

Review

Recombinant Allergen-Specific Antibody Fragments: Tools for Diagnosis, Prevention and Therapy of Type I Allergy

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Type I allergy represents a hypersensitivity occurring in almost 20% of the population that is based on the recognition of innocuous airborne antigens (pollen, mite, mould and pet allergens) by specific immunoglobulin E. Allergic symptoms (e.g. allergic rhinitis, conjunctivitis, asthma) are caused by the release of biological mediators from effector-cells after allergen-induced crosslink of receptor-bound IgE. Here we discuss strategies to obtain recombinant allergen-specific antibody fragments (Fabs) from mouse and human cell lines as well as directly from allergic patients lymphocytes via the combinatorial library technology. It is suggested to use recombinant allergen-specific Fabs for the standardization of allergen extracts currently used for diagnosis and treatment, to determine allergen contents in allergen sources and the environment to allow preventive measures and to use allergen-specific Fabs as therapeutic tools to interfere with the allergen-IgE interaction. The latter appears possible because IgE represents the least abundant class of immunoglobulins and there is increasing evidence for a limited diversity among allergens and their B-cell epitopes. Moreover, allergic effector reactions are mostly confined to accessible target organs so that a local application of competing Fabs prior to allergen exposure might represent a feasible therapeutic approach.

Key words: Allergen / Allergen-specific antibody fragments-Fab / Combinatorial libraries / Diagnosis / Prevention / Therapy / Type I allergy/IgE.

Introduction

Type I allergy represents an immunoglobulin-E (IgE)-mediated hypersensitivity reaction towards otherwise harm-

less antigens (e.g. pollen-, insect-, animal-, and mould proteins). With a prevalence of almost 20%, IgE-mediated diseases represent a health threat of increasing importance in industrialized countries (Miyamoto, 1993). In sensitized patients, innocuous environmental allergens can immediately induce a cascade of allergic reactions by crosslinking of effector-cell bound IgE-antibodies (Segal *et al.*, 1977). Allergic symptoms comprise allergic rhinitis, conjunctivitis, dermatitis, asthma and even anaphylactic shock. Diagnosis of Type I allergy is currently performed by serological measurement of specific IgE-antibody levels and *in vivo* testing (skin prick reactivity) using allergen preparations obtained from various allergen sources. These crude extracts are difficult to standardize regarding their allergen contents. Recent progress in the field of molecular allergen characterization by recombinant DNA technology has brought about two major achievements. First of all, sequence, structural and immunological similarities among the relevant allergens indicate a limited diversity of allergens and their B-cell epitopes. Second, a constantly increasing panel of recombinant allergens has become available for diagnosis and specific therapy of Type I allergy (reviewed in: Scheiner and Kraft, 1995; Valenta and Kraft, 1995). While diagnosis with allergen extracts at best permitted to identify the allergen source against which a patient is sensitized, recombinant allergens allow the precise determination of the patients individual IgE-reactivity profile (allergogram) (Valenta *et al.*, 1991; Valenta *et al.*, 1992; Menz *et al.*, 1996; Pauli *et al.*, 1996). The knowledge about a limited diversity of allergens together with the possibility to determine sensitivity to defined components with the aid of recombinant allergens led us to consider the interaction of allergens and patients IgE antibodies as a possible target for the treatment of Type I allergy. Allergen-specific IgG antibodies present in sera of patients who had received successful immunotherapy are long known as natural competitors of IgE antibodies and therefore represent suitable candidates for specific passive blocking therapy of Type I allergy. In the present contribution we describe strategies to obtain allergen-specific antibodies and recombinant antibody fragments and their use for diagnosis, prevention and therapy of Type I allergy.

Obtaining Allergen-Specific Antibodies and Antibody Fragments (Fabs)

For the production of allergen-specific antibodies and antibody fragments at least three approaches may be pursued. Animals (e.g. mice) can be immunized with allergens to establish hybridomas that secrete monoclonal antibodies. Alternatively it is possible to isolate directly peripheral lymphocytes from allergic patients to establish human allergen-specific cell lines or as a source to construct combinatorial libraries that can be screened for clones with specificity to a given allergen (Figure 1). Allergen-specific antibodies or recombinant antibody fragments (Fabs) may be used for different purposes. They can be applied for the detection and quantification of certain allergens in allergen-sources and extracts that are currently used for diagnosis and therapy, but also for the detection of allergens in the environment and food.

