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Anti-allergen antibodies can be neutralized by antibodies obtained against a peptide complementary to the allergen: towards a new peptide therapy for allergy

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Abstract

The concept of specific immune treatment against allergic diseases requires the development of antibodies capable of specifically neutralizing anti-allergen antibodies. The aim of the present study was to investigate whether a novel approach, consisting in raising anti-idiotypic blocking antibodies through peptide immunization, could be envisaged in the field of allergy. Using allergy to cow's milk as a model, we prepared polyclonal antibodies against a peptide that is complementary (i.e. hydropathically opposed) to a major epitope of bovine beta-lactoglobulin (BLG), one of the main allergens of bovine milk. Anti-complementary peptide antibodies were found to neutralize in vitro both well-characterized anti-BLG monoclonal antibodies from mice sensitized to BLG and anti-BLG IgE from two patients suffering from milk allergy. These results suggest a new strategy for the functional inhibition of specific disease-associated IgE that may be applicable to the specific treatment of various allergic disorders. © 2002 Published by Elsevier Science B.V.

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1. Introduction

In contrast to commonly used global immunosuppressive techniques, an ideal therapy for any allergic disorder would be one that specifically corrects the inadequate immune response to the allergen. In theory, anti-idiotypic antibodies (anti-Id) reactive with disease-causing idiotype (Id) IgE antibodies could represent such therapeutic agents, as discussed recently [1]. In practice, however, it is difficult to identify and isolate anti-allergen Id antibodies in order to prepare anti-Ids. Interestingly, it has been demonstrated that anti-Ids of predefined specificity can be

actively induced by using a peptide rather than the Id antibody as immunogen [2]. Furthermore, this procedure requires no theoretical knowledge of the Id antibody, only delineation of the disease-causing epitope. This approach is based on the observations, now reported in almost 40 different systems, that peptides displaying inverse hydropathic patterns (denoted complementary peptides, CP) can specifically bind to each other (see [3] for review). As a consequence, these interacting peptides can in turn induce formation of antibodies that interact at their combining sites, i.e. display Id/anti-Id interactions [4]. In a comparative study, this technique has even been shown to be more efficient in preparing anti-Ids than classical immunization with syngeneic Id antibodies [5]. Since this approach has yielded to date promising applications in various domains (as summarized in Section 4), including a novel experimental therapy for auto-immune

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diseases [6–9], the aim of the present work was to investigate whether this CP new approach could be successfully applied in the field of allergy.

Allergy to cow's milk provided an opportunity to test this approach because the disease-associated IgE antibodies found in allergic patients are mainly directed against a known protein (bovine beta-lactoglobulin, BLG), and especially against an epitope constituted by its 41–60 peptide sequence [10]. We report here that antibodies against a peptide complementary to this BLG epitope (and accordingly denoted GLB 06–14) were able to block in vitro not only well characterized murine anti-BLG monoclonal antibodies but also anti-BLG IgE found in the sera of two allergic patients.

2. Materials and methods

2.1. Chemicals and peptide synthesis

Unless otherwise stated, all chemicals were from Sigma and were of analytical grade. BLG 41–60 peptide (VYVEELKPTPEGDLEILLQK) was produced by tryptic hydrolysis of the native protein [10]. The complementary peptide (CP) GLB 06–14 (HIHLLDFG-WGLPLDLRDDVF) was synthesized using t-BOC chemistry, following the same protocol as that we previously described [11]. Three control peptides were used in this study: a 20-amino acid long peptide derived from the sequence of BLG: sequence 60–79 (KWEN-DECAQKKIIAEKTKIP), the neuropeptide substance P (SP: RPKPQQFFGLM) and a peptide PS complementary to SP (PS: HQSKELLRLGS). SP was obtained from Neosystem (France), PS and BLG 60–79 were already synthesized in the laboratory.

2.2. Immunizations

GLB 06–14 peptide (CP) was coupled to Keyhole Limpet Hemocyanin (KLH) with glutaraldehyde using standard protocols [11]. Three Blanc-du-Buscat rabbits were immunized intradermally with 0.7 mg of CP-KLH conjugate emulsified with Freund's complete adjuvant. Intradermal booster injections were given after 1, 8 and 12 weeks.

