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Allergenic epitopes of ovalbumin (OVA) in patients with hen's egg allergy: inhibition of basophil histamine release by haptenic ovalbumin peptide

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SUMMARY

We studied allergenic determinants that induce hypersensitivity to OVA, the major allergen in egg allergy, using immunoblot and histamine release assays. Immunoblot analysis demonstrated a part of the OVA epitope was in the C-terminal region comprising residues 347–385 (OVA347–385). Histamine was released from basophils of a patient with egg allergy upon stimulation with the OVA fragment corresponding to OVA347–385. Furthermore, detailed epitope mapping using overlapping peptides (residues 347–366, OVA-A; residues 357–376, OVA-B; and residues 367–385, OVA-C) in the OVA 347–385 region was carried out using the histamine release assay. In order for histamine release from basophils to occur, the allergen must possess two or more allergenic determinants located on the protein molecule at distances that would be equivalent to the distances between IgE molecules on the membrane surface. These results suggest that there are at least two epitopes that bind IgE antibodies on each OVA peptide. In addition, one epitope that binds IgE antibodies in two patients appears to reside in the haptenic peptide OVA357–366 (OVA-B1). The histamine release from basophils stimulated by OVA-B was completely inhibited by OVA-B1 in one of these patients. Similarly, OVA-B1 inhibited the histamine release produced by OVA-A in the other by more than 40%. These results suggest that haptenic synthetic peptides could regulate the allergic reaction in the effector phase if common epitope(s) recognized by IgE antibodies in the patients with egg allergy can be found. These are the first studies that provide an antigen-specific approach to inhibiting histamine release from basophils by a haptenic peptide recognized by IgE antibodies in an allergic disorder.

Keywords ovalbumin epitope IgE antibody food allergy histamine release

INTRODUCTION

Egg white is one of the most common food items to frequently induce hypersensitivity, particularly in atopic children. It has been shown that among 40 different egg white proteins, OVA is a major allergen in patients with egg allergy. OVA, which by weight accounts for 58% of whole egg white, is a water-soluble glycoprotein whose complete primary structure has been defined. Based on this knowledge, we deemed OVA a suitable protein for which to characterize the antigenic determinants relating to food allergy and for studies on IgE response regulation.

We previously reported that the IgE antibodies to OVA in the serum of patients with egg allergies differ from the IgG or IgA antibodies with respect to their binding activities with different preparations of denatured or fragmented OVA [1]. In addition, the finding that IgE antibodies bind to denatured or fragmented OVA suggested that IgE antibodies recognize determinants associated not only with the conformational structure but also with the primary structure. In fact, there have been several reports of the linear epitopes involved in the binding of OVA to IgE antibodies using synthetic peptides. Elsayed *et al.* and Kahlert *et al.* analysed the epitopes on OVA recognized by IgE antibodies using synthetic peptides or cyanogen bromide (CNBr)-cleaved OVA, and demonstrated that OVA1–11, which corresponds to amino acid residues 1–11 of OVA [2], OVA323–339 [3], OVA34–46, OVA47–55 [4] and OVA41–172, OVA301–385 [5], react with IgE antibodies in patients with egg allergy.

IgE-mediated allergic reactions involve chemical mediators, such as histamine. Histamine is released from basophils or mast cells stimulated with multivalent antigens. The binding between the allergen and the allergen-specific IgE on the surface of the

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mast cells or basophils imposes certain other structural requirements on the allergen. Namely, in order for bridging to occur between two IgE antibody molecules, the allergen must possess two or more allergenic determinants located along the protein molecule at distances equivalent to the distance between the IgE molecules on the membrane surface. Direct skin tests using OVA1–11 on patients with egg allergy showed no activity, and the site was therefore concluded to encompass one single IgE-binding haptenic epitope [2]. These results do not confirm that these linear epitopes are actively involved in the IgE bridging that results in histamine release from mast cells or basophils *in vivo*. The epitopes of OVA relating to food allergy should be analysed for their ability to cause the release of histamine from basophils or mast cells, in addition to their ability to bind IgE antibodies.

In this study, we isolated an OVA fragment recognized by the IgE antibodies to OVA in most patients with egg allergy enrolled in this study, and identified linear allergenic epitopes by sequencing the fragments causing histamine release. We found at least four epitopes residing in the fragment, and demonstrated that a haptenic OVA synthetic peptide that contains only one epitope can bind IgE antibodies to OVA and inhibit histamine release from basophils caused by stimulation with multivalent synthetic peptides.

