

Carbohydrate-based particles: a new adjuvant for allergen-specific immunotherapy

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SUMMARY

The occurrence of systemic anaphylactic side-effects in the course of allergen-specific immunotherapy has been strongly reduced by the adsorption of allergens to aluminium hydroxide, the most frequently used adjuvant in humans. Using the major timothy grass pollen allergen, Phl p 5b, in its recombinant form for immunization of mice, we demonstrate that carbohydrate-based particles (CBP) exhibit several potential advantages over aluminium-hydroxide as adjuvant for immunotherapy. Similar to alum-bound rPhl p 5b, CBP-bound rPhl p 5b induced a stronger antibody and cytokine response than unbound rPhl p 5b after subcutaneous injection in mice. The antibodies induced by CBP-bound rPhl p 5b, exhibited potentially beneficial activities as they cross-reacted with group 5 allergens from five other grass species and inhibited the binding of grass pollen allergic patients IgE to Phl p 5b. Alum-bound rPhl p 5b induced a preferential allergen-specific Th2-response characterized by high immunoglobulin G1 (IgG1) antibody levels and elevated interleukin (IL)-4 and IL-5 production in cultured splenocytes. By contrast, CBP-bound rPhl p 5b, but not rPhl p 5b alone or coadministered with CBP, induced a mixed allergen-specific T helper 1 (Th1)/Th2 immune response characterized by the additional production of allergen-specific IgG2a/b antibody responses and elevated interferon- γ production. Conjugation of rPhl p 5b to CBP yielded a stable vaccine formulation with preserved immunogenic features of the allergen and, in contrast to alum, induced no granulomatous tissue reactions. Based on these results, CBP is suggested as a potentially useful adjuvant for specific immunotherapy of IgE-mediated allergies.

INTRODUCTION

Allergen-specific immunotherapy which is mostly conducted by injecting allergen extracts into allergic patients was introduced 1911 by Leonard Noon.¹ The occurrence of severe anaphylactic side-effects caused by the injection of aqueous allergen extracts and the necessity to administer a great number of injections over long periods prompted the development of safe and efficacious allergen formulations. More than 60 years ago aluminium-hydroxide-adsorbed allergen extracts were introduced for depot vaccination, showing improved immune stimulatory as well as reduced anaphylactic properties.^{2,3} Even today, aluminium hydroxide is, despite its T helper 2 (Th2)-driving potential by far the most common and a very safe adjuvant for injection immunotherapy in humans.⁴ More recently several new adju-

vants capable of initiating Th1-immune responses (e.g. liposomes⁵ and CpG DNA⁶) have been included in experimental animal models (mice)^{6–8} and first immunotherapy studies in patients.^{5,9}

Allergen-specific immunotherapy is one of the few known causative treatments of IgE-mediated allergy and numerous clinical studies document its clinical efficacy.^{4,10} Common clinical practice includes the subcutaneous injection of allergen extracts adsorbed to aluminium hydroxide with gradually increasing doses to a maintenance level and treatment periods up to 5 years or more.⁴ Aluminium hydroxide is preferred to other adjuvants (e.g. oil emulsions, liposome formulations) for injection immunotherapy because it induces relatively little tissue reactions.⁴ Nevertheless aluminium hydroxide can cause local granuloma formation at the injection sites.^{11–15} Other major disadvantages of aluminium hydroxide are the unpredictable efficacy of adsorption of certain allergens/allergen extracts and stability of the adsorbates, the possibility that allergens are altered in the course of the undefined adsorption process and the difficulties in assessing quality and quantity of allergens once that they are adsorbed to the aluminium hydroxide.¹⁵

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Carbohydrate-based particles (CBP, 2 µm Sepharose particles) can covalently bind antigens at high density without dramatic alteration of their immunological properties. Therefore Sepharose-bound antigens, peptides or antibodies are used for a great variety of immunological assays.¹⁶ The coupling to CBP is based on the principle of cyanobromide activation, resulting in stable formation of amide bonds and can thus be applied for most proteins and peptides at high efficacy. It has further been shown that antigens bound to 2-µm particles were optimal for processing and presentation by antigen-presenting cells.¹⁷

In this study we performed experiments to investigate if CBP can be used as an alternative adjuvant for allergen-specific immunotherapy. Purified recombinant Phl p 5b, a major timothy grass pollen allergen, was coupled to CBP, adsorbed to aluminium hydroxide, mixed with CBP or used alone. The different preparations were used to immunize mice and the levels, kinetics and profiles of antibody responses were analysed. Furthermore we investigated the cytokine production in mouse spleen cell cultures and analysed the injection sites by histopathology. The CBP-p5-induced mouse antibodies were tested for cross-reactivity to natural group 5 allergens from various grasses and their ability to inhibit the IgE-binding of grass pollen allergic patients' to the allergen was studied.