2.3. Anti-BLG monoclonal antibodies, control antibodies and human sera from allergic patients

Anti-BLG mouse IgG (mAbs 92R and 83R) directed to the (41–60) epitope of the protein were prepared in our laboratory and characterized previously [12]. Control antibodies, i.e. an anti-SP IgG (mAb SP31) and anti-PS rabbit polyclonal antibodies, have already been described [11,13]. Sera from two patients with clinical symptoms of milk allergy were obtained from hospital

banks of sera. Both sera contained high levels of specific anti-BLG IgE (27.3 and 10.2 ng/ml for patients SX and SY, respectively), measured as described previously [14].

2.4. Detection and characterization of anti-complementary peptide (GLB 06–14) antibodies by enzyme immunoassay

Enzyme immunoassays (EIA) were based on the use of GLB 06–14 peptide (CP) coupled to the enzyme acetylcholinesterase (CP-AChE) as the tracer and a second antibody solid-phase as the separation method. Enzymatic tracer was prepared following a protocol that we described previously [15]. Different dilutions of anti-CP rabbit sera in PBS buffer were mixed with enzymatic tracer in wells of a microtiter plate coated with affinity-purified mAb against rabbit Ig. After 10 h incubation at 4 °C, plates were washed, AChE substrate was added and O.D. was measured after 0.5–2 h of enzymatic reaction [15].

To assess the affinity and specificity of the anti-CP antibodies, different concentrations of unlabeled CP or control peptides (SP, PS and BLG 60–79) were incubated together with rabbit sera (diluted 1/100 000) and the tracer. After 10 h incubation at 4 °C, plates were treated as above. The apparent mean affinity of the sera was taken as the concentration of unlabeled CP inhibiting 50% of the tracer-antibody maximal binding (IC_{50}).

2.5. Measurement of the neutralization of anti-BLG antibodies by anti-complementary peptide (GLB 06–14) antibodies

Anti-BLG 41–60 antibodies (mAbs 92R or 83R) directly labeled with AChE or crude sera samples from allergic patients were mixed with anti-CP sera at different dilutions (or with PBS buffer alone for measuring the maximal binding capacity: $Bo = 100\%$). After 5 h incubation at room temperature, the mixture was transferred into a plate coated (at 10 µg per well) with the peptide BLG 41–60 or with native BLG. In the case of anti-BLG mAbs, plates were incubated for 10 h at 4 °C, washed and AChE revealed as described above. In the case of human sera, plates were incubated for 24 h at 4 °C, washed and filled with AChE-labeled mAb against human IgE. After an additional 12 h incubation at 4 °C, plates were washed and AChE activity was measured after 5–10 h of enzymatic reaction.

To test the specificity of the neutralizing effect of anti-CP antibodies, AChE-labeled anti-BLG mAbs or human sera were mixed with anti-CP sera (diluted 1/4 or 1/2) which had been preincubated for 2 h with increasing concentrations of CP, or control peptides. All subsequent steps were identical to those described above.

3. Results

3.1. Design of complementary peptide (GLB 06-14)

As reviewed elsewhere [3], the sequence of the complementary peptide CP (GLB 06-14) could be designed either by simply using computer programs generating amino acid sequences whose hydropathic profiles are opposed to that of the 41-60 BLG sequence, or by reading the nucleotide sequence complementary to that of BLG mRNA encoding amino acids 41-60. Since BLG cDNA has recently been cloned and sequenced [16], the latter method was chosen: the sequence of CP accordingly obtained is given in Section 2. As expected, and illustrating a remarkable property of the genetic code [17], the hydropathic profile of CP appears perfectly matched with that of BLG 41-60 (Fig. 1).

3.2. Characterization of anti-complementary peptide (GLB 06-14) antibodies

Specific anti-CP antibodies were detected in significant amounts in the sera of two out of three rabbits immunized with GLB 06-14 peptide. Antibody titers, as defined by the dilution of antiserum giving 50% of maximal tracer binding, increased after booster injections and reached a value of approximately 1/200 000 for the last bleeds of both rabbits. These two bleeds exhibited a high affinity for CP (close to 0.3 nM), as estimated from IC₅₀ values in the competitive EIA (not shown). No cross-reactivity was detected with control peptides (SP, PS and BLG 60-79) or with BLG 41-60, demonstrating the specificity of anti-CP antibodies. Due to their high antibody titers, affinities and specificities, only these two last bleeds from both rabbits were selected for further experiments.

