

27639

Peter Steinberger
Dietrich Kraft
Rudolf Valenta

Institute of General and
Experimental Pathology, AKH,
University of Vienna, Austria

Expression in *Escherichia coli* of Human IgE Antibody Fragments with Specificity for Major Timothy Grass Pollen Allergens Using the Combinatorial Library Approach

Key Words

Immunoglobulin E · Recombinant IgE-Fabs · Combinatorial library ·
Major grass pollen allergens · Expression in *Escherichia coli*

Introduction

Although immunoglobulin E represents the least abundant immunoglobulin class present in serum, it is extremely powerful in the induction of immediate-type reactions (allergic rhinoconjunctivitis, asthma and anaphylactic shock) due to the Fcε-receptor-mediated activation of effector cells (mast cells, basophils) [1, 2]. The low concentration of IgE in serum (10–400 ng/ml) and the low frequency of IgE-producing cells [3] have made it difficult to characterize IgE antibodies at a molecular level. The heavy-chain constant region of human IgE has been well characterized by protein chemical and molecular biological techniques [4, 5]. In contrast, little information is available regarding the variable regions of human IgE antibodies and their usage in atopic individuals [6, 7]. So far, it has been impossible to grow and culture human B cells producing IgE antibodies with specificity for defined allergens. In order to characterize human IgE antibodies with specificity for major allergens and to investigate their interaction with the corresponding allergens at a molecular level, we have chosen the combinatorial library approach. Using the pComb3H plasmid, which is able to recombine an immunoglobulin heavy-chain fragment with a light chain so that the corresponding Fab fragment can also be presented on the surface of filamentous phage [8], it has become possible to express functional antibody fragments in *Escherichia coli* and to screen a large phage repertoire by panning to the given antigen. The pre-

sent contribution describes the construction of an IgE combinatorial library from a patient allergic to grass pollen and the isolation of recombinant human IgE-Fabs with specificity for major grass pollen allergens [9].

Results and Discussion

In order to determine the optimal source for B lymphocytes which produce allergen-specific IgE antibodies, we investigated the influence of cytokines, which, such as IL-4 and IL-13, represent well-established inducers of IgE synthesis in allergen-specific IgE synthesis. While IL-4 and IL-13 were able to upregulate of total IgE synthesis in short time cultures of human mononuclear cells, no influence on allergen-specific IgE synthesis was observed [10, 11]. B cells from allergic individuals showed spontaneous secretion of allergen-specific IgE antibodies which was neither upregulated by IL-4, IL-13 or in conjunction with anti-CD40 antibodies nor was it downregulated by a neutralizing IL-4 antibody. We therefore chose freshly isolated peripheral blood lymphocytes obtained from a patient allergic to grass pollen shortly after the grass pollen season as a source for the isolation of RNA (fig. 1). cDNAs coding for IgE heavy chain fragments and kappa and lambda light chains were reversely transcribed and PCR amplified using specific primers. A combinatorial library containing randomly recombined IgE heavy chain coding cDNAs and light chain

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34
<http://www.karger.ch>

© 1997 S. Karger AG, Basel
1018–2438/97/1133–0258 \$12.00/0

This article is also accessible online at:
<http://BioMedNet.com/karger>

Correspondence to: Dr. Rudolf Valenta
Institute of General and Experimental Pathology,
AKH, University of Vienna
Währinger Gürtel 18–20, A–1090 Vienna (Austria)
Tel. +43 1 40400 5108, Fax +43 1 40400 5130

Questa fotocopia viene fornita
per motivi strettamente personali
di studio e/o ricerca

Isolation of RNA from fresh peripheral blood lymphocytes of an allergic individual



Reverse transcription of the IgE-heavy chain, kappa light chain and lambda light cDNAs



PCR amplification of IgE-heavy chain fragment cDNAs and light chain cDNAs



Random combination of the heavy chain fragment and light chain cDNAs in plasmid pComb3H