Based on our finding that CBP-p5 elicited immune responses comparable to aluminium hydroxide, however, with a slightly pronounced Th1-shift, but without granulomatous tissue reactions and, that CBP-p5-induced antibodies blocked allergic patients' IgE binding to rPhl p 5b, we suggest CBP as a possible new adjuvant for specific immunotherapy.

MATERIALS AND METHODS

Patient sera

Nine patients with a documented clinical history of allergy to timothy grass pollen, sensitized to Phl p 5b and a timothy grass pollen CAP radioallergen sorbent test (RAST) class value of 2 or higher (Pharmacia CAP system, Pharmacia Diagnostics, Uppsala, Sweden) were included in the study.

Recombinant allergen cDNA coding for the major allergen rPhl p 5b was obtained by polymerase chain reaction (PCR) according to the published sequence.¹⁸ The Phl p 5b cDNA was subcloned into pET 17b (Novagen, Madison, WI) expressed, in *Escherichia coli* BL-21 (DE3) and purified to homogeneity as described.¹⁹ Endotoxin content as determined by the *Limulus* amoebocyte lysate QCL-1000 test (BioWhittaker, Walkersville, MD) was less than 6.4 EU/mg protein.

Preparation of conjugates and adsorbates

Cyanogen-bromide-activated²⁰ spherical Sepharose particles (CBP; beaded agarose, with a mean diameter of 2.1 µm) were provided by Pharmacia Diagnostics. Prior to conjugation, 4 mg of lyophilized rPhl p 5b was dissolved in 8 ml 0.1 M carbonate-buffer pH 8.0 and added to 110 mg of activated particles in 2.0 ml 0.1 M carbonate-buffer pH 8.0. The allergen was conjugated repeatedly to the particles by end-over-end mixing for 1 hr at room temperature. CBP-p5 was centrifuged at 1000 g for 5 min. Coupling efficiency was estimated to be between 75 and 95% by measuring unbound protein in supernatants after conjugation by light absorbance at 280 nm (Hewlett-Packard,

Waldbronn, Germany) and by BCA Protein Assay (Pierce, Rockford, IL). Remaining active groups were blocked by incubating resuspended particles in 5 volumes of 0.1 M glycine pH 8.5 by end-over-end mixing for 1 hr. The gel was further washed three times with 5 gel volumes, alternatively with 0.1 M sodium acetate containing 1.0 M NaCl pH 4.0 and 0.1 M Tris containing 1.0 M NaCl pH 8.0. Finally the CBP-p5 was transferred to 50 mM phosphate buffer, 0.15 M NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA) 0.02% NaN₃, 0.05% Tween 20 pH 7.5 and stored at +4° until use. Stability of the covalent link between rPhl p 5b and CBP was confirmed after 3 months of storage at +4° by analysis of rPhl p5b in the supernatant.

Aluminium hydroxide (alum) adsorbates were prepared freshly prior to injection as described.²¹ Briefly, aluminium hydroxide, AluGel-S (Serva, Heidelberg; Germany) was diluted 1 : 1 in phosphate-buffered saline (PBS) and mixed with rPhl p 5b to yield 5 µg per 100 µl gel.

Immunization of mice

Six groups, each consisting of eight female 6–8 weeks old BALB/c mice (Charles River, Kisllegg, Germany) were immunized with CBP-p5 (group 1), Alum-p5 (group 2), CBP + p5 (group 3), p5 alone (group 4), Alum alone (group 5) and CBP alone (group 6). The mice were immunized subcutaneously in the neck with 100 µl of each of the six preparations. Each of the injections given to groups 1–4 contained 5 µg of rPhl p 5b. Immunizations were given and blood was taken on days 0, 23 and 52. On day 52 a booster injection was administered and 7 days later the animals were killed and spleen cells were prepared for cytokine measurements. The animals were kept in the animal care unit of the Department of Pathophysiology of the University of Vienna according to the local guidelines for animal care.