Antibodies represent also valuable research tools to investigate the interaction of allergens and patients IgE antibodies. Those antibodies which compete with the binding of IgE antibodies either bind to/or close to IgE-epitopes or induce conformational changes in the allergen that prevent IgE-recognition. Unexpectedly mouse and human monoclonal antibodies were discovered which increased IgE-binding to allergens, most likely by causing a conformational change that makes additional IgE-epitopes available (Visco *et al.*, 1996; Laffer *et al.*, 1996; Lebecque *et al.*, 1997). While at present there is no evidence that such antibodies may cause disease exacerbation, the beneficial role of antibodies that compete with the allergen-IgE interaction is well established. In order to obtain blocking antibodies, mouse monoclonal antibodies may be tested *in vitro* for their ability to block IgE-binding (Lebecque *et al.*, 1997). If mouse monoclonal antibodies are considered for therapy it will be necessary to modify them by humanization or to produce them as Fabs to reduce their immunogenicity. Human monoclonal antibodies may represent even better candidates for antibody based therapy, however similar as for the mouse antibodies, production via tissue culture methods is rather labor intensive and sets limits as to the number of different antibodies that can be obtained and screened. We therefore suggest to employ combinatorial library technology for the production of recombinant allergen-specific antibody fragments. Using peripheral blood mononuclear cells from allergic individuals it is possible to generate cDNAs coding for heavy chain fragments and light chains by reverse transcription and PCR. Heavy chain and light chain cDNAs can then be combined and co-expressed in bacterial expression systems to represent the complete humoral diversity of the donor (Huse *et al.*, 1989). Clones producing allergen-specific Fabs can then be selected using defined allergens by direct immunoscreening (Huse *et al.*, 1989) or panning (McCafferty *et al.*, 1990; Barbas *et al.*, 1991). In the last decade considerable advances were achieved regarding the expression of functional antibody fragments in *Escherichia coli*. Basically two systems allow the presen-

tation of heavy and light chain variable regions on the surface of filamentous phage. The pCANTAB system (Pharmacia, Uppsala, Sweden) is designed to express single-chain variable fragments (Fvs) of heavy and light chain which are artificially joined with a neutral linker (Winter and Milstein, 1991). In contrast, the pCOMB system co-expresses heavy chain fragments including a portion of their constant region together with the light chain (Barbas *et al.*, 1991). Both systems allow the efficient isolation of antigen-specific scFvs or Fabs by panning of the phage to a given antigen. The advantage of both systems over conventional cell culture technique lies in the fact that both Fabs and scFvs can be produced in *E. coli* (Hoogenboom *et al.*, 1992; Plückthun 1992; Soderlind *et al.*, 1992; Winter *et al.*, 1994; Harrison *et al.*, 1996). The re-

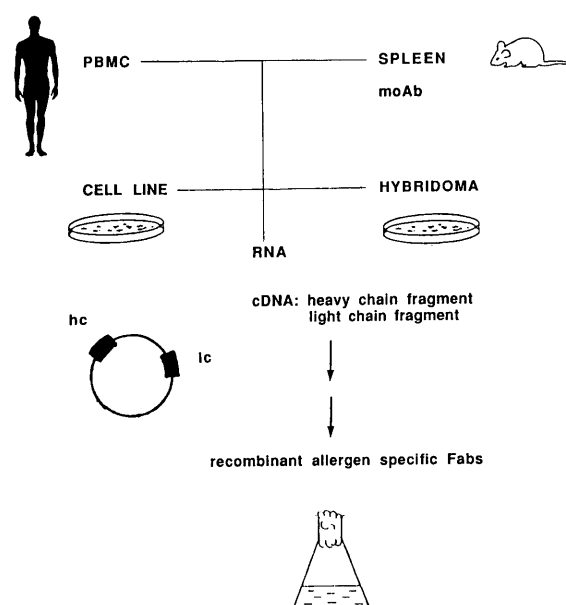


Fig. 1 Obtaining Allergen-Specific Recombinant Fabs. PBMC, peripheral blood mononuclear cells; hc, heavy chain; lc, light chain.

