Use of phage display technology to investigate allergen-antibody interactions

Janet M. Davies, PhD, Robyn E. O'Hehir, FRACP, PhD, and Cenk Suphioglu, PhD

Prahran, Australia

Phage display is an advanced technology that can be used to characterize the interactions of antibody with antigen at the molecular level. It provides valuable data when applied to the investigation of IgE interaction with allergens. The aim of this rostrum article is to provide an explanation of the potential of phage display for increasing the understanding of allergen-IgE interaction, the discovery of diagnostic reagents, and the development of novel therapeutics for the treatment of allergic disease. The significance of initial studies that have applied phage display technology in allergy research will be highlighted. Phage display has been used to clone human IgE to timothy grass pollen allergen Phl p 5, to characterize the epitopes for murine and human antibodies to a birch pollen allergen Bet v 1, and to elucidate the epitopes of a murine mAb to the house dust mite allergen Der p 1. The technology has identified peptides that functionally mimic sites of human IgE constant domains and that were used to raise antiserum for blocking binding of IgE to the FcεRI on basophils and subsequent release of histamine. Phage display has also been used to characterize novel peanut and fungal allergens. The method has been used to increase our understanding of the molecular basis of allergen-IgE interactions and to develop clinically relevant reagents with the pharmacologic potential to block the effector phase of allergic reactions. Many advances from these early studies are likely as phage display technology evolves and allergists gain expertise in its research applications. (J Allergy Clin Immunol 2000;105:1085-92.)

Key words: *Phage display, IgE, FAb, allergen, epitope*

Current clinical strategies for allergy treatment involve allergen avoidance by patients, pharmacotherapy, or immunotherapy. Only the third strategy has the potential to provide a long-term solution for a subject who has allergies. However, many allergen extracts used for immunotherapy are poorly defined, suffer from batch to batch variation, and have the potential to cause anaphylaxis. Safer and more effective allergen preparations would be helpful for use in clinical desensitization.

Abbreviations used

cDNA:	Complementary DNA
CDR:	Complementarity determining region
FAb:	Antibody fragment (light chain and the heavy chain variable and first constant region)
FcεRI:	High-affinity IgE receptor
mRNA:	Messenger RNA
scFAb:	Single-chain antibody fragment
scFv:	Variable fragments of an antibody formed as a single chain

The display of foreign proteins on the surface of bacteriophage has recently been used to understand allergen-antibody interaction at the molecular level. Achievement of better understanding of interactions of allergen with IgE may facilitate rational design of new generation therapies for the treatment of allergy based on desensitization with allergens modified to give a better profile for efficacy and safety.

FOREIGN PROTEIN DISPLAY BY FILAMENTOUS PHAGE

Filamentous M13 phage have been genetically modified to carry foreign DNA sequences in the bacteriophage genome, so that there is surface expression of the foreign protein fused to one of the phage coat proteins.[1] Each phage displays a unique foreign protein, depending on the inserted gene.

Because the probing of a phage library displaying myriads of different foreign peptides is conducted in solution or in semisolid phase, up to 100 billion phage can be screened simultaneously. During the screening process phage clones may be selectively captured by an immobilized ligand by the process known as biopanning, which may be repeated several times until phage of the desired purity and specificity have been isolated. Table I summarizes the types of M13 phage display systems that can be used to study allergen-antibody interaction.

IgE is produced at low levels in B cells (nanograms per milliliter of serum) and monoclonal IgE-producing B cells have proved difficult to immortalize by viral transformation. With the ability to amplify IgE gene reper-

From the Department of Allergy, Asthma, and Clinical Immunology, Alfred Hospital and Monash University Medical School, Prahran, Australia.

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Reprint requests: Janet M. Davies, PhD, Department of Allergy, Asthma, and Clinical Immunology, Alfred Hospital and Monash University Medical School, Commercial Road, Prahran, Victoria 3181, Australia.

