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## Heterogeneity in the IgE Binding to a Peptide Derived from the House Dust Mite Allergen *Der p II* Indicates that the IgE Response Is Highly Polyclonal

### Key Words

*Der p II*  
Peptides  
Idiotypic

### Abstract

The fine specificity of IgE antibody binding to peptide 65-78 of the house dust mite major allergen *Der p II* was examined by comparison with binding to two peptides in which the cysteines corresponding to cys<sup>73</sup> and cys<sup>78</sup> in *Der p II* were substituted by serines and methionines. Differences in binding behavior indicated that at least three different subpopulations of IgE antibodies bound to peptide 65-78. Even at the level of such a small fragment the IgE response in individual donors proved to be polyclonal.

### Introduction

Jerne [1] has postulated that the IgE synthesis is regulated by an idiotype network and several groups have concluded that IgE antibodies can elicit anti-idiotypic antibodies that are able to mimic the epitope on the allergen against which the IgE antibodies were directed [2-5]. In one study nonallergic donors were claimed to have higher levels of anti-idiotypic antibodies and the levels of anti-idiotypic antibodies in allergic donors were found to normalize following immunotherapy [6]. Two possible mechanisms have been suggested to account for the desensitizing effect of auto-anti-idiotypic antibodies. As anti-idiotypic antibodies mimic the allergen they were believed to inhibit binding of allergen to mast cell bound specific IgE without being able to induce mast cell degranulation, however several groups found that anti-idiotypic antibodies can induce histamine release from mast cells [7, 8]. Another

mechanism explains the desensitizing effect of anti-idiotypic antibodies by depletion of cell-bound IgE by continuous triggering of the mast cells by subsymptomatic doses of these antibodies. This type of immunotherapy is more likely to be successful if the IgE response is oligoclonal. The clonality of the IgE response was studied with synthetic peptides.

In our epitope mapping studies with synthetic peptides of the major allergen *Der p II* from the house dust mite *Dermatophagoides pteronyssinus* we found that IgE antibodies from 60% of a panel of 246 sera from patients with a high house dust mite sensitization bound to the overlapping peptides comprising residues 65-78 and 74-87 of *Der p II*. However, only 7 of these sera gave more than 10% binding of anti-IgE in the peptide-RAST, showing that strong binding to these peptides is uncommon. From 3 of these 7 sera larger quantities could be obtained for inhibition studies. For these 3 sera we found that IgE antibody

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binding to Sepharose-coupled peptide 65-78 was inhibited by native *Der p* II to less than 20% of the uninhibited value. This complete inhibition showed that binding to peptide 65-78 was specific. For only one of these three sera we found specific IgE antibody binding to the shorter peptide 69-77 [9].

To further investigate this difference in binding behavior, we synthesized and tested two derivatives of peptide 65-78 in which both cysteines corresponding to *cys*<sup>73</sup> and *cys*<sup>78</sup> in *Der p* II were substituted by serine or methionine residues, thus mimicking reduction or reduction/alkylation, respectively.

## Material and Methods

### Peptide Synthesis and Testing

The peptide VPGIDPNACHYMKCK (peptide 65-78 of *Der p* II with an additional C-terminal lysine), the *ser*<sup>73</sup>/*ser*<sup>78</sup> and *met*<sup>73</sup>/*met*<sup>78</sup> variants were synthesized using the T-bag method and 9-fluorenylmethoxycarbonyl/*t*-butyl-strategy as described elsewhere [9–11]. They were coupled to CNBr-activated Sepharose-4B (Pharmacia, Uppsala, Sweden) without further purification (4 mg of peptide to 500 mg of Sepharose). The purity of the peptides was verified with reversed-phase HPLC [Delta Pak C-18, gradient from 0.1% trifluoroacetic acid (TFA) in water to 0.1% TFA in acetonitrile, UV detection at 214 and 280 nm] and varied from 70 to 80%. The chromatograms showed one or two secondary peaks. The most likely contaminants are byproducts formed by degradation of the Asp-Pro bond between residue 69-70 during cleavage from the solid-phase synthesis resin with TFA. For peptide 65-78 we found that the IgE antibodies from the three sera bound to the major component with the expected amino acid composition [9]. In order to direct the coupling to Sepharose the peptides were synthesized with the additional C-terminal lysine and an acetylated N-terminus. In the peptide-RAST these solid-phase antigens (0.5 mg) were incubated 16 h with IgE serum (50  $\mu$ l) in PBS-AT<sup>1</sup> (500  $\mu$ l). After 5  $\times$  washing with PBS-T, 16 h incubation with <sup>125</sup>I-radiolabeled sheep antihuman IgE antibodies in 500  $\mu$ l PBS-ATS (1 ng, 400 Bq/test) and 5  $\times$  washing with PBS-T the gamma-radiation was counted. Binding was expressed as percentage of added radiolabel [12]. As blank Sepharose blocked with 0.5 M glycine pH 8.5 and a Sepharose-coupled irrelevant peptide (GVTHDQLNNFRAGFD) containing an IgE epitope from the nonrelated allergen *Chi t* I [13] were used.