MATERIALS AND METHODS

Reagents

OVA (grade VII) was purchased from Sigma (St Louis, MO) and repurified before use by chromatography on a diethylaminoethyl cellulose column (DEAE) eluted with a linear gradient of 0–0·2 mol/*l* NaCl in 10 mmol/*l* phosphate buffer pH 6·0, according to the method of Bernhisel-Broadbent *et al.* [6]. Purity of the collected fractions was evaluated by ELISA reactivity against OVA- and ovomucoid (OVM)-specific MoAbs generated in BALB/c mice. MoAbs to OVA (F1H37) and OVM (F1H34) were kindly provided by Dr Akihiko Kato (Dainabot Ltd., Matsudo, Japan).

ELISA with monoclonal antibodies to OVA and OVM

To evaluate the purify of OVA, ELISA was used as previously described with some modifications [1]. Microtitre plates (Nunc, Roskilde, Denmark) with 1 μg/ml of either DEAE-repurified OVA, commercial OVA (grade VII) or commercial OVM (Sigma) were incubated with diluted mouse MoAb (1 : 500, 1 : 5000) overnight at 4°C. The amount of antibodies bound to the well was detected by alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma). The background of this assay was determined using a plate coated with 0·3% gelatin instead of antigens, and was subtracted from all data. The background optical density (OD) values were lower than 0·07 in all of the ELISAs.

Patients

Serum was obtained from 12 Japanese patients (seven males, five females, age range 1–11 years, mean 3·6 years) with egg allergy, displaying clinical symptoms of atopic dermatitis, diarrhoea, vomiting, or anaphylactoid shock after consuming eggs. They were diagnosed on the basis of food elimination and challenge tests [7], and their RAST scores for egg white classes 2–4 were positive. No patients had been treated with systemic corticosteroids. Among the 12 patients described, three patients, P1, P2, and P3, were involved in studies of stimulation and inhibition by multivalent peptide of histamine release from basophils using synthetic OVA peptide. The RAST scores for egg white of P1, P2, and P3 were 3. In addition, two healthy children with no allergic disorders, H1 and H2, were included as controls.

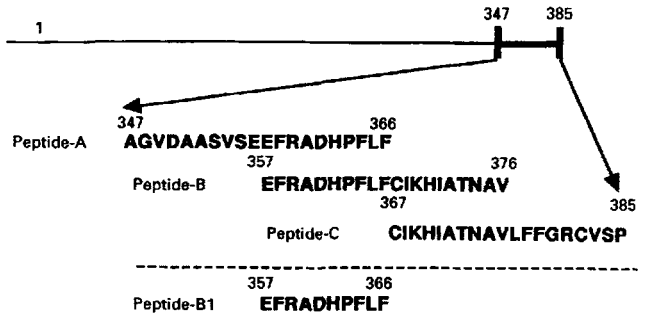

Fig. 1. Primary amino acid sequences of OVA peptides, OVA-A, OVA-B, OVA-C and OVA-B1.

Synthetic peptides of OVA

Four OVA peptides, OVA-A (residues 347–366), OVA-B (residues 357–376), OVA-C (residues 367–385), and OVA-B1 (residues 357–366) were synthesized on an Applied Biosystems 430A Peptide Synthesizer (Foster City, CA) (Fig. 1). Peptide purities were determined by high performance liquid chromatography (HPLC) and amino acid analysis.

Preparation of OVA fragments

A mixture of OVA (5 mg/ml) and *Staphylococcus aureus* strain V8 protease (0·5 mg/ml) was incubated at 4°C for 12 h. A second incubation in buffer containing SDS (62·5 mM Tris–HCl, 2% SDS, 20% glycerol) was then carried out at 20°C for 30 min to bind SDS to the mixture. This sample was boiled for 2 min and stored at −20°C until use [8].

SDS–PAGE

SDS–PAGE was performed by the method of Laemmli, with some modifications [9]. The samples (50 μg of protein/lane) were loaded onto a SDS–polyacrylamide slab gradient gel (84 × 90 × 1 mm, 10–20% acrylamide gradient) and electrophoresis was performed at a constant current of 25 mA. After electrophoresis, the gel was stained with coomassie brilliant blue, or used in immunoblot analysis as described below. Molecular weight protein standards (95·5, 66·2, 45, 31, 21·5 and 14·4 kD) were purchased from BioRad Labs (Richmond, CA).

Extraction of OVA fragments from gel

Gels containing OVA fragments with an approximate size of 10 kD were placed in seamless cellulose tubes (18/32; Sanko Pure Chemical Inc., Tokyo, Japan) and the OVA fragments were extracted at a constant current of 75 mA for 5 days. After extraction, the gels were removed from the tubes and the OVA fragments were dialysed against PBS.

