bundestag.

Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

ABBREVIATIONS

BCR	=	B-cell receptor
CDR	=	complementary determining region
IVIg	=	intravenous immunoglobin
mIg	=	membrane immunoglobulin
PBS	=	phosphor buffered saline
PEG	=	polyethylene glycol
SB-medium	=	super broth medium
SIT	=	specific immunotherapy
SPT	=	skin prick test
wt	=	wild type

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Received: November 05, 2010 Revised: January 18, 2011 Accepted: January 19, 2011