In the inhibition studies the antisera (50  $\mu$ l) were preincubated with dilution series of inhibitor (50  $\mu$ l) for at least 2 h. After addition of the Sepharose-coupled peptides in an end volume of 600  $\mu$ l PBS-AT, the RAST procedure described above was followed. As inhibitors a *Dermatophagoides pteronyssinus* extract (Commonwealth Serum Laboratories, Australia: a whole-body extract containing 2,750 nM *Der p* II by comparison with the international reference NIBSC 82/518), a *Dermatophagoides farinae* extract (a gift from Dr. M.D.

Chapman, U Va, Charlottesville, Va., USA, containing 16 nM of the crossreactive major allergen *Der f* II from the related house dust mite *D. farinae* by comparison with NIBSC 82/518) and the three peptide derivatives were used. As negative control the *Chi t* I related peptide was used.

As control for the inhibition studies IgE(Ha) was incubated in dissociation experiments with the three Sepharose-coupled peptide derivatives (of both the serine and methionine peptides 1 mg was coupled to 250 mg CNBr-activated Sepharose to obtain a similar percentage initial binding as for unmodified Sepharose-coupled peptide 65-78) for 16 h. After washing away unbound antibodies, mite extract (0.14 pmol *Der p* II/test) was added to neutralize antibodies dissociating from the solid phases. After 1, 4 and 25 h the solid phases were washed 5  $\times$  with PBS-T and radiolabeling was performed as described above. As initial binding no mite extract was added.

Specificity of binding was also demonstrated by comparison with unrelated IgE antibodies. We tested a panel of 25 IgE sera from non-allergic subjects with an increased IgE content (200–1,300 U/ml) for nonspecific binding. None of these sera showed significant IgE binding in the three peptide RASTs. In a similar panel of 25 subjects with > 20% binding in the mite RAST (IgE content 200–2,300 U/ml), we found 1 serum with strong IgE binding to Sepharose-coupled peptide 65-78 and weak binding to the substituted peptides and 7 sera with only weak binding to Sepharose-coupled peptide 65-78. One of these sera gave weak IgE binding to both substituted peptides, one only to the methionine peptide.

## Results

The results of binding and inhibition studies with the three Sepharose-coupled 14-mer peptides and IgE(Bi), IgE(Bo) and IgE(Ha) are summarized in table 1. A distinct difference in binding properties was found for IgE (Bi) and IgE(Bo) versus IgE(Ha). For all three sera specificity of IgE binding to these Sepharose-coupled peptides was demonstrated by inhibition by *Der p* II and *Der f* II. Binding was not inhibited by the negative control peptide.

The results from dissociation experiments with IgE(Ha) are given in figure 2. The less steep slope for Sepharose-coupled peptide 65-78 compared to both substituted derivatives correspond with a higher overall affinity of IgE(Ha) to the unmodified peptide.

## Discussion

Substitution of both cysteines of peptide 65-78 of *Der p* II by serine and methionine had different effects on the antibody responses of IgE(Bi) and IgE(Bo) compared to that of IgE(Ha). In order to simplify the discussion only the most extreme differences, i.e. the effects of substitution by methionines on the antibody behavior of IgE(Bi) and IgE(Ha), are discussed.