Immunoblot and amino acid sequence analysis of OVA fragments

After electrophoresis, the buffer in the gels was replaced with

blotting buffer (20% methanol containing 125 mM Tris and 960 mM glycine) by shaking the gels in blotting buffer three times for 10 min [10]. The peptides in the gel were then transferred to a polyvinylidene difluoride (PVDF) membrane (BioRad) in the same buffer at a constant current of 100 mA for 2 h, using a semi-dry blotting apparatus (Nihon Eido, Tokyo, Japan). After transfer and blocking with 3% gelatin in PBS pH 7·4 at 30°C for 16 h, the membrane was immersed in a solution containing the serum from patients diluted with PBS containing 0·2% gelatin (1 : 2) for 72 h at 4°C. The membranes were then washed extensively with PBS–T (PBS containing 0·05% Tween-20), and the membrane was soaked in a solution containing biotin-labelled goat anti-human IgE (Kirkegaard & Perry Labs, Gaithersburg, MD) diluted with PBS (1 : 800) at 20°C for 1 h. The membranes were washed again with PBS–T, and the antibodies on the membrane were detected with a Vectastain avidin–biotin peroxidase complex kit (Vector Labs, Burlingame, CA) using 4-chloro-1-naphtol as the substrate for peroxidase. Specific antibody-binding sites were visualized under ultraviolet light (254 nm). Amino acid sequence analysis of fragments was performed on a 477A Applied Biosystem Automatic Sequence Analyzer.

Radioimmunoassay of histamine

Histamine radioimmunoassay (RIA) was performed according to the instructions enclosed with the kit supplied by Immunotech. Whole blood (100 μl) diluted 1 : 4 in N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (20 mmol/*l*)-Tyrode's buffer, was challenged for 30 min at 37°C with 50 μl of various dilutions of allergens. The reaction was stopped by centrifugation at 1500 *g* at 4°C for 5 min. Total histamine was measured after cell lysis with distilled water, freezing and thawing, and acylation. Histamine was quantified in 100 μl of supernatant. Supernatant (100 μl) or standard solution was placed into designated 'acylation tubes'. Next, 50 μl of acylation buffer were added to each tube, and the solutions mixed by vortex until all the powdered acylation reagent was solubilized. After the addition of 1000 μl iodinated (^{125}I) histamine, the materials were again mixed by vortex, and 500 μl aliquots of these test solutions were transferred to MoAb-coated tubes and incubated at 4°C for 18 h. The solutions were aspirated and discarded, and the radioactivity in the antibody-coated tubes was counted in a gamma counter (Wallac, Turku, Finland) for 1 min. The resulting ct/min for 0·5–150 nmol/*l* histamine (assay standards enclosed with the kit) were used to construct standard curves. Histamine values for unknown samples were determined by comparison. The per cent of histamine release was determined using the formula: $(a/b) \times 100$, where *a* represents histamine in the supernatant released from basophils upon stimulation with antigen, and *b* represents total histamine. The asays were performed in duplicate. Spontaneous histamine release from cells, usually < 5%, was subtracted from the calculated histamine release. Results are expressed as means of percentage of histamine release and represent the mean of duplicate samples.

Inhibition of histamine release from basophils

Whole blood (50 μl) diluted 1 : 2 in N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (20 mmol/*l*)-Tyrode's buffer was mixed at 4°C for 24 h with 50 μl of synthetic peptides (OVA-B1 at the concentrations of 28–14 000 μg/ml and OVA-A at the concentrations of 56–28 000 μg/ml) dissolved in the same buffer. Blood pretreated with OVA-B1 or OVA-A was challenged for 30 min at 37°C with 50 μl of various dilutions of allergen. The reaction was stopped by centrifugation at 1500 *g* at 4°C for 5 min. Histamine RIA was performed according to the instructions enclosed with the kit supplied by Immunotech. The per cent inhibition of histamine release was determined using the formula: % inhibition $= (1 - (a/b)) \times 100$, where *a* represents histamine released from basophils preincubated with OVA-B1 in P2 and P3 or OVA-A in P2 and stimulated by OVA-B in P2 or by OVA-A in P3, and *b* represents histamine released by OVA-B stimulation in P2 or by OVA-A stimulation in P3 from basophils not preincubated with OVA-B1. The assays were performed in duplicate. Spontaneous histamine release from the cells, usually < 5%, was subtracted from the calculated histamine release. Results are expressed as means of percentage of histamine release and represent the mean of duplicate samples.

RESULTS

Purification of OVA

The results of an ELISA using mouse MoAbs to OVA or OVM are shown in Table 1. No binding activities of IgG antibodies could be detected using an OVM-specific MoAb when DEAE-repurified OVA was used as the antigen. On the other hand, when the commercially available OVA was used, a large amount of IgG antibodies was detected using an OVM-specific MoAb. This result indicated that the DEAE-repurified OVA used in our study was considerably more pure than commercial OVA.

Immunoblot of OVA fragments bound by IgE antibodies from patients with hen's egg allergy

Serum from 12 patients with hen's egg allergy was examined for IgE antibody binding to OVA fragments obtained by digestion with *Staphylococcus aureus* strain V8 protease (Fig. 2a,b).