Display of IgE Fabs on the surface of filamentous phage and isolation of phage expressing allergen specific IgE Fabs by panning to recombinant allergens



DNA sequence analysis of the cDNAs coding for allergen specific IgE Fabs and expression of soluble IgE Fabs in *E. coli*

cDNAs was then constructed in plasmid pComb3H and subjected to panning using ELISA plates coated with major timothy grass pollen allergens Phl p 1, Phl p 5 and Phl p 2 [9]. Phage expressing IgE Fabs with specificity for the three major grass pollen allergens were obtained and soluble IgE Fab fragments were expressed in *Escherichia coli* which could be purified by affinity chromatography using the immobilized allergens. Recombinant IgE Fabs cross-reacted with homologous allergens present in different grass species and partly inhibited IgE binding to the allergen. In conclusion, we suggest to consider recombinant IgE Fabs with specificity for major allergens as useful tools for a number of different purposes, among them: (1) investigation of the interaction of IgE and allergens on a molecular level; (2) for passive therapy to interfere with the IgE-allergen interaction in the allergic effector organs; (3) standardization of currently used allergen extracts; (4) environmental allergen analysis and (5) for the selection of hypoallergenic plant cultivars and food.

Acknowledgment

This study was supported by in part by grants S06703 and F0506 of the Austrian Science Foundation.

Fig. 1. The combinatorial library approach to isolate IgE antibody fragments with specificity for allergens.

References

- 1 Ishizaka K, Ishizaka T: Human reaginic antibodies and immunoglobulin E. *J Allergy* 1968; 42:330-363.
- 2 Bennich HH, Ishizaka K, Johansson SG, Rowe DS, Stanworth DR, Terry WD: Immunoglobulin E: A new class of human immunoglobulin. *Immunology* 1968;15:323-324.
- 3 MacKenzie T, Dosch HM: Clonal and molecular characteristics of the human IgE-committed B cell subset. *J Exp Med* 1989;169:407-430.
- 4 Flanagan JG, Rabbits TH: The sequence of a human immunoglobulin epsilon heavy chain constant region gene, and evidence for three non-allelic genes. *EMBO J* 1982;1:655-660.
- 5 Seno M, Kurokawa T, Ono Y, Onda H, Sasada R, Igarashi K, Kikuchi M, Sugino Y, Nishida Y, Honjo T: Molecular cloning of human immunoglobulin epsilon chain cDNA. *Nucleic Acid Res* 1983;11:719-726.
- 6 van der Stoep N, van der Linden, Logtenberg T: Molecular evolution of the human immunoglobulin E response: High incidence of shared mutations and clonal relatedness among ϵV_H5 transcripts from three unrelated patients with atopic dermatitis. *J Exp Med* 1993;177:99-107.
- 7 Snow RE, Chapman CJ, Frew AJ, Holgate ST, Stevenson FK: Analysis of Ig VH region genes encoding IgE antibodies in splenic B lymphocytes of a patient with asthma. *J Immunol* 1995; 154:5576-5581.
- 8 Barbas CF III, Lerner RA: Combinatorial immunoglobulin libraries on the surface of phage (Phabs): Rapid selection of antigen-specific Fabs. *Methods* 1991;2:119-124.
- 9 Steinberger P, Kraft D, Valenta R: Construction of a combinatorial IgE library from an allergic patient. *J Biol Chem* 1996;271:10967-10972.
- 10 Steinberger P, Bohle B, di Padova F, Wrann M, Liehl E, Scheiner O, Kraft D, Valenta R: Allergen-specific IgE production of committed B cells from allergic patients in vitro. *J Allergy Clin Immunol* 1995;96:209-218.
- 11 Dolecek C, Steinberger P, Susani M, Kraft D, Valenta R, Boltz-Nitulescu G: Effects of IL-4 and IL-13 on total and allergen specific IgE production by cultured PBMC from allergic patients determined with recombinant pollen allergens. *Clin Exp Allergy* 1995;25:879-889.