

Promiscuous Use of Light Chains by Human IgE Antibodies Specific for Three Major Grass Pollen Allergens

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Key Words

IgE, monoclonal human · Grass pollen allergens · Antibody expression · Heavy chain shuffling · Light chain shuffling

IgE antibodies are the key molecules responsible for the immediate and also late reactions in type I allergy, a genetically determined hypersensitivity disease [1]. Cross-linking of effector cell-bound IgE antibodies by allergens leads to the release of biological mediators (e.g., histamine, leukotrienes) and thus to the immediate symptoms of disease (allergic rhinoconjunctivitis, asthma, anaphylactic shock) [2, 3]. Furthermore, presentation of allergens via IgE antibodies strongly induces the proliferation of allergen-specific T cells and the release of proinflammatory cytokines [4, 5]. Despite the central role of IgE in the pathogenesis of allergic diseases, the characterization of the IgE molecule has been hampered by the extremely low concentration of IgE in serum and the low precursor frequency of IgE-producing cells. After the immunological and biochemical characterization of IgE in the late 60s [6, 7], the cDNA and deduced amino acid sequence of the IgE constant region was revealed [8, 9]. However,

due to the impossibility of immortalizing allergen-specific human IgE-producing cells, combinatorial library technology in conjunction with phage display technology was required to isolate human monoclonal IgE antibodies with specificity for a major grass pollen allergen [10].

We have isolated human IgE-Fabs with specificity for three major timothy grass pollen allergens, PhL p 1, PhL p 2 and PhL p 5, out of a combinatorial library constructed from lymphocytes of a grass pollen-allergic patient. Sequence analysis of four independently obtained PhL p 5-specific IgE-Fabs indicated that one PhL p 5-specific heavy chain could combine with four different light chains without loss of specificity [10]. To investigate whether IgE recognition of PhL p 5 and other allergens may primarily depend on the heavy chain, shuffling experiments were performed.

We recombined in plasmid pComb 3H [11] cDNAs coding for heavy chain fragments of IgE antibodies specific for PhL p 1, PhL p 2 and PhL p 5 with light chain-encoding cDNAs derived from antibodies specific for immunologically unrelated allergens. Figure 1 details the shuffling experiments for a PhL p 5-specific IgE Fab. The cDNA coding for the heavy chain fragment of a PhL p 5-specific IgE Fab was recombined with light chains from IgE Fabs specific for unrelated grass pollen allergens (PhL

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Shuffling experiment

<u>Light chain cDNA</u>		<u>Heavy chain fragment cDNA</u>	
1	human IgE anti-Phl p 5	+	human IgE anti-Phl p 5
2	human IgE anti-Phl p 1		
3	human IgE anti-Phl p 2		
4	human IgG ₄ anti-Bet v 1		
5	mouse IgG ₁ anti-Bet v 1		

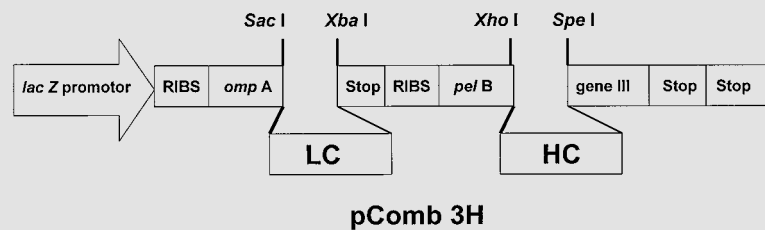


Fig. 1. Illustration of the antibody heavy chain fragment and light chain shuffling experiments. Heavy chain fragment-encoding cDNAs (HC) and light chain-encoding cDNAs (LC) can be recombined in plasmid pComb 3H and expressed as functional Fabs in *Escherichia coli*. RIBS = Ribosome-binding site. *ompA* and *pelB* code for leader peptides required for periplasmic expression.

p 1, PhL p 2) as well as with light chains from human and mouse IgG antibodies with specificity for the major birch pollen allergen, Bet v 1, using plasmid pComb 3H [11]. The identity of the obtained constructs was verified by sequencing. Replacement of light chains in IgE Fabs specific for PhL p 1, PhL p 2 and PhL p 5 with light chains from antibodies with specificity for unrelated allergens maintained the binding to the allergens whereas similar experiments performed with allergen-specific IgG led to a

loss of recognition. These results indicate that at least certain allergen-specific IgE antibodies are characterized by a promiscuous use of light chains.

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