Table 1. The binding activities of OVA or OVM specific monoclonal antibodies to OVA with or without purification

	ΔOD (405 nm)*			
	F1H37		F1H34	
	1 : 500	1 : 5000	1 : 500	1 : 5000
Commercial OVM	0·000	0·000	1·490	0·287
Commercial OVA	1·171	0·539	1·369	0·354
DEAE-repurified OVA	1·274	0·556	0·000	0·000

Binding activities of IgG antibodies to DEAE-repurified OVA, commercial OVA or commercial OVM were measured by ELISA with the OVA or OVM-specific MoAbs (1 : 500 or 1 : 5000). The background of this assay was determined using a plate coated with 0·3% gelatin instead of antigens and was subtracted from all data. The background OD values were lower than 0·07 in all ELISAs.

* Mean absorbance at 405 nm of duplicate samples.

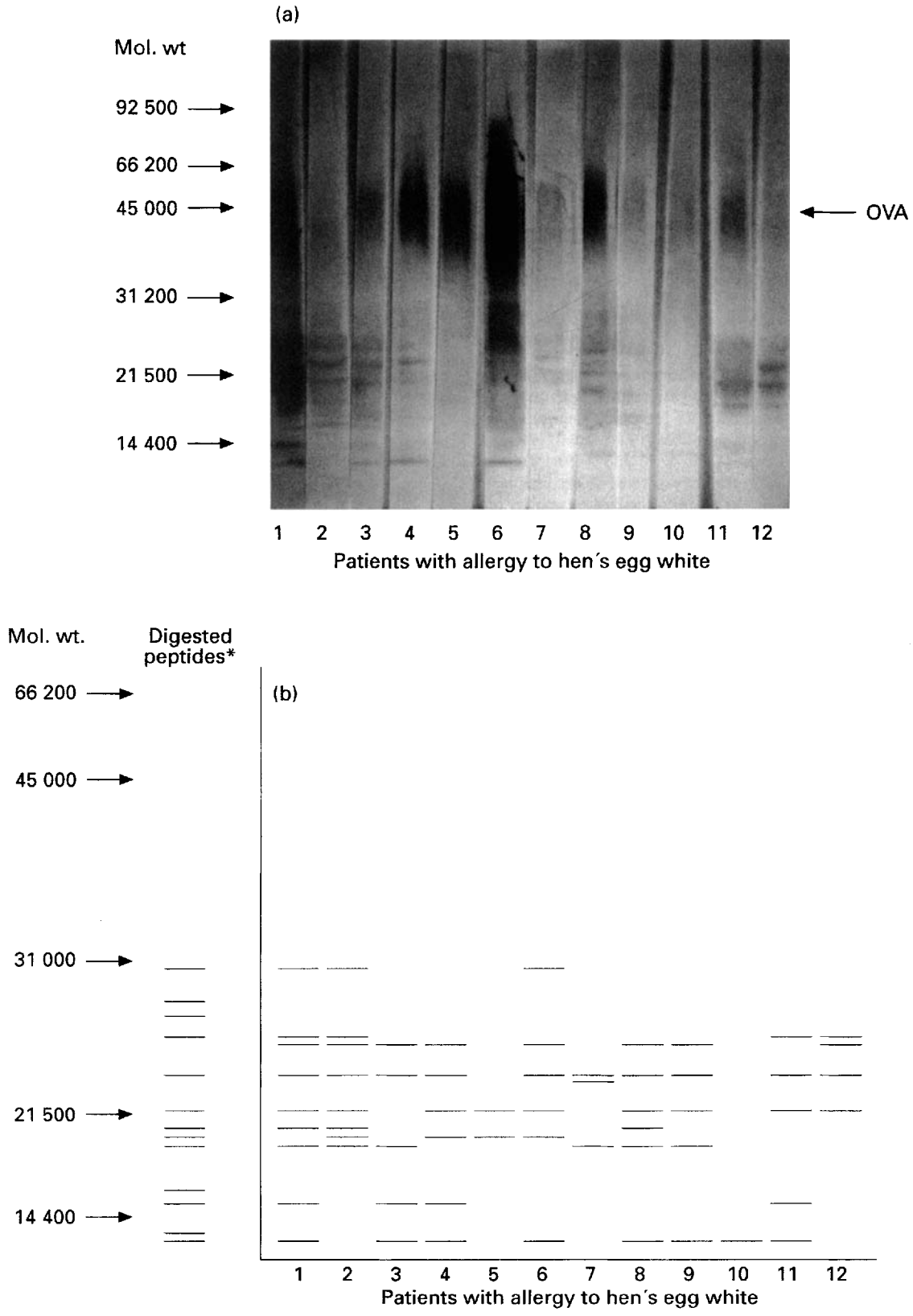

Fig. 2. (a) Western blot analysis using OVA fragments digested with V8 protease. OVA fragments were separated by SDS–PAGE under non-reducing conditions. Native OVA was treated with V8 protease as described in Materials and Methods. (b) Scheme for Western blot analysis using OVA fragments digested with V8 protease (a).