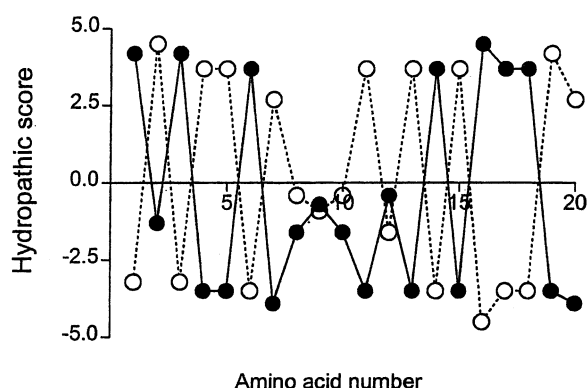


Fig. 1. Hydropathic profiles of the BLG 41-60 peptide (●) and its complementary peptide (CP) GLB 06-14 (○). Hydropathic score is based on Kyte and Doolittle values. Scale: most hydrophobic, +4.5, to least hydrophobic, -4.5.

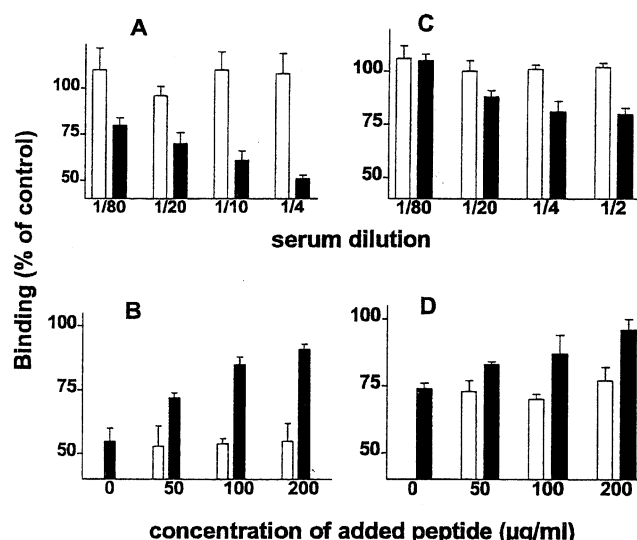


Fig. 2. Neutralization of anti-BLG mAb from mouse sensitized to BLG by anti-CP (GLB 06-14) antibodies. The binding of mAb 93R to the BLG 41-60 peptide (panel A) and native BLG (panel C) was measured after preincubation in the presence of buffer alone (100%), anti-CP antisera (black bars) or control sera (white bars) at different dilutions. Experiments were performed using the last bleeds from two rabbits immunized against CP and four control rabbit sera (two pre-immune bleeds, a serum from a non-immunized rabbit and a serum from a rabbit immunized against a control complementary peptide: PS). Results were obtained in two independent experiments, each value for each serum being determined in duplicate. Individual data from anti-CP antisera on the one hand and from all control sera on the other hand were pooled and values correspond to the mean $\pm$ S.D. of 6–12 determinations. In panels B and D, anti-CP antisera (diluted 1/4) were preincubated in the presence of increasing concentrations of CP (black bars) or two control peptides (white bars), before measuring their capacity to neutralize mAb 93R from binding BLG epitope (B) and native BLG (D). Results were obtained in two independent experiments, each value for each peptide being determined in duplicate. Data referring to both control peptides (SP and PS) were pooled and values correspond to the mean $\pm$ S.D. of 4–8 determinations.

3.3. Anti-complementary peptide (GLB 06-14) antibodies neutralize anti-BLG mouse monoclonal antibodies

Competition experiments were performed in order to investigate whether anti-CP antibodies could behave as neutralizing antibodies as regards well-characterized anti-BLG mouse monoclonal antibodies. As shown in Fig. 2A, anti-CP sera from both rabbits, but not control sera, dose-dependently inhibited the binding of an anti-BLG antibody (mAb 92R) to its epitope, the BLG 41-60 peptide. Similar results were obtained using another anti-BLG 41-60 antibody (mAb 83R, not shown).

As seen in Fig. 2B, this inhibition was largely reversed when anti-CP sera were preincubated with increasing concentrations of CP, but not of control peptides. This indicates that the blocking effect was actually due to specific anti-CP antibodies present in

rabbit sera, and not for instance to heterophilic anti-isotypic or anti-allotypic antibodies. Together, the results of both experiments (i.e. inhibition of binding to BLG epitope and CP-induced reversal of this inhibition) very strongly suggest that anti-CP polyclonal antibodies and anti-BLG mAbs specifically recognize each other through their respective paratopes, i.e. in an idiotypic/anti-idiotypic fashion.

