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Possible therapeutic potential of a recombinant group 2 grass pollen allergen-specific antibody fragment

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Abstract

Background—The induction of blocking IgG antibodies that compete with IgE for allergen binding is one important mechanism of allergen-specific immunotherapy. The application of blocking antibodies may be an alternative treatment strategy.

Methods—A synthetic gene coding for a single chain fragment (ScFv) specific for the major timothy grass pollen allergen Phl p 2 was inserted into plasmid pCANTAB 5 E, and the recombinant ScFv was expressed in *Escherichia coli* and purified by affinity chromatography. The ScFv was tested for allergen binding by ELISA and its association and dissociation was measured by surface plasmon resonance (Biacore) technology. The ability of the ScFv to inhibit allergic patients IgE binding to Phl p 2 and Phl p 2-induced basophil degranulation was studied by ELISA competition and basophil activation (CD203c) assays.

Results—We report the expression, purification, biochemical and immunological characterization of a monomeric single chain fragment (ScFv) of human origin specific for the major timothy grass pollen allergen, Phl p 2. The Phl p 2-ScFv shows high affinity binding to the allergen and blocked the binding of allergic patients' polyclonal IgE to Phl p 2 up to 98%. Furthermore, it inhibited allergen-induced basophil activation.

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Authors Contributions: Gadermaier E, Flicker S and Valenta R designed the study. Gadermaier E generated and characterized the ScFv fragment, Flicker S assisted in purification. All three authors contributed to the manuscript by analysing the data, writing and reviewing the manuscript. Blatt K and Valent P performed or analysed basophil activation studies and contributed to the manuscript by reviewing.

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Conclusions—The Phl p 2-ScFv inhibited allergic patients IgE binding to Phl p 2 as well as Phl p 2-induced basophil activation and might be useful for passive immunotherapy of grass pollen allergy.

Keywords

Allergy; grass pollen; recombinant single chain fragment; therapy

Grass pollen is one of the most important allergen sources worldwide (1). Allergen-specific immunotherapy (SIT) is a clinically effective, disease-modifying treatment for grass pollen allergy and has long lasting effects (2). However, it has been demonstrated that current allergen extract-based SIT induces protective IgG responses mainly against group 1 and group 5 allergens but not sufficiently against group 2 allergens which represent major allergens for more than 60% of grass pollen allergic patients (3, 4). Moreover, it has been demonstrated in clinical studies that the group 2 allergen from timothy grass pollen, Phl p 2, is besides Phl p 5 the most potent allergen in eliciting immediate type skin and respiratory symptoms (5, 6).

Using combinatorial cloning technology we have previously identified a Phl p 2-specific IgE Fab (7) which after conversion into a human IgG₁ antibody inhibited allergic patients' IgE binding to Phl p 2 and Phl p 2-induced basophil activation and therefore may be useful for treating patients by passive immunization (7, 8).

However, in the case of grass pollen allergy the major manifestations of disease are allergic rhinitis, conjunctivitis and asthma for which topical forms of treatment would be advantageous. For such topical application large amounts of antibodies which are easy and inexpensive to produce and which should have low immunogenicity would be needed. Unfortunately, complete antibodies may elicit neutralizing antibodies in treated patients even when they are fully human and their production is very expensive because cell culture systems are needed (9). In order to decrease costs and immunogenicity it has therefore been suggested to use antibody fragments (*i.e.*, Fabs) (9).

Methods, Results and Discussion

We report the expression, purification and characterization of a ScFv fragment in *E. coli* derived from a Phl p 2-specific IgE Fab (7) designated Phl p 2-ScFv. Major advantages of the Phl p 2-ScFv over the previously described IgE Fab are that it consists of one, always correctly assembled polypeptide chain and that it can be expressed in *E. coli* as soluble, monomeric ScFv (28 kDa) in good yields (~ 300 µg/l at lab scale conditions without re-engineering or fed batch fermentation) (Fig 1A).

The Phl p 2-ScFv specifically recognized group 2 allergens (*i.e.*, Phl p 2) but no other unrelated timothy grass pollen allergens (*e.g.* Phl p 1, Phl p 5, Phl p 6, Phl p 7, Phl p 12, Phl p 13) or the major birch pollen allergen Bet v 1 (data not shown). Importantly, surface plasmon resonance studies revealed a similar affinity constant of the ScFv fragment to Phl p 2 as was found for the complete antibody (8). The affinity constant (K_D) for Phl p 2-ScFv:

3.88×10^{-11} M could be compared to that of the Phl p 2 IgG (i.e., 1.14×10^{-10} M) since the latter was determined in a 1:1 interaction model (8) (Fig 1B).