ELISA detection of allergen-specific antibodies

Immunoglobulin G1 (IgG1), IgG2a/b and IgG3 specific to rPhl p 5b were analysed for each blood sample in duplicates by enzyme-linked immunosorbent assay (ELISA).²² Ninety-six well microtitre plates (Nunc, Roskilde, Denmark) were coated with 100 µl PBS containing 5 µg/ml rPhl p 5b over night at +4°. The plates were washed twice with PBS containing 0.05% Tween 20 (PBS-T). To reduce unspecific binding, 200 µl PBS-T with 1% bovine serum albumin (BSA) was added for 2.5 hr at room temperature. One hundred µl mouse serum diluted in PBS-T containing 0.5% BSA (PBS-TB) (1 : 1000 for IgG1, 1 : 100 for IgG2a/b and IgG3, respectively) was added per well and incubated over night at +4°, followed by washing 5 × 250 µl with PBS-T. One hundred µl rat anti-mouse IgG1, IgG2a/b and IgG3 (PharMingen, San Diego, CA), respectively, diluted 1 : 1000 in PBS-TB were added to each well over night at +4° followed by washing. One hundred µl horseradish peroxidase-labelled sheep anti-rat IgG diluted 1 : 1000 in PBS-TB, was added for 2 hr at room temperature followed by washing 5 × 250 µl with PBS-T. 3,2'-Azino-di-(3-ethyl-benzthiazolin-sulfonate; ABTS, Sigma, St. Louis, MO) was used as substrate and the colour reaction was read at 405 nm with a Dynatech microplate reader (Denkendorf, Germany).

Group 5 allergens in different grass pollen were detected by ELISA. Pollen allergen extracts were prepared from *Phleum*

pratense, *Lolium perenne*, *Poa pratensis*, *Anthoxanthum odoratum*, *Triticum sativum*, *Avena sativa*, *Cynodon dactylon*, *Zea mays* and *Pharagmites antralis* (Allergon AB, Vålänge, Sweden) as described²¹ dissolved in PBS (100 µg/ml) and coated (100 µl/well) to 96-well ELISA plates (Nunc). After blocking and washing, the plate-bound extracts were exposed to sera from five mice immunized with CBP-p5 analysed individually in duplicates diluted 1 : 100 in PBS-TB. For control purposes, the corresponding preimmune sera were used in dilutions of 1 : 100. Bound IgG antibodies were detected with horseradish peroxidase-labelled sheep anti-mouse IgG antiserum (Amersham, Little Chalfont, UK) diluted 1 : 1000 in PBS-T.

Measurement of cytokine production in spleen cell cultures

For determination of interleukin 4 (IL-4), IL-5 and interferon- γ (IFN- γ) production, spleen cell suspensions from each group of immunized mice were cultured in 48-well plates (Costar, Cambridge, MA) with and without timothy grass pollen extract (25 µg/well) at a concentration of 5×10^6 /well. Supernatants were taken 24 hr for IL-4 and IL-5 and 48 hr for IFN- γ after antigen stimulation and stored at -20° until analysis.²³ Levels of IL-4 and IL-5 were measured by ELISA (Endogen, Cambridge, MA). The sensitivity of the tests were <5 pg/ml. IFN- γ levels were measured in 96-well plates (Nunc, Maxisorp) coated with rat anti-mouse IFN- γ (Endogen, Woburn, MA) at a concentration of 0.5 µg/ml carbonate buffer pH 9.6 for 6 hr at room temperature. Thereafter biotin-labelled rat anti-mouse IFN- γ antibodies (Endogen) were applied at a concentration of 0.1 µg/ml, followed by peroxidase-conjugated streptavidin (1 : 10 000 in PBS/4% BSA; Endogen) for 30 min. For colour development 3,3',5,5' tetramethylbenzidine base (TBM) substrate (Chemicon, Temecula, CA) was used and the absorbance was measured at 450 nm (Dynatech). The sensitivity of the assay was <15 pg/ml. Results reflect the measured cytokine levels in pg/ml after subtraction of baseline levels determined in nonstimulated cultures.