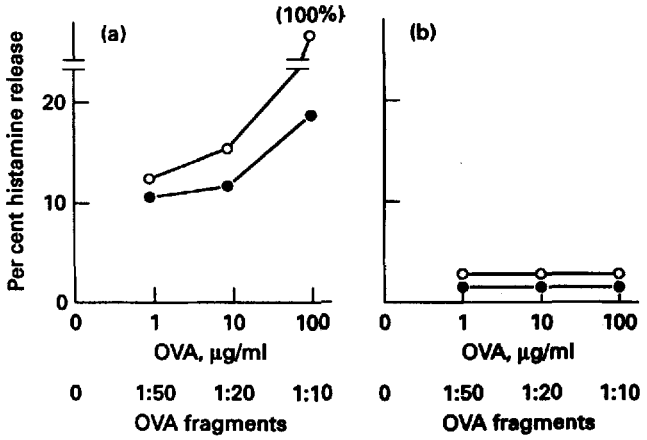

Fig. 3. Basophil histamine release in a patient with egg allergy (P1) (a) and healthy individual (H1) (b) following stimulation with an OVA fragment (OVA347–385) digested with V8 protease or native OVA. Blood was incubated with three concentrations (100–1 μg/ml) of native OVA (○) or three concentrations (1:10, 1:20 and 1:50) of OVA fragment (OVA347–385) (●). Results are expressed as per cent histamine release. The data represent the means of duplicate samples.

Twelve fragments from 31 kD to 10 kD were detected by protein staining and transferred to PVDF membranes. Various fragments were recognized by IgE antibodies when serum samples from patients with hen's egg allergy were screened individually. The specificities of IgE antibodies for OVA were not homogeneous among individuals. OVA fragments of 25, 23, 21·5 and 10 kD were bound by serum from patients more often than other fragments. The amino acid sequence of the 10-kD fragment was determined with an Applied 477A Sequence Analyzer, since this fragment was bound by IgE antibodies most strongly and was the smallest. This fragment corresponded to OVA347–385 (Fig. 1).

Ability of the 10-kD OVA fragment obtained by V8 protease digestion to induce histamine release from basophils in patient with hen's egg allergy

The histamine release from peripheral blood cells (PBC) of one patient, P1, with hen's egg allergy upon stimulation by the isolated OVA fragment corresponding to OVA347–385 at 1:10, 1:20, and 1:50 dilution or by native OVA at concentrations from 1 to 100 μg/ml, was dose-dependent and > 10% (Fig. 3). On the other hand, histamine was not released from PBC of healthy control, H1, upon stimulation by the OVA fragment or native OVA.

Ability of three synthetic peptides corresponding to the C-terminal region OVA347–385 to induce histamine release from basophils in patients with hen's egg allergy

To investigate the epitopes recognized by IgE antibodies in greater detail, histamine release from PBC of patients with egg allergy was determined upon stimulation with synthetic OVA peptides (Fig. 4). In P2, the synthetic OVA peptides OVA-B and OVA-C, and native OVA at a concentration of 1000 μg/ml, produced 27·4%, 14·0%, and 17·1% histamine release from PBC, respectively. However, OVA-A at a concentration of 1000 μg/ml produced no histamine release (< 10%) in P2. On the other hand, OVA-A at concentrations of 100 and 1000 μg/ml produced 45·5% and 25% histamine release, respectively, in P3. In addition, native OVA at concentrations of 100–1 μg/ml produced more than 25% histamine release, while OVA-B and C produced no histamine release (< 10%) in P3. Histamine was not released from PBC of healthy individuals, H1 and H2, stimulated with the same concentrations of OVA peptides or native OVA.