IgE-Fab α Phl p 2

Ant o	Ave s	Cyn d	Lol p	Phr a	Poa p	Sec c	Tri s	Zea m	Phl p	kD
										← 14.2
										← 6.5

Fig. 2 A Phl p 2-Specific Human IgE Fab Crossreacts with Group 2 Allergens in Pollen from Different Grass Species. Comparable amounts of pollen extracts from ten monocots (Ant o: *Anthoxanthum odoratum*, Ave s: *Avena sativa*, Cyn d: *Cynodon dactylon*, Lol p: *Lolium perenne*, Phr a: *Phragmites australis*, Poa p: *Poa pratensis*, Sec c: *Secale cereale*, Tri s: *Triticum sativum*, Zea m: *Zea mays*, Phl p: *Phleum pratense*) were separated by SDS-PAGE, blotted onto nitrocellulose and probed with a human IgE-Fab with specificity for the major timothy grass pollen allergen Phl p 2 (Dolecek *et al.*, 1993).

combinant antibody fragments are small and hence less immunogenic and can be subjected to *in vitro* manipulation (*in vitro* affinity maturation by mutagenesis, shuffling of heavy and light chains or the corresponding variable regions or other manipulations) (Gram *et al.*, 1992; Pack *et al.*, 1995; Low *et al.*, 1996; Hoogenboom, 1997). Since recombinant antibody fragments lack the major part or the complete constant region they will not elicit effector reactions which are mediated via Fc receptors. Using appropriate oligonucleotide primers, recombinant antibody fragments can be produced starting from spleen, cell lines, hybridomas of human or animal origin or by constructing combinatorial libraries directly from patients B-lymphocytes.

Recombinant Allergen-Specific Fabs as Tools to Explore the Allergen-IgE Interaction: Cloning Allergen-Specific IgE

The understanding of the interaction of allergens and patients IgE-antibodies requires structural analysis of the allergen and its IgE-epitopes as well as the characterization of allergen-specific IgE-antibodies (Fedorov *et al.*, 1997). The latter has been a major obstacle because of the extremely low levels of serum IgE, and the low frequency of IgE-secreting B-cells. In order to characterize allergen-specific human IgE antibodies on a molecular level, we have chosen the combinatorial library approach. A combinatorial IgE library was constructed from peripheral blood lymphocytes obtained from a grass pollen allergic patient during the grass pollen season (Steinberger *et al.*, 1996). Using recombinant major timothy grass pollen allergens Phl p 1 (Laffer *et al.*, 1994), Phl p 2 (Dolecek *et al.*, 1993) and Phl p 5 (Vrtala *et al.*, 1993) for panning, phage expressing specific IgE-Fabs could be isolated. cDNAs coding for the IgE heavy chain fragments and their corresponding light chains were sequenced and recombinant soluble IgE

Fabs with specificity for the major grass pollen allergens could be expressed and purified up to milligram amounts. As is shown in Figures 2 and 3, human IgE-Fabs with specificity for the major timothy grass pollen allergens Phl p 2 and Phl p 5 crossreact with homologous allergens present in several other grass species, thus explaining allergic symptoms of grass pollen allergic patients after contact with different grass species as being based on immunological crossreactivity. Further molecular and structural analysis of the interaction of IgE-Fabs and their corresponding allergens will provide knowledge regarding their mode of interaction, and help to devise specific forms of therapy that are based on the interruption of this contact. Recombinant allergen-specific IgE Fabs lack most parts of their constant region and in particular their Fc ϵ receptor I binding site and therefore are unable to activate allergic effector cells (e.g. mast cells, basophils) to release mediators. As IgE-Fabs contain the variable region needed for allergen-binding they will compete with complete IgE-antibodies for binding to allergens and represent possible therapeutic tools.

Recombinant Allergen-Specific Fabs for the Standardization of Natural Allergen Extracts Used for Diagnosis and Therapy

Diagnosis and specific therapy (specific immunotherapy) of Type I allergy are currently performed with natural allergen extracts. Natural allergen extracts are used for biological (i.e. elicitation of immediate type skin reactions) as well as for serological diagnosis (i.e. detection of IgE-antibodies with specificity for a certain allergen extract) of Type I allergy. The application of increasing doses of allergen extracts by injection (i.e. specific immunotherapy) is known since 1911 as an effective treatment of Type I allergy (Noon, 1911). For both, diagnosis and treatment of Type I allergy, natural extracts are still prepared from the allergen sources and, in addition to allergens, contain a series of other ill defined components. Moreover, extracts prepared from complicated tissues lack certain allergens due to inefficient extraction and degradation. The estimation of the contents of individual allergens in allergen extracts represents therefore a difficult problem that needs to be addressed by standardization procedures. Allergen extracts are currently standardized by biological methods (i.e. by testing their capacity to elicit immediate type skin reactions) and their allergen content is evaluated with antibodies (e.g. serum IgE from allergic patients, polyclonal antisera and monoclonal antibodies raised against defined allergens). Recombinant allergen-specific Fabs will represent extremely useful tools for the standardization of natural allergen extracts. Among the recombinant Fabs, human IgE-Fabs are of particular interest for standardization procedures because they mimic the reactivity of patients serum IgE most closely.