Histopathological analysis of skin sections

Skin was excised exactly from the injection areas, cut into 4-mm thick strips, fixed in 7.5% formalin, pH 7.5, and embedded in paraffin. Five µm sections were stained with haematoxylin-eosin or Giemsa.

ELISA competition assay

ELISA plates were coupled with rPhl p 5b (0.1 µg/well), blocked and washed as described for the ELISA assay above. Plates were exposed first to pooled sera of mice immunized either with CBP-p5 (group 1) or alum-p5 (group 2) or, for control purposes, with the corresponding preimmune serum pools diluted 1 : 100 overnight at 4° . After washing, the plates were incubated with sera from grass pollen allergic patients diluted 1 : 5. Bound human IgE antibodies were detected with an alkaline-phosphatase conjugated mouse monoclonal antihuman IgE antibody (PharMingen).²⁴ The inhibition of allergic patients' IgE binding to rPhl p 5b by the mouse immune sera was calculated using the formula $100 - (\text{OD second bleeding} / \text{OD preimmune serum}) \times 100$.

Statistical analysis

The non-parametric test Kruskal-Wallis ANOVA was used for evaluation of allergen specific antibody responses, IFN- γ , IL-5

and IL-4 cytokine levels. Outliers and extreme values were defined according to Tukey Box and Whisker plot. Mann-Whitney *U*-test was used for evaluation of the blocking of human IgE binding. $P < 0.05$ was considered statistically significant.

RESULTS

CBP-bound rPhl p 5b induces strong allergen-specific antibody responses

In order to compare levels and kinetics of rPhl p 5b-specific antibody responses we analysed sera from all groups of mice and bleedings separately for each IgG subclass on one ELISA plate. Mice from groups 1–4 showed increasing rPhl p 5b-specific antibody responses in the course of the immunizations, which peaked after the second immunization (Fig. 1). CBP-p5, alum-p5 and CBP + p5 immunized mice showed significantly higher antibody levels compared to mice who had received rPhl p 5b alone. Mice from groups 5 and 6 who had received only CBP and alum, showed no antibody response to rPhl p 5b (data not shown). The levels of rPhl p 5b specific IgG2a/b antibodies were significantly higher in the CBP-p5 group compared to the other groups (Fig. 1). The kinetics and magnitude of rPhl p 5b-specific IgG₁ and IgG2a/b antibody responses were similar in groups 2 (alum-p5) and 3 (CBP + p5).

Mice immunized with CBP-bound rPhl p 5b show mixed Th1/Th2 cytokine responses to natural Phl p5

The profile of IFN- γ , IL-5 and IL-4 secreted by spleen cells from mice of the six immunization groups cultivated in the presence of natural timothy grass pollen extract is shown in Fig. 2. The cytokine levels in groups 1–3 were similar, with the exception that mice who had received CBP-conjugated rPhl p 5b (group 1) mounted a significantly stronger IFN- γ production than mice immunized with alum-p5 (group 2) or CBP + p5 (group 3). Lower release of a similar cytokine profile, as observed in groups 2 and 3 was found in spleen cell cultures from mice who had received rPhl p 5b alone (group 4). Cell culture supernatants from mice receiving adjuvans only (i.e. group 5: alum and group 6: CBP) showed no significant cytokine production (Fig. 2).

CBP-bound rPhl p 5b induces no granulomatous tissue reaction

In order to analyse the tissue reactions at the injection sites, skin sections were taken from the mice belonging to groups 1–6 and processed for histological examination. In representative skin sections, a mixed cellular inflammatory reaction with prominent eosinophilic granulocytes in the deep dermis was observed, Fig. 3(a–e). Additional granulomatous responses could be seen with granular debris in the centre and predominantly foam cells and eosinophils at the cellular outer rim (Fig. 3b,d, between the arrows) with the alum-p5 and alum treated mice. The inflammatory tissue reactions of mice immunized with CBP-p5 and CBP alone (Fig. 3a,e) as well as with p5 alone (Fig. 3c) were smaller and contained less granular debris than those of the alum-treated mice. Close ups of the tissue reactions are shown in the right hand panels. A mixed cellular inflammatory infiltrate, including macrophages