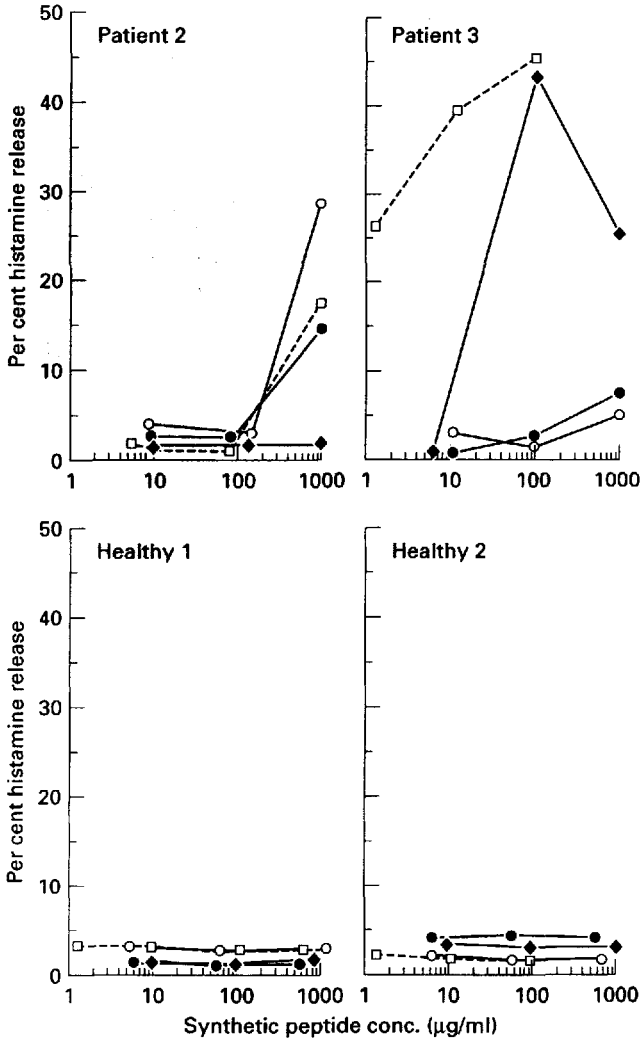

Fig. 4. Histamine release from basophils in patients with egg allergy (P2 and P3) or healthy individuals (H1 and H2) following stimulation by OVA synthetic peptides. Blood was incubated with OVA-A (◆), OVA-B (○), OVA-C (●) or native OVA (□). Blood was incubated with three concentrations (10–1000 μg/ml) of synthetic peptides in P2, P3, H1, and H2, three concentrations (10–1000 μg/ml) of native OVA in P2, four concentrations (1–1000 μg/ml) of native OVA in H1, and H2, or three concentrations (1–100 μg/ml) of native OVA in P3. Results are expressed as per cent histamine release. The data represent the means of duplicate samples.

Inhibition of histamine release from human basophils by haptenic peptide

Histamine release from PBC is elicited in response to the bridging of IgE molecules that bind to specific receptors on basophils. Therefore, we investigated whether haptenic syn-

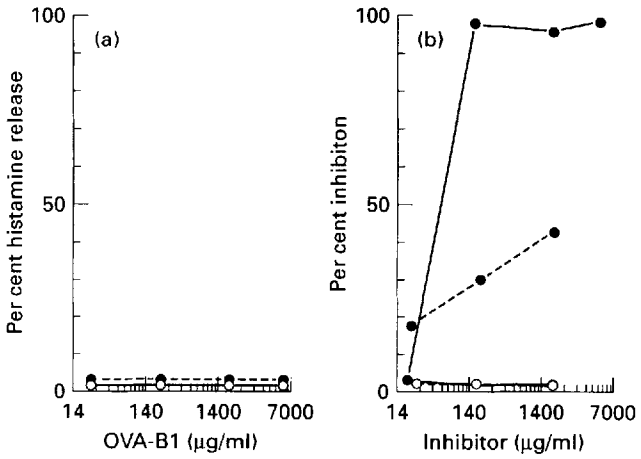

Fig. 5. (a) Histamine release from basophils in patients with egg allergy (P2 and P3) following stimulation by OVA-B1. Blood was incubated with four concentrations (14–7000 μg/ml) of synthetic peptides in P2 (○), P3 (●). Results are expressed as per cent histamine release. The data represent the means of duplicate samples. (b) Inhibition of histamine release from basophils stimulated by OVA-B in P2 (——) and OVA-A in P3 (-----) by preincubation with the haptenic OVA peptide, OVA-B1 (●) or OVA1–11 (○). Fifty microlitres of whole blood, diluted 1 : 2 in N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (20 mmol/*l*)-Tyrode's buffer were mixed at 4°C for 24 h with 50 μl of the synthetic peptide, OVA-B1, diluted to various concentrations. Blood pretreated with OVA-B1 was challenged for 30 min at 37°C with 50 μl of 1000 μg/ml OVA-B in P2 or OVA-A in P3. The per cent inhibition of histamine release by OVA-B1 was calculated as described in Materials and Methods. The per cent histamine release from basophils stimulated by OVA-B in P2 or OVA-A in P3 without OVA-B1 pretreatment was 16% and 82%, respectively.