Using a competitive IgE ELISA (8) performed in excess of Phl p 2-ScFv (i.e., 20 µg/ml) it was demonstrated that the purified Phl p 2-ScFv inhibited significantly ($p < 0.05$) the binding of grass pollen allergic patients ($n=20$) IgE to Phl p 2 yielding a maximal inhibition of up to 98% in certain patients (40 % median inhibition) (Fig 2A). Pilot experiments showed that full IgE inhibition was obtained already at a concentration of >4 µg/ml of purified Phl p 2-ScFv indicating that a possible therapeutic effect may be achieved with a relatively low concentration of ScFv (data not shown). This extent of inhibition is quite remarkable considering that the patients' IgE response (10, 11) is polyclonal and that the ScFv format may cause less steric hindrance than a complete antibody. Perhaps more important, we found that the purified Phl p 2-ScFv also inhibited Phl p 2-induced basophil activation measured by up-regulation of CD203c in each of the tested patients more than 100-fold (Fig 2B) (12). In fact, Fig 2B shows that after pre-incubation of Phl p 2 with the specific ScFv a more than 100-fold dose of allergen gave a similar SI compared to pre-incubation with the control ScFv.

Due to the fact that the Phl p 2-ScFv lacks IgG constant regions, co-crosslinking of IgE and IgG receptors can be excluded as underlying mechanism for inhibition of basophil activation (13). Possible mechanisms for the inhibition of IgE binding to the allergen therefore comprise direct competition of the ScFv with IgE for the same epitope and/or portions thereof, steric hindrance or induction of conformational changes leading to loss of IgE binding. This latter mechanism can be excluded based on the study by Padavattan *et al.* (8). Therefore, competition of the ScFv with IgE antibodies for their binding sites on the allergen must be the mechanism underlying the inhibition of basophil activation. This competition may occur either through direct competition of the ScFv for the binding site of IgE or through steric hindrance of IgE binding. This finding that a ScFv strongly inhibits IgE binding and basophil activation underlines that the blocking activity of antibodies or antibody derivatives resides in their variable region and not in the constant regions and therefore is not necessarily associated with a certain immunoglobulin class or subclass (e.g., IgG₄).

The more than 100-fold inhibition of basophil activation indicates that the Phl p 2-ScFv has therapeutic potential and may be considered for systemic and topical application. Suitable patients may be selected by testing for IgE reactivity to Phl p 2 and the inhibition of this IgE binding to Phl p 2 by the Phl p 2-ScFv. The systemic route may require additional engineering of the antibody fragment which would increase serum half-life (14) and the size and therefore the inhibiting potential of the antibody fragment due to additional sterical inhibition of IgE binding, but could also increase immunogenicity and costs of production. The non-modified ScFv can be easily produced in *E. coli* at low costs and hence seems particularly suitable for repeated topical application but this assumption needs to be substantiated by clinical studies.

The Phl p 2-ScFv can now be manufactured under conditions of good manufacturing practice for clinical trials investigating whether topical application to the eyes and

respiratory tract can inhibit group 2 allergen-induced allergic symptoms. Although the inhibition of IgE binding to Phl p 2 by Phl p 2-ScFv was not always complete a more than 100-fold reduction of basophil activation was obtained with the purified Phl p 2-ScFv (Fig 2B). However, in certain patients (approx. 10%) no inhibition of IgE binding was found but inhibition does not need to be an absolute prerequisite for topical application. It is possible that capturing of the allergen by the Phl p 2-ScFv reduces its penetration through the epithelial barrier and allergen-induced activation of inflammatory cells.

The Phl p 2-ScFv may be considered as add on local therapy for grass pollen allergic patients who are sensitized to group 2 allergens and do not produce sufficiently protective group 2 allergen-specific IgG antibodies in the course of SIT. Furthermore, the Phl p 2-ScFv may be part of a cocktail of protective grass pollen allergen-specific ScFvs for passive therapy of grass pollen allergy. All of these possible therapeutic applications will however require clinical testing in proof of principle studies.