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TABLE I. Summary of M13 phage display systems

Protein displayed	Type of library	Carrier protein	Genetic system
Antibody	scFv	pIII	Phagemid
	FAb	pIII	Phagemid
		pVIII	Phagemid
	scFAb	pIII	Phagemid
Antigen	Gene-specific	pIII	Phage
		pVIII	Phagemid
	Random peptide	pIII	Phage
		pVIII	Phagemid
	cDNA expression	pIII	Phagemid

scFv, Variable fragments of an antibody formed as a single chain; *FAb,* antibody fragment (light chain and the heavy chain variable and first constant region); *scFAb,* antibody fragment formed as a single chain; *cDNA,* complementary DNA.

toires from messenger RNA (mRNA) of peripheral blood B cells and express them on the surface of bacteriophage, antibody specificity can be investigated for those that are present at very low levels. Antibodies present in a phage-displayed library with specificity for a particular allergen can be selected, propagated, and characterized. Phage display technology also lends itself to the characterization of allergen epitopes.

Display of foreign proteins on M13 phage coat proteins pIII or pVIII

M13 phage consist of a DNA genome packaged as a long filament covered in the major coat protein pVIII. This coat protein, together with one of the minor coat proteins at the tip of the phage particle pIII, has been used to display foreign proteins.[2] Both pIII and pVIII have been used in allergy research. The basic structure of M13 displaying antibody fragments and peptides on pIII are depicted in Fig 1. Although there are other protein display systems, including λ phage libraries and libraries generated on the tip of flagellin of bacteria[3] or in T7 phage,[4] most allergy work has used M13 phage and we will focus on this system in this article.

Antibody display

Antibodies are naturally formed by the union of a light and heavy chain, and several of the antibody domains forming these two chains can be incorporated into phage display systems. The major differences in the display systems are the number of domains expressed by the phage and how they are linked. Antibody fragment (FAb) systems include the two light chain domains, variable and constant, and the variable and first constant domain of the heavy chain.[5] For single-chain variable domain (scFv) systems only the variable domains of each chain are present, and hence less of the natural antibody is displayed.[6]

The light and heavy chains of a FAb are cloned separately into the phage genome with the heavy chain physically fused to one of the phage coat protein genes to anchor the FAb to the phage surface. Diagrammatic representation of the structure and formation of a phage-displayed FAb fragment is given in Fig 2. The two separate antibody chains become joined during assembly by a disulfide bond that forms in the periplasm of the bacteria. For the scFv the two variable domains are each cloned into the phage genome to form one molecule linked by a small synthetic sequence. The complete scFv is fused to the gene of the phage coat protein, and thus it can be expressed on the surface. Although FAb antibody fragments contain more of the native antibody sequence and may represent the natural antibodies better, recombinant scFv should be more stable because the size of the foreign gene inserted into the vector is smaller than for FAb.

Although pVIII has been used to display antibody fragments,[7] nearly all antibody display research is currently conducted with use of pIII. A further development in antibody display that has been applied in allergy research involves the generation of phage-displayed single-chain FAb (scFAb) libraries.[8] There are several aspects inherent to phage-display antibody technology that have an impact on its application that are worthy of consideration here.

When a phage-displayed antibody library is prepared, the Ig genes for the light and heavy chains are amplified by PCR separately with use of cDNA generated from mRNA of cultured B cells, total peripheral blood lymphocytes of allergic subjects, or spleen cells from immunized mice. In this way a library of antibody specificities that mirrors those present in vivo can be generated in vitro and expressed by phage: this is known as repertoire cloning.[9] From such a repertoire the scFv or FAb specific for a particular allergen can be selected by biopanning and the Ig genes encoding allergen-specific antibody fragments can be determined by DNA sequencing of the insert in the bacteriophage genome.

Notably for either FAb and scFv libraries, the pairing of light and heavy chain genes in vitro is random and may not represent the pairing that occurs in vivo. It is assumed that functionality for Ig genes in vivo will be conserved in the library; subsequent biopanning with the allergen of interest will only select recombinations of heavy and light chains that maintain the in vivo specificity for the given allergen. This may not always be a valid assumption, a phenomenon that must be considered when the aim is to clone the natural repertoire of allergen-specific IgE.