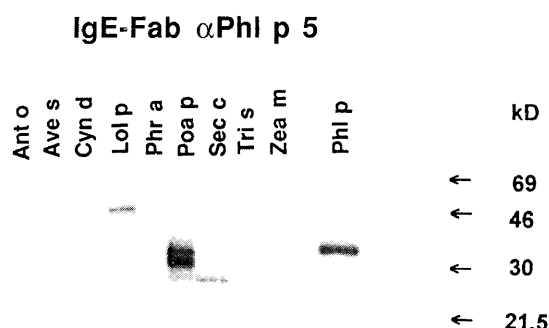


Fig. 3 A Phl p 5-Specific Human IgE Fab (Steinberger *et al.*, 1996) Crossreacts with Group 5 Allergens in Pollen from Different Grass Species.

Comparable amounts of pollen extracts from ten monocots were separated by SDS-PAGE, blotted onto nitrocellulose and probed with a human IgE-Fab with specificity for the major timothy grass pollen allergen Phl p 5 (Vrtala *et al.*, 1993). Abbreviations are as in Figure 2.

Use of Recombinant Allergen-Specific Fabs for the Measurement of Allergens in Allergen Sources and the Environment; Tools to Prevent Allergen Exposure

Sensitization is believed to occur early in childhood in genetically prone individuals after increased exposure to allergens. The exact measurement of the presence of allergens in our environment (i.e. air, buildings, and food) might therefore represent a relevant first line for allergy prevention. The generation of allergen-specific antibodies and recombinant antibody fragments will allow the precise detection of allergens and their amount in the environment and in various food products. Preventive measures may comprise the elimination of certain allergen sources (e.g. mites), the selection of 'hypoallergenic' plants and crops and the identification of food products with low allergen contents. The latter application may be also of interest regarding the analysis of food which has been modified by genetical engineering.

Allergen-Specific Antibodies and Antibody Fragments for Passive Therapy

There is good reason to expect that allergen-specific Fabs which interfere with the allergen-IgE interaction might be used for therapeutic purposes. Experimental data which indicate a limited diversity of allergens and their B-cell epitopes (reviewed in: Valenta and Kraft, 1995; Valenta *et al.*, 1996) as well as the observation that the IgE-reactivity profile in allergic patients is subjected to little variations encourage to consider passive therapy with blocking antibodies. IgE antibodies represent the least abundant class of immunoglobulins and it should therefore be possible to apply excess doses of competitors. It is further notable that allergic symptoms (allergic rhinitis, conjunctivitis, asthma, dermatitis, food intolerance) are mostly confined to certain target organs that are easily accessible and hence allow local therapy instead of systemic application.

Our contention that therapy with blocking allergen-specific Fabs might be a realistic approach is further supported by a body of experimental data on the effectiveness of blocking antibodies. The occurrence of allergen-specific antibodies which did not belong to the 'reaginic' class was reported early (Cooke *et al.*, 1935). It was demonstrated that certain allergen-specific IgG antibodies were able to inhibit anaphylactic reactions (Loveless, 1940; Lichtenstein *et al.*, 1968). Moreover it was speculated that blocking antibodies, in particular those belonging to the IgG₄ subclass might be induced by specific immunotherapy (Aalberse *et al.*, 1983). It was recently shown in a defined experimental system that human monoclonal antibodies with specificity for the major birch pollen allergen, Bet v 1 (Breiteneder *et al.*, 1989), blocked IgE-binding to Bet v 1 and inhibited the Bet v 1-induced histamine release (Visco *et al.*, 1996).

We suggest to use recombinant allergen-specific Fabs, either human IgG or IgE Fabs, for passive (blocking) therapy of Type I allergy. Bacterially expressed Fabs can be modified by standard molecular biological techniques and engineered according to the desired application. To reduce the immunogenicity of the Fabs it is possible to further reduce their size as well as to remove immunogenic parts. Alternatively, mouse monoclonal antibodies can be humanized to reduce their immunogenicity. By *in vitro* maturation, using mutagenesis or CDR rearrangements it is possible to improve the affinity of a recombinant Fab to its antigen. Such a manipulation might even increase the blocking activity of allergen-specific Fabs. Molecular and structural analysis of blocking Fabs might finally provide templates to build synthetic competitors of the allergen-IgE interaction.

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