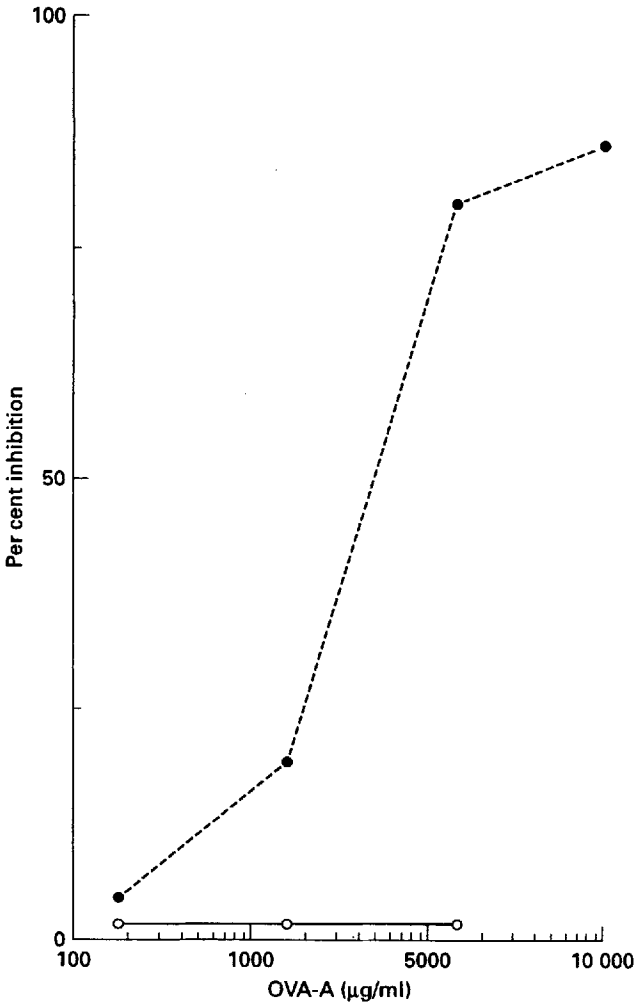

Fig. 6. Inhibition of histamine release from basophils stimulated by OVA-B in P2 by preincubation with the OVA peptide, OVA-A (●), OVA1–11 (○). Fifty microlitres of whole blood, diluted 1 : 2 in N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (20 mmol/*l*)-Tyrode's buffer were mixed at 4°C for 24 h with 50 μl of the synthetic peptide, OVA-A, diluted to various concentrations. Blood pretreated with OVA-A was challenged for 30 min at 37°C with 50 μl of 1000 μg/ml OVA-B in P2. The per cent inhibition of histamine release by OVA-A was calculated as described in Materials and Methods. The per cent histamine release from basophils stimulated by OVA-B in P2 without OVA-A pretreatment was 15%.

thetic peptide OVA-B1 in P2 and P3 or OVA-A in P2, which can be considered to contain only one epitope, could inhibit histamine release from human basophils by blocking the connection between IgE molecules and the multivalent synthetic peptides (Fig. 5). OVA-B1 or OVA1–11 as a control peptide at concentrations of 14–7000 μg/ml produced no histamine release (< 10%) in both patients (Fig. 5a). This result indicated that OVA-B1 which overlaps with OVA-A and OVA-B had only one epitope. In P2, histamine release from PBC upon stimulation with OVA-B was inhibited more than 90% by preincubation of the PBC with OVA-B1 at concentrations of 7000–140 μg/ml. Similarly, histamine release by stimulation with OVA-B in P2 was inhibited after preincubation of basophils with OVA-A which includes OVA-B1 (Fig. 6). Furthermore, OVA-B1 at a concentration of 1400 μg/ml caused a 42% inhibition of histamine release from PBC stimulated with OVA-A in P3. The inhibition of histamine release was demonstrated to occur in a dose-dependent manner. In P2 and P3, OVA1–11, which is not a determinant for binding to IgE antibodies in either patient, at concentrations from 1400 μg/ml to 14 μg/ml, did not inhibit histamine release from PBC stimulated with OVA-B in P2 or OVA-A in P3 after preincubation of the PBC with OVA1–11.

DISCUSSION

The experiments described provide new information about the allergenic epitopes recognized by IgE antibodies to OVA and about histamine release from basophils stimulated with peptides corresponding to OVA fragments from patients with egg allergy. Furthermore, we demonstrated that a haptenic OVA synthetic peptide that contains only one epitope for IgE antibody binding inhibits histamine release from basophils in patients with egg allergy.

We focused on the smallest fragment that was recognized by IgE antibodies from most patients with egg allergy. The molecular size of this fragment was determined to be 10 kD, based on SDS–PAGE analysis. However, since this fragment corresponds to OVA347–385, its molecular weight is estimated to be approximately 5000 based on its 39 residues. This fragment was located near the end of the gel in SDS–PAGE.

Therefore, the molecular weight estimation based on SDS–PAGE analysis was probably inaccurate.