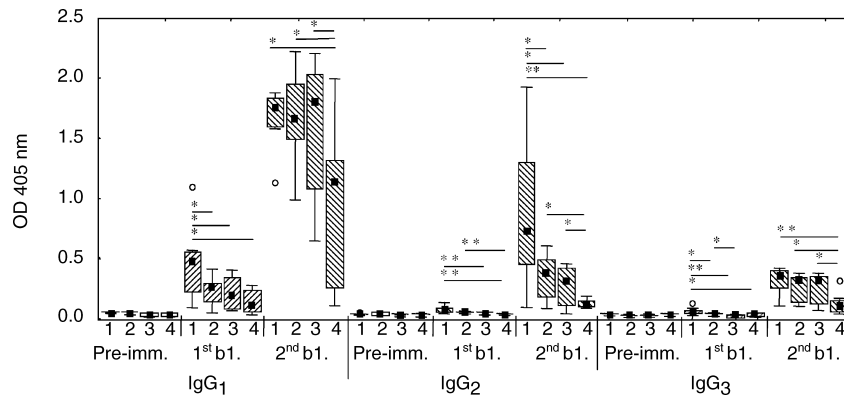


Figure 1. IgG subclass responses of mice to ELISA plate-bound rPhl p 5b. The optical density values (OD 405 nm) displayed on the y-axis correspond to the levels of Phl p 5b-specific IgG1, IgG2, and IgG3 antibodies in the sera of the four mouse groups (1: CBP-p5; 2: alum-p5; 3: CBP + p5; 4: p5). Results are displayed for preimmune sera (Pre-imm.), the first (1st bl.) and second (2nd bl.) bleeding as box plots where 50% of the values are within the boxes and nonoutliers between the bars. Filled squares denote median values and open circles outliers of each group, respectively. Kruskal–Wallis ANOVA: * $P < 0.05$; ** $P < 0.01$.

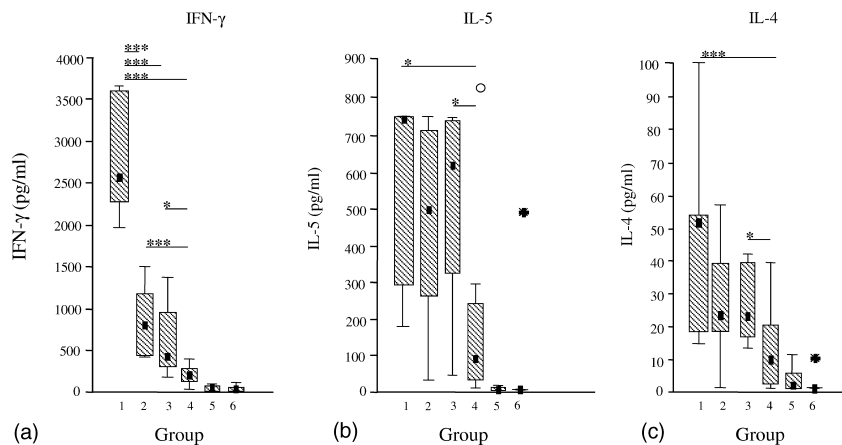


Figure 2. *In vitro* cytokine production in spleen cell cultures. IFN- γ , IL-5 and IL-4 levels were measured in supernatants of antigen-stimulated spleen cells of each group of mice (CBP-p5: group 1; alum-p5: group 2; CBP + p5: group 3; p5: group 4; alum: group 5; CBP: group 6). In the plot 50% of the values are within the boxes and non-outliers between the bars. Filled squares denote median values, open circles outliers and stars extremes of each group, respectively. Kruskal–Wallis ANOVA, * $P < 0.05$; *** $P < 0.001$.

and lymphocytes with occasional mast cells and eosinophils was observed in the injection sites in the deep dermis.