However, if the aim is to generate an antibody library containing sequences with increased affinity or specificity for other purposes, such as the development of a FAb for in vivo therapeutic use as a blocking agent, this phenomenon can be used to advantage. Such a strategy has already been used to develop an allergen-specific FAb to compete for binding to the high-affinity IgE receptor (FcεRI). In another phage display system the heavy and light chain Ig gene fragments have been engineered on separate vectors that recombine inside a bacterium infected with multiple copies of each vector. This is referred to as combinatorial infection.[10]

To increase the diversity of antibody fragment libraries further, semisynthetic FAb genes have been prepared in which the framework regions of the antibody

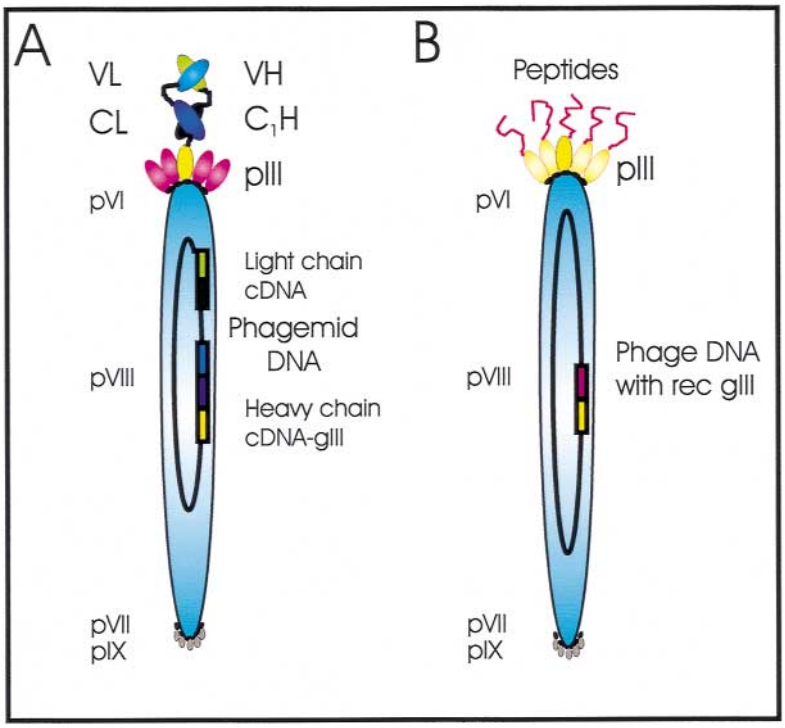

FIG 1. Display of foreign proteins by filamentous phage. **A,** Display of antibody fragments (light chain and the heavy chain variables and first constant region) (FAb) on a single copy of recombinant pIII encoded by a phagemid vector. **B,** Display of random peptides on each copy of pIII encoded within the phage genome. The phage coat proteins, antibody domains, and recombinant genes of the DNA are marked. *VL,* Variable domain of the light chain; *VH,* variable domain of the heavy chain; *CL,* constant domain of the light chain; *C_1H,* first constant domain of the heavy chain.

chains are left intact but the naturally occurring antigen complementarity determining regions (CDRs) have been substituted by synthetic random sequences.[11] The natural process of antibody affinity maturation has then been mimicked in vitro by mutagenizing libraries of antibody fragments.[12]

Allergen display

To address the allergen component of antibody-allergen interaction, several approaches may be used including screening of phage-displayed gene-specific libraries, random peptide libraries, or cDNA expression libraries. The cDNA encoding a particular allergen can be fragmented to generate overlapping sequences that are expressed as peptides on the phage surface and used to select epitopes for a given antibody. To date, this approach of gene-specific display has not been successfully applied in allergy research.

Allergen characterization is an essential prerequisite for understanding the interaction between allergen and antibody. The λ bacteriophage libraries have been extensively used to clone and characterize diverse types of allergens; the area of allergen discovery has been reviewed elsewhere.[13] To capitalize on the advantage of solution-phase screening afforded by M13 phage systems, cDNA expression libraries have been shuttled into the M13 and used for the purpose of allergen discovery: novel fungal and peanut allergens have been cloned in this way.[14,15]

The generic method of mapping epitopes for a given antibody is to screen phage-displayed random peptide libraries. These may be of various lengths, and libraries of peptides with 6, 7, 8, 9, 12, and 15 amino acids are available.[16] The peptides displayed on the surface of the phage may be constrained by the insertion of cysteine residues on both sides of the peptide insert, causing a cyclic peptide to be formed by disulfide bonds between the cysteine residues.[16] This may be advantageous and yield peptides with higher affinity for an antibody than unconstrained peptide libraries.