<sup>1</sup> PBS = Phosphate-buffered saline; PBS-AT = phosphate-buffered saline with 0.3% bovine serum albumin and 0.1% Tween-20; PBS-ATS = PBS-AT with 4.5% normal bovine serum and 0.5% normal sheep serum; PBS-T = phosphate-buffered saline with 0.1% Tween-20.

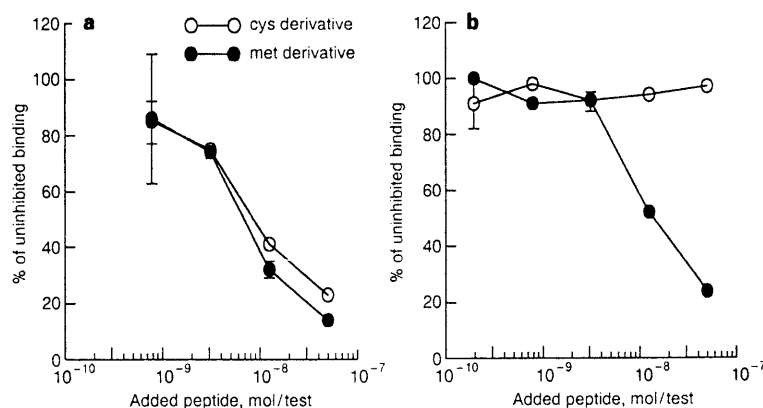


**Fig. 1.** Homologous and heterologous inhibition of IgE(Ha) binding to Sepharose-coupled cysteine peptide (**a**) and methionine peptide (**b**) by both peptides.

For serum Bi, all anti-peptide antibodies reacted well with the cysteine peptide, but heterogeneity was found in the reactivity to the methionine peptide. Part of the IgE antibodies reacts equally well with the methionine peptide and with the cysteine peptide, as indicated by the similar inhibitory activities of these two peptides in the methionine peptide RAST. Another part of the IgE antibodies reacts preferentially with the cysteine peptide, as indicated by the higher inhibitory activity of the cysteine peptide in the cysteine peptide RAST. This also fits with the higher uninhibited binding found in the cysteine peptide RAST compared to the methionine peptide RAST (table 1).

For serum Ha, the situation was reversed: most if not all peptide antibodies reacted well with the methionine peptide, but heterogeneity was found in the reactivity to the cysteine peptide: the majority of the methionine peptide-binding antibodies did not react well with the cysteine peptide (fig. 1). The finding of IgE antibodies with a higher reactivity to the 'unnatural' methionine peptide can be explained by assuming that these antibodies are directed against the N-terminal part of the peptide. In the cysteine peptide cystine formation (cys<sup>73</sup>-cys<sup>78</sup>) in the C-terminal side results in a more folded structure that changes the configuration or accessibility of the N-terminal side. However, in the intact protein the epitope recognized by the IgE antibodies specifically reactive with the methionine peptide is available, as indicated by the efficient inhibition of the methionine peptide-RAST by the allergen (table 1). For both patients binding to this peptide was inhibited by *Der p* II to less than 20% of the uninhibited value [data not shown].

Higher binding to methionine peptide compared to cysteine peptide was found for IgE(Ha). In view of the dis-