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References

1. Suphioglu C. What are the important allergens in grass pollen that are linked to human allergic disease? *Clin Exp Allergy*. 2000; 30(10):1335–1341. [PubMed: 10998006]
2. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med*. 1999; 341(7):468–475. [PubMed: 10441602]
3. Mothes N, Heinzkill M, Drachenberg KJ, Sperr WR, Krauth MT, Majlesi Y, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy*. 2003; 33:1198–1208. [PubMed: 12956739]
4. Gadermaier E, Staikuniene J, Scheibelhofer S, Thalhammer J, Kundi M, Westritschnig K, et al. Recombinant allergen-based monitoring of antibody responses during injection grass pollen immunotherapy and after 5 years of discontinuation. *Allergy*. 2011; 66(9):1174–1182. [PubMed: 21480924]
5. Westritschnig K, Horak F, Swoboda I, Balic N, Spitzauer S, Kundi M, et al. Different allergenic activity of grass pollen allergens revealed by skin testing. *Eur J Clin Invest*. 2008; 38(4):260–267. [PubMed: 18339006]
6. Niederberger V, Stubner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K, et al. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol*. 2001; 117(4):848–851. [PubMed: 11676821]
7. Flicker S, Steinberger P, Norderhaug L, Sperr WR, Majlesi Y, Valent P, et al. Conversion of grass pollen allergen-specific human IgE into a protective IgG(1) antibody. *Eur J Immunol*. 2002; 32(8):2156–2162. [PubMed: 12209627]
8. Padavattan S, Flicker S, Schirmer T, Madritsch C, Randow S, Reese G, et al. High-affinity IgE recognition of a conformational epitope of the major respiratory allergen Phl p 2 as revealed by X-ray crystallography. *J Immunol*. 2009; 182(4):2141–2151. [PubMed: 19201867]
9. Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of therapeutic antibodies. *Nat Rev Immunol*. 2010; 10(5):345–352. [PubMed: 20414207]
10. Levin M, Ohlin M. Inconclusive evidence for or against positive antigen selection in the shaping of human immunoglobulin E repertoires: a call for new approaches. *Int Arch Allergy Immunol*. 2013; 161(2):122–126. [PubMed: 23343692]

11. Gadermaier E, Levin M, Flicker S, Ohlin M. The human IgE repertoire. *Int Arch Allergy Immunol*. 2014; 163:77–91. [PubMed: 24296690]
12. Hauswirth AW, Natter S, Ghannadan M, Majlesi Y, Schernthaner GH, Sperr WR, et al. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol*. 2002; 110(1):102–109. [PubMed: 12110828]
13. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIB cross-linking. *J Clin Invest*. 2006; 116(3):833–841. [PubMed: 16498503]
14. Kontermann RE. Strategies for extended serum half-life of protein therapeutics. *Curr Opin Biotechnol*. 2011; 22(6):868–876. [PubMed: 21862310]

A

$$K_d = 3.26 \times 10^{-5} \text{ s}^{-1}$$

$$K_a = 8.41 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$$

$$K_D = 3.88 \times 10^{-11} \text{ M}$$

B

FIG 1.

A, Coomassie blue-stained SDS-PAGE. Protein molecular mass marker (lane M) (kDa) and purified Phl p 2-ScFv (lane 1). **B**, Sensor chip-based studies of the interaction between Phl p 2 and different concentrations of Phl p 2-ScFv. Signal intensities (RU) are shown (y-axis) versus the time (s) (x-axis). Association (k_a), dissociation rate constants (k_d) and affinity constant (K_D) are indicated.

Patient	Control ScFv (20 µg/ml)	Phl p 2-ScFv (20 µg/ml)	% Inhibition
1	1.54	0.83	45.7
2	1.47	0.84	42.6
3	0.22	0.15	32.7
4	0.25	0.16	38.0
5	0.49	0.33	33.2
6	0.18	0.16	13.3
7	0.16	0.17	-5.2
8	0.47	0.30	36.8
9	0.76	0.52	30.8
10	0.60	0.23	62.0
11	0.24	0.11	53.0
12	0.72	0.43	41.1
13	0.27	0.17	38.8
14	0.45	0.29	35.2
15	1.61	0.75	53.6
16	0.49	0.18	62.4
17	0.44	0.26	41.4
18	0.38	0.24	36.8
19	0.51	0.01	97.6
20	1.01	0.54	46.9
Median	0.48	0.25	40.0

A

B

FIG 2.

A, Patients' (#1-20) IgE binding (OD values) to plate-bound Phl p 2 after pre-incubation with purified Phl p 2-ScFv or an unrelated ScFv and percentages of inhibition are shown. Median IgE binding and median percentages of inhibition are shown in the bottom line. **B**, Blood leukocytes from three Phl p 2-allergic patients (numbering as in Fig 2A) were exposed to increasing doses of Phl p 2 (x-axis) that had been pre-incubated with purified the Phl p 2-ScFv or an unrelated ScFv. CD203c up-regulation was calculated from mean fluorescence intensities (MFIs) obtained with stimulated (MFI_{stim}) and unstimulated

(MFI_{control}) cells and is expressed as stimulation index (SI= MFI_{stim}/MFI_{control}) (mean +/– SD) that is displayed on the y-axis.