Phage-displayed random peptide libraries frequently yield peptides that mimic the binding site for an antibody but do not correspond to the linear sequence of the cognate antigen. These peptides are known as mimotopes and can represent linear and conformational peptide epitopes as well as nonprotein epitopes. Although interpretation of the peptide sequences of mimotopes may be difficult, critical amino acids common to the selected peptides are revealed that help to identify key residues contained within a conformational epitope.[17]

Genetic mechanisms of phage display

The mechanisms that have been developed for foreign gene expression by the phage may be loosely divided into two types, phage and phagemid. In the simpler phage system, the foreign cDNA is inserted directly into the phage genome; every copy of pIII or pVIII displays

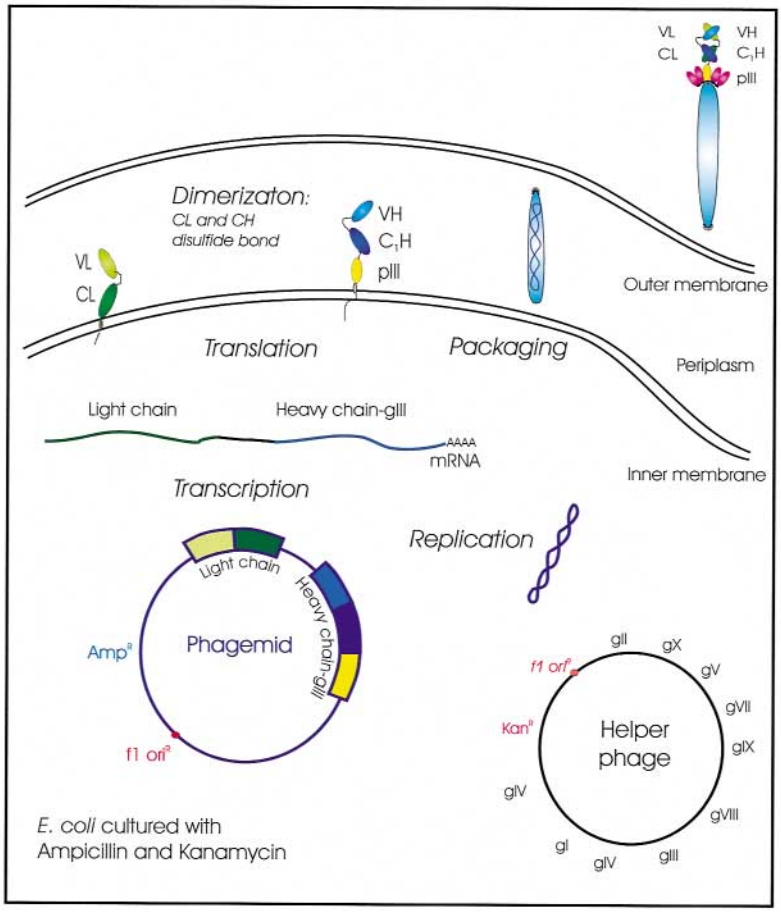

FIG 2. Generation and display of a FAb encoded by a phagemid vector. The phagemid is maintained in the *Escherichia coli* by a selection marker for ampicillin resistance *(Amp^R)* and carries the Ig light chain (*green*) and heavy chain (*blue*) fused to the phage gIII (*yellow*). The mRNA is transcribed and translated. The antibody light chain and heavy chain-pIII fusion then combine with a disulfide bond and are packaged onto the surface of the growing phage particle. Production of the phage particle is facilitated by the gene products (gI-gX) of the helper phage that is maintained by the selection marker for kanamycin resistance *(Kan^R).* The single-strand DNA of the phagemid is preferentially replicated from the f1 origin of replication (f1 ori^R) because the equivalent site has been mutated *(f1 ori^R)* in the helper phage.

the foreign protein. If the inserted peptide disrupts the pIII or pVIII protein functionally, the phage will not be viable and the peptide will not be displayed.