Mice immunized with CBP-bound rPhl p 5b exhibit IgG cross-reactivity with natural pollen extracts from grasses containing group 5 allergens

To study whether immunization with CBP-p5 induces IgG antibodies, which cross-react with group 5 allergens from pollen of other grass species, ELISA experiments were performed with preimmune sera and immune sera from the second bleeding. rPhl p 5b-specific IgG antibodies reacted with natural group 5 allergens from timothy grass (*Phleum pratense*) and from five grass species (*Lolium perenne*, *Poa pratensis*, *Anihoxanthum odoratum*, *Triticum sativum*, *Avena sativa*). The highest levels were recorded for *Poa pratensis* (Fig. 4). No IgG reactivity to pollen extracts from grasses (*Cynodon dactylon*, *Zea mays*, *Phragmites australis*) lacking group 5-related allergens was

found. The preimmune sera showed no significant IgG reactivity to any of the nine grass pollen extracts (Fig. 4).

Sera from mice immunized with CBP-bound rPhl p 5b inhibit the binding of allergic patients' IgE to the allergen

Previously it had been shown that sera from mice immunized with alum-adsorbed rPhl p 5b can inhibit the binding of grass pollen allergic patients IgE antibodies to the allergen.²⁰ We therefore studied whether sera from mice immunized with CBP-p5, can also inhibit the binding of grass pollen allergic patients' IgE to rPhl p 5b by ELISA competition experiments. Microtitre-bound rPhl p 5b was preincubated with serum pools taken on day 52, obtained either from mice immunized with CBP-p5 or alum-p5 and, for control purpose, with the corresponding pools of preimmune sera and then exposed it to serum IgE from nine grass pollen allergic patients. Sera from mice immunized with

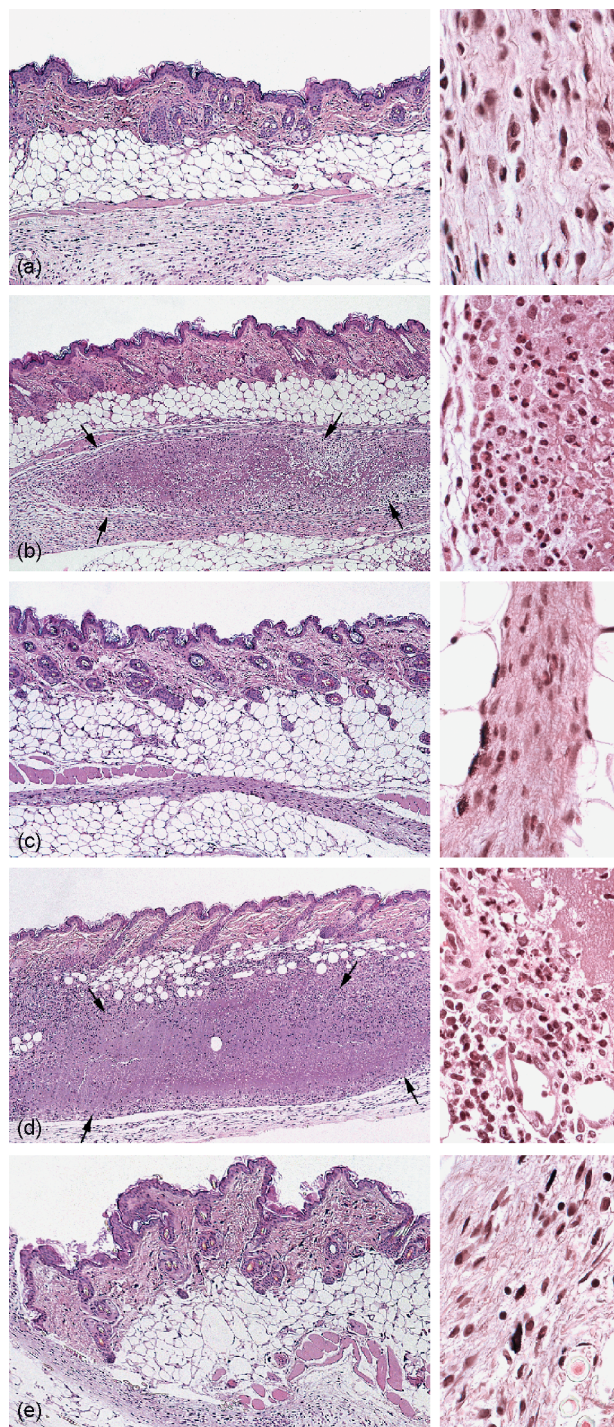


Figure 3. Histopathological analysis of injection sites. On the left side representative skin sections (10-fold magnification) from CBP-p5 (a), alum-p5 (b), p5 (c), alum (d) and CBP (e) immunized mice are shown. The arrows indicate the margins of the granuloma at the injection sites. On the right side sections close ups (100-fold magnification) of the tissue reactions are displayed.

