

Lessons from the Antibody Recognition of the Major Timothy Grass Pollen Allergen Phl p 1

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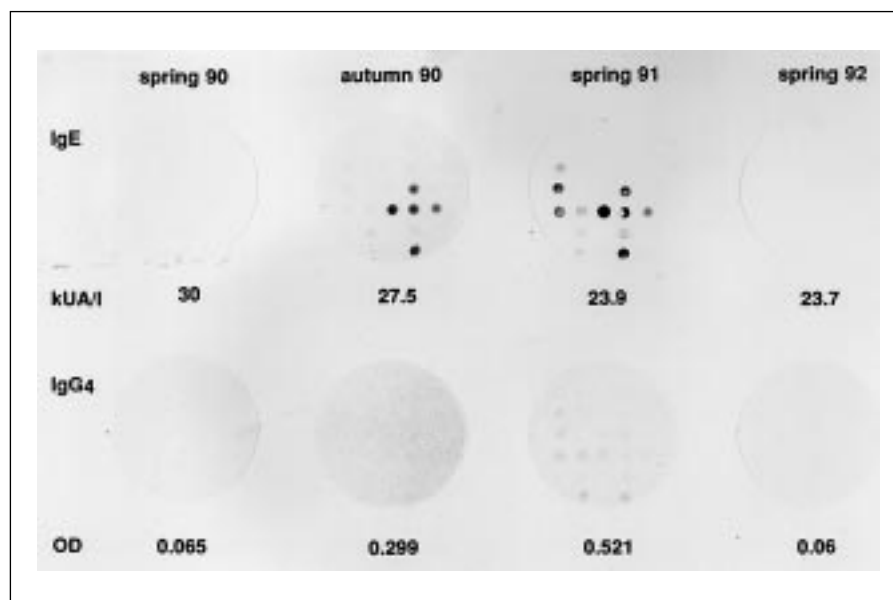
Key Words

B cell epitopes · Timothy grass pollen allergen, major · Phl p 1

At least 40% of type I allergic patients are sensitized against grass pollen allergens [1], of which the major allergenic component, the group 1 allergens, are recognized by more than 95% of grass pollen-allergic patients [2]. Molecular cloning revealed high sequence conservation among group 1 allergens from divergent grass species [3], thus representing the basis for extensive cross-reactivity among the group 1 allergens [4]. The group 1 allergen of timothy grass, Phl p 1 [3], was selected to investigate the interaction of IgE and IgG4 antibodies with a prominent allergen at the molecular level. In order to define continuous (sequential) epitopes, we randomly fragmented the Phl p 1 cDNA, to construct an epitope expression library [5]. By screening of the epitope expression library with IgE and IgG4 antibodies of timothy grass pollen allergic patients we identified five epitopic areas on Phl p 1, which are conserved among group 1 allergens from various species. Comparison of the IgE- and IgG4-defined fragments of Phl p 1 revealed that IgE and IgG4 antibodies bind to the same, but also to different fragments with varying intensity. In order to estimate the percentage of Phl p 1-specific IgE that is directed against the recombinant fragments, RAST-based IgE inhibition experi-

ments were performed. A combination of recombinant Phl p 1 fragments inhibited the IgE binding of 13 grass pollen-allergic patients to complete rPhl p 1 from 11 to 69%, indicating the relevance of the defined continuous epitope-containing fragments in IgE recognition. When we analyzed serum samples of grass pollen allergic patients without immunotherapy that were collected over several years for their IgE reactivity with purified recombinant timothy grass pollen allergens and with recombinant Phl p 1 fragments, we found no evidence for a change in the qualitative allergen recognition pattern. Likewise the IgE reactivity pattern to recombinant Phl p 1 fragments remained stable in the natural course of the disease. Thus, no evidence was obtained that 'epitope spreading', a phenomenon described for various autoimmune diseases, could play a role in atopic diseases. However, when we analyzed sera obtained from patients before and during grass pollen immunotherapy, we found an induction of rPhl p 1 fragment-specific IgE and IgG4 response in the course of therapy (fig. 1). This finding suggests that allergen-specific immunotherapy can induce antibody responses against previously not recognized epitopes. Support for a blocking activity of these antibodies comes from the finding that increases of rPhl p 1-specific IgG4 were accompanied by decreases in rPhl p 1-specific IgE levels. Our additional observation that IgE and IgG4 responses were directed to partly different epitopes and followed different kinetics indicates that allergen recognition by different antibody isotypes in allergic individuals is

Fig. 1. Induction of IgE and IgG4 antibodies to recombinant Phl p 1 fragments during immunotherapy. Serum samples from a Phl p 1 allergic patient were obtained before (spring 90) and during hyposensitization treatment (autumn 90, spring 91, spring 92) and tested for IgE and IgG4 reactivity to recombinant Phl p 1 fragments. The levels of rPhl p 1-specific IgE and IgG4 antibodies in each serum sample are shown in kUA/I and optical density (OD), respectively.



poorly synchronized, and may be interpreted as a footprint of a preferentially nonsequential class switch event [6]. Focusing IgG4 responses to IgE epitopes by using allergen fragments may thus represent a promising immunotherapy strategy [7].

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