To meet this problem, phagemid systems were developed. The foreign cDNA is carried on a separate plasmid, known as a phagemid, that has only the phage gene, gIII or gVIII, into which the foreign sequence is to be inserted. To produce phage particles from this type of vector, a bacterium must also carry a helper phage with all the genes necessary for phage production, including a wild-type copy of the same gene used for display of the foreign protein in the phagemid (Fig 2). Therefore both recombinant and wild-type protein will be produced and be incorporated into the phage particle. For pIII approximately one of the five copies, and for pVIII from 1% to 30% of the 2700 copies, will display the foreign protein.[18] The phage particle preferentially caries the phagemid DNA because the helper phage DNA is mutated so that it replicates more slowly.

The type of genetic system used, phage or phagemid, will affect the copy number of display and in turn the avidity or affinity of interaction between the selecting ligand and the phage-displayed molecule. The type of system will also govern the size of the protein that may be displayed and the effects of the foreign protein on phage biology: infection, replication, and packaging. When a library of random peptides or foreign genes is created, not all sequences of the library will be tolerated by the phage or bacterium equally well.

USE OF PHAGE DISPLAY SYSTEMS IN ALLERGY RESEARCH (TABLE II)

Phage display technology is the most satisfactory approach for cloning specific variants of human monoclonal IgE. When the interaction between serum IgE and an allergen has been characterized, it will be possible to develop novel therapeutic agents on the basis of the dis-

TABLE II. Summary of phage display technology applied in allergy research

Protein displayed	Type of M13 library	Selecting agent	Result	Reference
Antibody display				
Murine IgE scFAb	pIII	Trichosanthin	Trichosanthin-specific FAbs, model of FAb-allergen interaction	8, 19
Human-based semisynthetic IgG FAb	pIII	Rye grass allergen rec Lol p 2	FAb that competed with serum IgE for reactivity with Lol p 2, blocking of histamine release in vivo	20
Murine monoclonal IgG FAb	pIII	Not appropriate, mAb specific for Bet v 1	Ig gene sequences, epitope mapped, structural studies	21
Human IgE FAb	pIII	Timothy grass allergen rec Phl p 5	FAbs to Phl p 5 that cross-react with group 5 allergens from other grasses	22
Allergen display				
Random peptides	Cyclic and noncyclic nonapeptides on pVIII	Affinity-purified human antibodies to mugwort, birch, celery tuber extracts	Mimotopes that inhibit binding with native allergen	23
Fcε gene-specific, random peptides	pVIII, cyclic nonapeptides and octapeptides on pVIII	Murine mAb BSW17 to human IgE	No specific phage from Fcε library, mimotopes from random peptide library that inhibit reactivity of BSW17 with IgE and that induce antisera to IgE	24
Random peptides	Cyclic and noncyclic nonapeptides on pVIII	Murine mAbs, BIP1 and BIP4, to birch allergen Bet v 1	Mimotopes that inhibit reactivity to mAb with Bet v 1 and induce IgG to Bet v 1	25
Random peptides	Pentadecapeptides on pVIII and cyclic nonapeptides on pX of T7 phage	Murine mAb to dust mite allergen Der p 1 2C7	Mimotopes similar in sequence to surface-exposed loop of Der p 1, Der p 1 blocks 2C7 capture of mimotopes	26
cDNAs of fungus *Aspergillus fumigatus*	pIII	Human sera reactive with fungal allergens	9 Allergens, some used in skin prick tests, 2 novel allergens with diagnostic utility	14, 27
cDNAs of peanut *Arachis hypogeae*	pIII	Human sera reactive with peanut allergens	4 Novel allergens, reactivity profiles with allergic subjects	15

covery of high-affinity competing antibodies. FAb fragments from such reagents, which lack the Fc portion, could be administered as blocking agents to mop up allergen in vivo and prevent FcεRI-mediated triggering of histamine release from basophils or mast cells.

The competing FAb would not need to be IgE in origin; it would only need to have a much higher affinity than IgE for the IgE-binding epitope of the allergen. This approach is dependent on the specificity of the monoclonal FAb and would be allergen specific. This strategy would be most likely to succeed if the immunodominant epitopes are targeted, implying that the same epitopes would need to be recognized by IgE from many individuals for such a FAb to become an effective therapeutic agent.

A more generic approach would be to block the interaction of the IgE with the FcεRI receptor on the basophil. The FAb in this case would need to block IgE binding in vivo and also prevent signal transduction from the receptor so that it failed to release histamine. Each of these approaches has been attempted (Fig 3).