Additional experiments showed that anti-CP antibodies were also able to block the interaction between anti-BLG mAbs and the entire native BLG protein (Fig. 2C). This inhibitory effect was weaker than that observed for the binding of the same anti-BLG antibody (mAb 92R) to the 41-60 peptide (Fig. 2A). This is probably due to the fact that the binding affinity of mAb 92R is much higher for the 41-60 epitope in the native conformation of BLG (which was used as immunogen) than for the corresponding free peptide. Importantly, however, the neutralizing effect of rabbit sera here again was almost totally reversed by preincubation in the presence of increasing concentrations of CP but not of control peptides (Fig. 2D), demonstrating the specificity of the blocking effect of anti-CP antibodies.

3.4. Anti-complementary peptide (GLB 06-14) antibodies neutralize anti-BLG IgE from two allergic patients

Since anti-CP antibodies did recognize the paratope of different mouse antibodies to a major epitope of BLG, they might also recognize quite well human antibodies with similar specificity. This was confirmed using sera from two patients suffering from cow's milk allergy, in which high levels of anti-BLG IgE were detected. As shown in Fig. 3(A and C), anti-CP antibodies dose-dependently prevented IgE from both allergic patients from binding the BLG 41-60 peptide. No significant inhibition was measured when using different control sera. Interestingly, the efficiency of the blocking effect exerted by anti-CP antibodies was related to the concentration of specific anti-BLG IgE found in these two allergic patients since greater inhibition was observed for patient SY (Fig. 3A), whose serum contained lower levels of specific IgE (10.2 ng/ml) than that of patient SX (27.3 ng/ml, figure 3C). Most importantly, the specificity of the neutralizing effect of anti-CP antibodies was demonstrated in both cases (Fig. 3B and D) by its complete reversal following preincubation of anti-CP sera in the presence of CP but not of control peptides. Due to the tiny amounts of human sera available, no attempt was made here to investigate whether anti-CP antibodies could also prevent the binding of human IgE to native BLG protein, as was demonstrated for mouse mAbs.

4. Discussion

This report documents the feasibility of a new procedure of eliciting neutralizing antibodies that might be useful for the therapy of some allergic disorders. This approach derives from the molecular recognition theory (MRT, reviewed in [3]) stating that peptides or proteins interact through hydropathically inverted amino acid sequences. Although, as said above, such interactions have been reported between nearly 40 CP pairs [3,18], MRT has met with real skepticism, essentially due to the incomplete understanding of the molecular basis of these interactions. Different mechanisms have been tentatively proposed [19,20] and we have also recently suggested a dynamic model, based on studies using surface plasmon resonance technology, according to which hydropathy could play a critical role in the very first steps of the interaction process between peptides or proteins [21]. Moreover, the generality and biological importance of such interactions is suggested by multiple observations that endogenous naturally occurring pep-