CBP-p5, inhibited allergic patients ($n = 9$) IgE binding to rPhl p 5b from 37 to 80% (mean 59.1%) whereas an inhibition between 51 and 90% (mean 67.9%) was observed with sera from group 2

(alum-p5) (Fig. 5). Although the inhibition of IgE binding obtained with the sera from mice treated with alum-bound-rPhl p 5b was somewhat higher than that obtained with sera from mice immunized with CBP-p5, no significant difference between the groups 1 in terms of blocking capacity was observed ($P = 0.31$; Fig. 5).

DISCUSSION

We have demonstrated that CBP may represent a useful adjuvant for allergen-specific immunotherapy yielding comparable immune responses as aluminium-hydroxide, the most commonly used adjuvant for injection immunotherapy.⁴ We found that immunization with purified rPhl p 5b allergen covalently bound to CBP induced strong antibody responses ($\text{IgG1} > \text{IgG2a/b} > \text{IgG3}$) in mice. The CBP-p5-induced mouse IgG antibodies maintained a capacity to cross-react with natural group 5 allergens from 5 grass indicating that the covalent coupling of rPhl p 5b to CBP did not significantly alter the structure of the cross-reactive epitopes. Perhaps more important is that the CBP-p5-induced mouse antibodies competed with the binding of grass pollen allergic patients' IgE antibodies to Phl p 5b. The latter finding suggests that the CBP-allergen conjugate induced antibodies with desirable features of blocking antibodies^{25,26} similar to the alum-p5 formulation, which was included for comparison. In this context it should be noted that several studies performed with alum-adsorbed allergens provided evidence that blocking antibodies induced in the course of immunotherapy have beneficial effects as they can inhibit the allergen-induced effector cell activation and the IgE-mediated presentation of allergens to T cells.²⁷⁻²⁹

We suggest considering CBP as potential adjuvant for allergen-specific immunotherapy because it seems to resemble many of the beneficial features of alum but will have several advantages regarding the production of vaccine formulations for immunotherapy. First, the coupling of proteins to CN-Br activated Sepharose, is a well-described and reproducible procedure known to give high density, high yield and stable bonding of proteins or peptides.³⁰ In this context it should be emphasized that a great variety of allergen extracts, purified allergens and allergen-derived peptides have been routinely coupled to Sepharose^{31,32} for immunological assays, indicating that the coupling procedure preserves the immunological properties of the conjugated allergens. For the latter reason, CBP may facilitate the production and quality control of allergy vaccine formulations because of the precise and reliable coupling chemistry and the possibility to perform immunological stability tests.

Another important advantage of Sepharose is its biocompatibility, which will be relevant in clinical practise when it comes to administration of vaccination formulations via different routes (e.g. injection, oral immunotherapy or nasal administration). Sepharose is known to be well tolerated when exposed to a variety of cell types in tissue culture and as column matrix in clinical *ex vivo* treatments.³³ This is supported by our finding that immunization of mice with CBP induced no granulomatous reactions compared to aluminium hydroxide under comparable treatment.

Several *in vitro* data also document that antigens that are covalently conjugated to particles are processed and presented in a very efficient way by antigen-presenting cells^{17,34,35} a