The delineation of immunodominant epitopes of allergens for serum IgE provides complementary knowledge to the approach described above. Phage display technology achieves this in two ways. First, it provides the means of cloning the in vivo repertoire of allergen-specific IgE, from which the specificity can be fine-mapped. Second, it allows mapping of epitopes of allergens, or derivation of mimics of allergens (ie, mimotopes), by selection from phage-displayed random peptides or gene-specific libraries. Such mimotopes might have therapeutic potential to block allergen cross-linking of IgE bound to FcεRI receptor and subsequent release of inflammatory mediators from effector cells.

Timothy grass pollen is an important cause of seasonal allergy in Europe and North America. An IgE FAb library has been generated from a subject with timothy grass pollen allergy who also displayed serum IgE cross-reactivity with allergens from a number of other grasses.[22] Four FAb clones that were specific for the major allergen Phl p 5 were selected. Interestingly, each of these uses the same ε heavy chain. The CDR3 region in the light chains showed the most sequence variation and was rich in aromatic residues. In other studies, CDR regions that were rich in aromatic amino acids were found to sustain the allergen cross-reactivity of specific IgE.[28] Cross-reactivity of these clones was demonstrated with rye grass and also with an allergen of timothy grass that migrated at the molecular weight corresponding to group 6 allergens. The corresponding FAbs partly reduced the reactivity of human serum IgE with Phl p 5. As might be expected, culture of PBMCs in the presence of IL-13 and IL-4 increased the total level of IgE from B cells, but

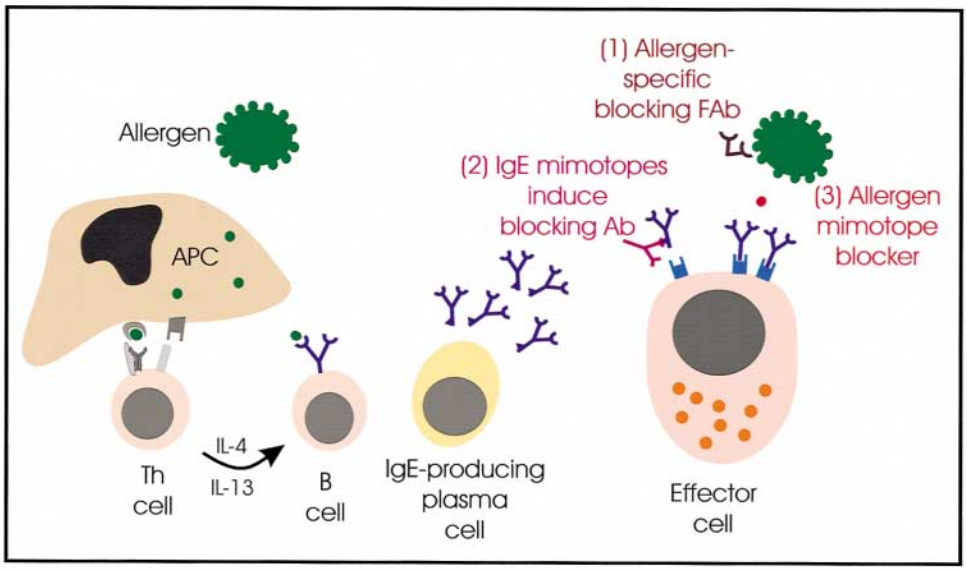

FIG 3. Strategies for intervention in allergic reactions with reagents discovered by phage display technology. Primary allergen exposure leads to processing of allergenic epitopes by antigen-presenting cells *(APC).* APCs prime T helper cells, which provide activating cytokine signals to allergen-specific B cells that develop into IgE-producing plasma cells. IgE is captured by the FcεRI on the surface of effector cells, such as basophils, and are thus primed for a secondary allergen exposure and subsequent release of inflammatory mediators. *(1),* Allergen-specific FAb can compete with IgE for allergen, reduce FcεRI-IgE cross-linking on effector cells and thus block mediator release. *(2),* IgE mimotopes can induce antibodies that react with IgE and block interaction of IgE with FcεRI. *(3),* Allergen-specific mimotopes can compete with allergen and block binding with IgE.

allergen-specific IgE was not increased.[29] The authors concluded that the best source of B cells for the cloning of allergen-specific IgE as FAbs was fresh PBMCs. It would be interesting to establish the usage of Ig ε genes from other individuals allergic to timothy grass to determine whether the preferential usage of a single IgE chain is observed in other subjects.