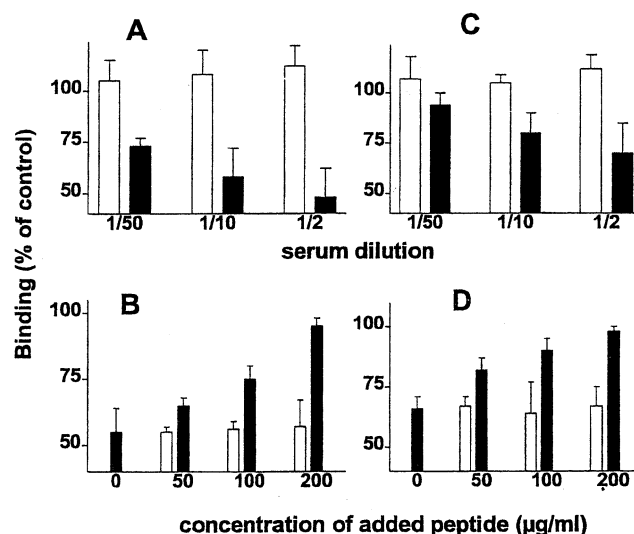


Fig. 3. Neutralization of anti-BLG specific IgE from two allergic patients by anti-CP (GLB 06-14) antibodies. The binding of IgE from allergic patients SY (panel A) and SX (panel C) to the BLG 41-60 peptide was measured after preincubation of human sera in the presence of buffer alone (100%), two anti-CP antisera (black bars) or four different control sera (white bars, see legend to Fig. 2 at different dilutions. Results were obtained in two independent experiments, each value for each serum being determined in duplicate. Individual data from anti-CP antisera on the one hand and from all control sera on the other hand were pooled and values correspond to the mean $\pm$ S.D. of 6–12 determinations. In panels B and D, anti-CP antisera (diluted 1/2) were preincubated in the presence of increasing concentrations of CP (black bars) or two control peptides (white bars, SP and PS), before measuring their capacity to neutralize IgE from patients SY (B) and SX (D). Results were obtained in two independent experiments, each value for each peptide being determined in duplicate. Data referring to both control peptides (SP and PS) were pooled and values correspond to the mean $\pm$ S.D. of 4–8 determinations.

tides or proteins could interact through hydrophatically inverted sequences [3,22,23] or, accordingly, that endogenous proteins with similar hydrophathy could display similar binding properties [24].

Aside from the generation of anti-Id antibodies by immunization with CP [4,7], the concept underlying the MRT has led to date to various important applications, including for example: (i) the localization of interactive sites of ligands and receptors by identification of complementary sequences [25]; (ii) the production of anti-CP polyclonal or monoclonal antibodies which recognize the ligand-binding domains on a dozen different receptors (see [26] for review); (iii) the recent discovery of novel receptors (e.g. [27]); or (iv) the characterization of novel ligands for receptors by designing peptides complementary to sequences from biological receptors [28,29]. In terms of the clinical applications of the MRT, much success has been achieved in the domain of autoimmunity: in animal models of three different auto-immune diseases (myasthenia gravis, multiple sclerosis and Guillain-Barr   syndrome), it has been shown that antibodies to a synthetic peptide, designed to be complementary to a dominant B or T epitope, were capable of specifically blocking auto-antibodies or disease-associated T cell receptors, respectively, and significantly to lessen the severity of the symptoms [6–9].

In this context, the data reported here indicate, for the first time to our knowledge, that this strategy is also applicable in the field of allergy, i.e. that antibodies against a peptide complementary to an allergen are able specifically to neutralize in vitro disease-associated anti-allergen IgE. Nevertheless, in the present case, a significant neutralization was only observed for relatively low anti-CP sera dilutions, although the same sera displayed high antibody titers. This could mean that only a small fraction of polyclonal anti-CP antibodies behave as anti-idiotypic blocking antibodies or that the affinity of the interaction of anti-CP antibodies with IgE is low compared with that of IgE with the allergen. The enhancement of the neutralizing effect of the anti-CP antibodies could require their purification by affinity chromatography or the production of monoclonal antibodies. The availability of such reagents would also allow a more detailed and systematic characterization of their blocking properties towards IgE from much larger series of allergic patients.

Conventionally prepared anti-Id have been clearly demonstrated to modulate the release of inflammatory products [30]. As a consequence, the next step of the present study is obviously to check that the IgE-blocking capacity of the anti-CP antibodies similarly leads to a reduction of mast cell/basophil in vitro degranulation upon challenge with BLG. It will be also worth investigating in vivo whether the passive administration of anti-CP antibodies (or perhaps more interestingly their

induction by vaccination with CP) reduces the level and/or the deleterious effect of anti-BLG antibodies in animals sensitized with the bovine protein. Considering the moderate blocking effect of the anti-CP antibodies observed under our experimental conditions, it may be anticipated that the best results would be obtained using antibodies prepared against a mixture of peptides complementary to different epitopes of BLG (including minor epitopes) that were identified as targets of disease-associated human IgE [10]. A positive result of such experiments would extend the data from a recent study showing that administration of conventional anti-Id to mice, prior to challenge with a pollen allergen, actually decreased the level of allergen-specific IgE [31]. It would also comfort the results of other studies describing the beneficial role of auto anti-Id which appear in vivo in the course of desensitization in allergic patients [32] and, more generally, pointing to the potential therapeutic value of anti-Id in modulating allergic responses both in animals [31] and in humans [33,34].

In conclusion, the results reported here suggest that a new strategy for the specific neutralization of disease-associated IgE could be rationally envisaged. Providing that further in vitro and in vivo experiments confirm the functional properties of anti-CP antibodies, the present study can be simply regarded as a promising first step towards the design of peptide vaccines of potential value in the treatment of some allergic disorders.

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