It is known that the most accessible surfaces of any globular protein appear to be potentially immunogenic. The composition of the immunoreactive fragment based on the crystal structure of OVA [11] reveals that the C-terminal fragment 347–385 is located on the surface as a part of this fragment. Thus, this fragment, which is expressed on the surface of the OVA molecule, might be reactive with IgE antibodies in patients with egg allergy.

IgE-mediated allergic reactions are dependent on chemical mediators, such as histamine. Histamine is released from basophils or mast cells that IgE antibodies bind to upon stimulation with multivalent antigen. When receptor aggregation is induced by a reaction of the cell-bound IgE with a multivalent antigen, chemical mediators are released from the cells. In fact, the determination of the allergen in patients suffering from allergic disorders is often performed by histamine release experiments in basophils from the patient [12,13]. OVA347–385, as described in this study, is an allergenic region of OVA, since histamine was released from the basophils of P1, upon stimulation by this fragment. Furthermore, the epitopes on OVA347–385 were investigated by histamine release assay using overlapping synthetic OVA peptides, OVA-A, B, and C. The results show that histamine was released from basophils stimulated with OVA-B or C in P2 and by OVA-A in P3. In order for bridging to occur between two IgE antibody molecules, the allergen must possess two or more allergenic determinants located along the protein molecule at distances that would be equivalent to the distances between the IgE molecules on the membrane surface [14]. Therefore, our results suggest that there are at least two epitopes recognized by IgE antibodies from P2 on OVA-B or C and from P3 on OVA-A (Fig. 4). On the other hand, these peptides do not polymerize and induce degranulation of basophils in an antigen-specific manner, since histamine was not released from basophils from healthy individuals or other patients with egg allergy (data not shown) stimulated with the same synthetic peptides. It is considered that the minimum amino acid length bound by IgE antibodies is six to eight residues [15–17]. In addition, Taylor *et al.* reported that a certain interval is needed between epitopes recognized by IgE antibodies due to the aggregation event [18]. Consequently, we conclude that epitopes that are bound by IgE antibodies exist on OVA347–356, OVA357–366, OVA367–376, and OVA377–385. Epitopes on OVA357–366 are among the epitopes that were recognized by the IgE antibodies of both P2 and P3. These epitopes are considered to play an important role in the IgE-mediated allergic reactions of humans.

According to Kanner *et al.* [19], the calcium flux that occurs upon stimulation with the multivalent antigen DNP-IgG as an initial event in the release of histamine from basophils or mast cells is completely inhibited after preincubation with the univalent hapten, dinitrophenol (DNP). Thus, we investigated whether a haptenic peptide, OVA357–366 (OVA-B1), that most probably has only one IgE epitope, can inhibit histamine release from human basophils by blocking the reaction between IgE molecules and multivalent synthetic peptides, including the relevant epitope of OVA-B1. After preincubation of basophils with OVA-B1, histamine release by stimulation with OVA-B in P2 or OVA-A in P3 as multivalent peptide was inhibited in a dose-dependent manner. Similarly, histamine release by stimulation with OVA-B in P2 was completely inhibited after preincubation of basophils with OVA-A (data not shown). Peptide OVA-A includes OVA-B1 (Fig. 1). On the other hand, histamine release by OVA-B or OVA-A was not inhibited by preincubation of basophils with OVA1–11, which does not bind anti-OVA IgE antibodies from P2 or P3. This indicates that the haptenic synthetic peptide OVA-B1 inhibits histamine release that follows stimulation by OVA-B or OVA-A specifically and competitively. Inhibition of histamine release following stimulation with OVA-A in P3 was 42% after preincubation of basophils with OVA-B1, whereas inhibition following stimulation by OVA-B in P2 was 100%. There may be two possible explanations for the fact that inhibition by OVA-B1 in P3 was less than in P2. The affinity of the IgE antibodies for OVA-B1 may differ between P2 and P3. The amino acid sequences of OVA-A or OVA-B other than those corresponding to OVA-B1 may influence the binding activity of IgE antibodies to the epitope on OVA-B1. If this is the case, OVA-B1 may inhibit histamine release from basophils induced by OVA-A or OVA-B differently. Alternatively, the number of IgE antibodies to OVA-B1 that bind to basophils in P3 may be greater than in P2, since the per cent histamine release resulting from stimulation with native OVA in P3 was much higher than in P2. In this case, more OVA-B1 as a blocking peptide would be needed to inhibit histamine release in P3 than in P2.

In conclusion, our studies suggest that a haptenic synthetic peptide could regulate the allergic reaction in the effector phase if common epitope(s) could be found in patients with egg allergy. This method of regulation of an allergic reaction is epitope-specific and does not influence the rest of the immune system. Furthermore, such regulation could provide epitope-specific treatments for food allergies.

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