With the aim of producing blocking antibodies to the rye grass pollen allergen Lol p 2, De Lalla et al[20] screened a scFAb library of semisynthetic human IgG antibodies.[11] scFAbs with high affinity for Lol p 2 (K_a = 2.63 nmol/L) that compete with serum IgE for reactivity with Lol p 2 by ELISA were derived. This level of affinity is approximately 10 times that expected of natural antibodies, which is a desirable characteristic of a therapeutic reagent. Four scFAbs were isolated that used identical λ light chains and heavy chains that differed by only a few amino acids. These IgG scFAbs are specific for the Lol p 2 allergen of rye grass because no cross-reactivity with other grass allergens was observed. Synthetic peptides spanning the entire length of the allergen were used to map the epitope for serum IgE and the scFAb to residues 39-51. Serum IgE also recognized another peptide spanning residues 64-88, which would account for the remaining reactivity with the whole allergen that could not be blocked by the scFAb. Most important, the scFAb blocks serum IgE induction of histamine release from basophils, exemplifying its potential as a blocking agent for use in vivo. However, Lol p 2 is one of the minor allergens of rye grass.

Rye grass pollen is the predominant grass allergen in Australia and a major allergen in some other countries with temperate climates. It would be interesting to generate IgE FAb from rye grass–allergic individuals that reflects the in vivo responses to these grass pollen allergens. Selection of IgE FAbs to the major rye grass allergens, Lol p 1 and p 5, would be particularly informative and useful, because more than 90% of rye grass pollen–allergic individuals recognize these allergens.[13] Although IgG scFAbs[20] are of high affinity, IgE FAbs may be more likely to recognize the immunodominant epitopes targeted in vivo, confer cross-reactivity with related allergens, and therefore increase potential clinical efficacy as novel therapeutics.

By screening a random dodecapeptide library displayed on pIII with a specific mAb, we have identified a mimotope of grass pollen (Suphioglu, unpublished observation). Moreover, a synthetic peptide corresponding to the amino acid sequence of this mimotope significantly inhibited the interaction between the allergen and the specific mAb as well as human IgE.

Trichosanthin is an important allergen in China because it is derived from a medicinal plant and is used for inducing abortion.[30] It is now receiving attention because of its activation of chemokines and enhancement of signaling from chemokine receptors, suggesting a use for treatment of HIV infection.[31] It can elicit anaphylaxis mediated by IgE that limits its therapeutic potential. Li and Yeh[8] have expressed murine IgE scFAb phage-dis-

played from four murine IgE clones with specificity to trichosanthin.[30] Once again, aromatic amino acids were contained in the CDR3 region of the scFAb and were predicted by molecular modeling to interact with the antigen.[19] The scFAb-trichosanthin complex is an excellent candidate for structural studies because the scFAbs are monoclonal and can be purified to homogeneity at high concentrations and trichosanthin is a small molecule whose structure has already been resolved.

Birch pollen is another important aeroallergen responsible for seasonal rhinitis in Europe. Phage-display technology has been used to express a murine mAb 4A6 to the major birch allergen profilin (Bet v 1).[21] The Ig gene sequences were derived and the epitope for the FAb was mapped to continuous hexapeptide Bet v $1_{42\text{-}47}$ (PQFKPQ) of which the P_{42} and F_{44} are buried in the structure of the native antigen, whereas the remaining amino acids contact the FAb. Timothy grass profilin, with which 4A6 does not react, only differs from birch profilin in this sequence by the presence of a glutamate residue in place of Q_{47}.

Phage-displayed random peptide libraries were screened similarly with two other mAbs to Bet v1: BIP1 and BIP4.[25] Mimotopes that inhibited the reactivity of the mAbs with the native allergen were selected that are capable of inducing an IgG antibody response to the cognate allergen. In a study using antibodies from human serum affinity-purified on mugwort, birch, and celery tuber extracts, mimotopes from phage-displayed random peptide libraries were selected.[23] The phage-displayed peptides mimicked conformational epitopes that did not resemble the amino acid sequences of known plant allergens but were capable of inducing an antibody response to the natural allergen on immunization.