**Table 1.** Inhibition studies with the three IgE sera and the three Sepharose-coupled peptide derivatives

| Inhibitor/Sepharose | cys-peptide | ser-peptide | met-peptide |
|---------------------|-------------|-------------|-------------|
| <i>IgE(Bi)</i>      |             |             |             |
| Uninhibited         | 13.45       | 3.90        | 7.50        |
| <i>Der p</i>        | 0.04        | 0.02        | 0.02        |
| <i>Der f</i>        | 0.10        | 0.05        | 0.04        |
| cys-peptide         | 5,000       | 7,000       | 3,000       |
| ser-peptide         | 39,000      | 21,000      | 13,000      |
| met-peptide         | 39,000      | 11,000      | 4,000       |
| <i>IgE(Bo)</i>      |             |             |             |
| Uninhibited         | 7.50        | 3.55        | 3.80        |
| <i>Der p</i>        | 0.05        | 0.02        | 0.01        |
| <i>Der f</i>        | 0.09        | 0.03        | 0.01        |
| cys-peptide         | 5,000       | 2,000       | 2,000       |
| ser-peptide         | > 100,000   | 13,000      | 12,000      |
| met-peptide         | > 100,000   | 21,000      | 12,000      |
| <i>IgE(Ha)</i>      |             |             |             |
| Uninhibited         | 15.30       | 28.50       | 27.30       |
| <i>Der p</i>        | 0.01        | 0.01        | 0.02        |
| <i>Der f</i>        | 0.03        | 0.02        | 0.03        |
| cys-peptide         | 8,000       | 50,000      | > 100,000   |
| ser-peptide         | 8,000       | 9,000       | 20,000      |
| met-peptide         | 7,000       | 11,000      | 14,000      |

The amount of inhibitor that gave 50% inhibition is given in pmol.

sociation results (fig. 2) this unexpected finding is unlikely to be caused by an affinity difference. We therefore assume that this higher binding indicates/reflects a higher concentration of antibodies reactive with the methionine peptide. We therefore assume a subpopulation of antibodies that does not bind to the cysteine peptide well.

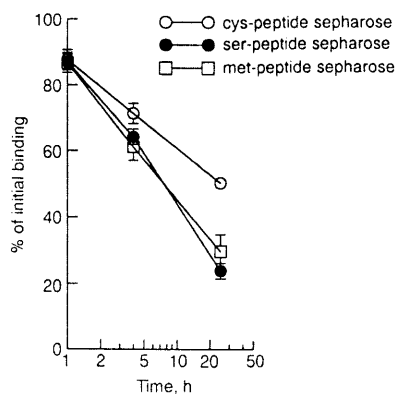


**Fig. 2.** Dissociation experiment with the three Sepharose-coupled peptide derivatives and IgE(Ha). Antibodies dissociating from the solid-phases were scavenged by an excess *Der p* II. The antibodies bound to Sepharose-coupled cysteine peptide dissociated more slowly from the solid-phase than antibodies bound to the Sepharose-coupled substituted peptides, showing a larger overall affinity of IgE(Ha) antibodies for the unmodified peptide.

The occurrence of these subpopulations could also be an explanation for the different binding behavior towards peptide 69-77 that we found earlier [9]. The assumption that most IgE antibodies from serum Bi are directed against the N-terminal part of peptide 65-78 explains why IgE(Bi) did not bind significantly to peptide 69-77.

Auto-anti-idiotypic antibodies against specific IgE antibodies against *D. pteronyssinus* allergens have been described by Saint-Remy and co-workers [14-16] who sug-

gested that immunotherapy with antigen-antibody complexes, formed by small amounts of allergen and a large excess of autologous antibodies, was effected by increased levels of auto-anti-idiotypic antibodies. They found that, contrary to the immunization of rabbits with monoclonal antibodies, the majority of the auto-anti-idiotypic antibodies resulting from immunotherapy were paratope-associated [17]. As these auto-anti-idiotypic antibodies carried the 'internal image' of the *D. pteronyssinus* allergens they were able to compete with these allergens for anti-*D. pteronyssinus* antibody binding. The paratope-associated idiotopes of these auto-anti-idiotypic antibodies had a public character and were shared by unrelated individuals [17-20]. However, our peptide studies indicated the existence of at least three different antibody subpopulations binding to a small stretch of amino acid residues. The strong difference in binding behavior between these subpopulations makes it improbable that they share the same idiotypic.

As Saint-Remy and co-workers already suggested, certain subpopulations of auto-anti-idiotypic antibodies may be lost during the complicated multistep isolation procedure. On the other hand, the three IgE sera we studied may have exceptional distributions of anti-*Der p* II IgE subpopulations and closer examination of larger numbers of IgE sera is necessary to substantiate our theory that the antibodies binding a short stretch of amino acid residues are polyclonal.

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