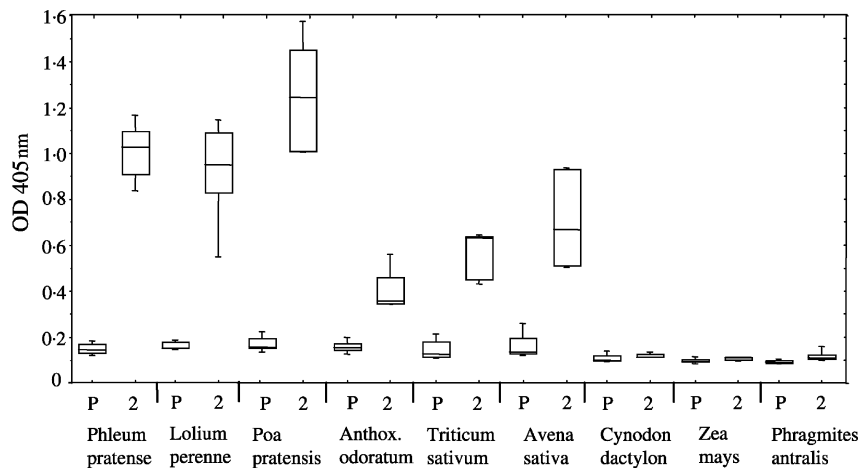


Figure 4. Cross-reactivity of IgG antibodies from CBP-p5-immunized mice ($n = 5$) with natural group 5 allergens from various grass species. Optical density values (y-axis, OD 405 nm) corresponding to the serum IgG antibody levels to pollen extracts from nine grass species (*Phleum pratense*, *Lolium perenne*, *Poa pratensis*, *Anthoxanthum odoratum*, *Triticum sativum*, *Avena sativa*, *Cynodon dactylon*, *Zea mays*, *Phragmites antralis*) are displayed for sera from five mice collected before (P) the immunizations and during the second bleeding (2) at the x-axis. Boxes and horizontal bars denote 50% of the values and nonoutlier range, respectively. Mean values are indicated in the boxes by horizontal bars.

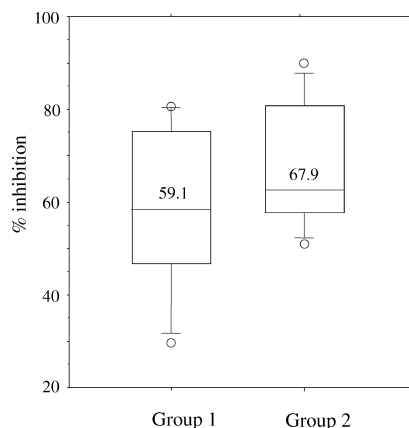


Figure 5. Inhibition of grass pollen allergic patients' IgE binding to rPhl p 5b by murine sera. ELISA plate-bound rPhl p 5b was preincubated with a serum pool from CBP-p5 (Group 1) or from alum-p5 (Group 2) immunized mice. The percentage inhibition of IgE binding determined for sera from nine grass pollen allergic patients is displayed on the y-axis. Boxes and horizontal bars denote 50% of the values and non-outlier range, respectively. Mean values are indicated in the boxes by horizontal bars and open circles denote outliers.

finding that is supported by our notion that the CBP-p5 immunized mice showed a more vigorous IFN- γ response than (CBP + p5), p5 or alum-treated mice. In contrast to soluble antigens it has been suggested that 2- μ m particles, upon phagocytosis by monocytes can act as a potent signal for IL-12 and IFN- γ production,³⁶ thus skewing the T-cell responses toward Th1.

Also in our study CBP seemed to favour a balanced Th1/Th2 response. The comparable antibody level and cytokine production in allergen cultured spleen cells in the mice immunized with CBP + p5 and alum-p5 indicates that non-antigen-conjugated CBP may promote a Th2 response. However, the IgG2a/b level and IFN- γ productions were significantly elevated in the

group receiving CBP-p5, which is indicative for a trend towards a Th1 response, a feature which has also been proposed for other new adjuvants.^{5,6} On the other hand, immunization with CBP-p5 also demonstrated strong IL-4 and IL-5 production indicative of a Th2 response. In this context it has been reported that Th2 responses can have a positive impact on the clinical development of established allergic sensitization.³⁷ Moreover, concerns have been raised whether the induction of a too strong Th1 response should be the major objective in immunotherapy. Some reports highlight the potential danger that Th1-driving adjuvants may initiate autoimmune responses^{38,39} while others do not consider Th 1-responses to be a major drawback in immunotherapy as long as the response is allergen-specific.^{4,40} In conclusion we think that CBP resembles many of the desirable features of an adjuvant for allergen-specific immunotherapy and, thus may be soon evaluated for safety and efficacy in patients.

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