These studies exemplify that mimotopes can be used to vaccinate against allergens and may be safer than the current use of natural allergens, which evoke IgE responses. Depending on their ability to induce protective antibody synthesis or alter the cytokine milieu elicited against an allergen, mimotopes could form the basis for novel immunogens for use in desensitization.[32]

An alternative approach is to develop reagents that target the IgE itself and can be used to block the effector phase of allergen-IgE interaction with the FcεRI on basophils.[24] A mAb BSW17 that recognizes human IgE was initially used to screen an IgE gene-specific phage-displayed library. No selection of clones was observed that would indicate that the epitope for BSW17 is conformational and that the library of overlapping linear peptides of IgE did not contain the complete epitope for the mAb. Libraries of phage-displayed random cyclic peptides were then screened and mimotopes that inhibited the reactivity of BSW17 with IgE were derived. These mimotopes were used to immunize rabbits and an anti-IgE response directed against the C3 or C4 was elicited. When monkeys were vaccinated with these peptide mimotopes, this suppressed systemic cutaneous reactions induced by the hapten allergen 4-hydroxy-3-nitro-4-hydroxy-iodophenyl acetic acid conjugated to BSA,[33] illustrating their therapeutic potential.

House dust mite allergens are extremely important perennial allergens associated with clinical allergy. The selection of mimotopes from phage-displayed random peptide libraries with use of a murine mAb to the major house dust mite allergen Der p 1 has recently been reported.[26] The mAb 2C7 inhibits up to 70% of the reactivity of human IgE with Der p 1, and the Ig genes were similar to members of human Ig gene families. It was argued that the epitope for 2C7 could also represent a major epitope for serum IgE of allergic individuals.

Because the majority of Der p 1 epitopes are conformational and phage-display studies can yield mimotopes that represent nonlinear epitopes, it was reasoned that screening of phage-displayed libraries would be highly informative. Phage were selected from libraries of cyclic nonapeptides displayed on the major capsid protein pX of T7 phage and of libraries of noncyclic pentadecapeptides displayed on the surface of pIII of M13 phage. Many of the phage-displayed peptides that were selected by 2C7 contain the motif DxxxR within a surface-exposed loop, Val_{140}-Tyr_{169}, of the cognate Der p 1 allergen. Because there is the possibility that the murine mAb inhibits reactivity of human serum IgE reactivity with Der p 1 by steric hindrance rather than by specific competition with serum IgE for the same epitope, confirmation is necessary to map epitopes recognized by human IgE FAbs specific for Der p 1 from dust mite–allergic individuals.

Other studies using the pJuFo system have defined novel allergens of the fungus *A fumigatus*[14] and peanut.[15] The newly cloned fungal allergens have allowed development of a panel of allergens that uses serum IgE reactivity to distinguish between subjects who are asthmatic with coincidental fungal reactivity and those who are primarily responding to the fungal antigens.[27,34] Employment of such strategies would be useful for the development of novel diagnostic tools and in the future for the generation new therapeutics.

CONCLUSION

The most remarkable aspect of the 15-year history of phage-display technology has been the length of time it has taken to use this very powerful tool in combination with human molecular genetics and drug discovery to study conditions such as atopy. Because phage display is an enabling technology that can facilitate study of allergen-IgE interaction at the molecular level for any allergen in any individual, it has already provided leads for the development of new-generation therapeutics.

To date there has been a bias toward use of phage display only for delineation of antibody-allergen interaction, and even this has only been pursued for seasonal plants, in particular grass or birch pollen allergens. The first study of the perennial allergen Der p 1 has been reported, but there remains the need to develop strategies for other allergens, particularly those where subjects have chronic exposure. Further investigation is also required for allergens of high anaphylactic potential, such as peanut, for

which the only currently available treatment is allergen avoidance. Because exposure to allergens such as bee venom is usually infrequent and the current desensitization regimens are highly effective, detailed investigation of these allergen-antibody interactions is of lower priority. However, when treatment regimens have less than ideal efficacy or safety, it would be remiss not to improve our basic understanding of the scientific basis of the problem and the ways in which existing treatments could be modified on the basis of scientific evidence rather than ad hoc attempts to provide one-off solutions.

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