

Allergen-Specific Polyclonal Antibodies Reduce Allergic Disease in a Mouse Model of Allergic Asthma

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Key Words

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Abstract

Background: Recombinant allergen-specific immunoglobulin G (IgG) antibody therapy can reduce allergic asthma symptoms by inhibiting the immunoglobulin E (IgE)-mediated allergic response. This study investigated the effect of intranasally administered allergen-specific monoclonal (mAb) and polyclonal (pAb) antibody on airway inflammation and hyperresponsiveness (AHR) in a mouse model of human asthma. **Methods:** Ovalbumin (OVA)-specific IgG2b antibodies were generated by phage display using spleens from OVA-immunized mice, and screening against OVA and finally expressed in CHO cells. Sensitized mice were treated intranasally with either a recombinant anti-OVA mAb (gc32) or a polyclonal preparation comprising seven selected antibodies (including gc32). Control mice received diluent only, OVA only, a control polymeric IgG or dexamethasone. Following challenge with nebulized OVA, investigators assessed airway inflammation by histology and cellular composition of the bronchoalveolar fluid, and methacholine-induced airway hyperresponsiveness (AHR). Serum levels of total and OVA-specific IgE were measured by ELISA. **Results:** Sensi-

tized mice developed airway inflammation and AHR in response to OVA challenge. Intranasally administered OVA-specific murine polyclonal or monoclonal IgG2b antibodies both reduced OVA-induced lung inflammation. Polyclonal, but not anti-OVA mAb, also reduced AHR and eosinophil influx into the airway lumen. Both anti-OVA antibody preparations reduced levels of specific IgE with no effect on total IgE levels. **Conclusions:** Intranasal treatment with allergen-specific pAb reduces pulmonary inflammation and AHR in a mouse model of allergic asthma, but allergen-specific mAb reduces inflammation only. Allergen-specific recombinant pAb offers a potentially valuable therapeutic approach to the management of allergic asthma.

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Introduction

The treatment of allergic asthma aims to reduce airway inflammation and airway hyperresponsiveness (AHR), and is largely based on bronchodilator therapy and/or corticosteroid agents that have a nonspecific anti-inflammatory effect. Specific allergy vaccination offers the potential to modify the underlying disease pathology, and was first reported almost a century ago [1]. However, ear-

ly techniques were relatively unsophisticated and involved injecting allergic rhinitis sufferers with subcutaneous pollen extracts [1]. The mechanism for allergy vaccination remained unclear until Cooke et al. [2] published their classical work demonstrating inhibitory antibody production in response to allergen-specific inoculation. Further studies by Loveless [3, 4] showed that the mechanism for allergen-specific vaccination involved competitive inhibition.

Loveless also distinguished between sensitizing and blocking antibodies [3]. Subsequent studies by Lichtenstein et al. [5, 6] and Djurup et al. [7] have identified these blocking antibodies as immunoglobulin G (IgG), and established that such blocking antibodies correlate with reduced specific immunoglobulin E (IgE) levels following immunotherapy.

IgE antibody binding to allergen on the surface of mast cells and eosinophils is a crucial step in type I hypersensitivity reactions, which are important in the pathology of allergic disease but have little or no defensive role against infection [8]. It appears that IgE may also act directly on bronchial epithelial cells of people with allergic asthma [9]. Passive antibody therapy, i.e. administration of exogenous antibodies, is well-established in a range of conditions such as cancer, viral infection, and neutralization of bacterial toxins [10]. Hence, the potential benefits of targeted passive antibody therapy for allergic asthma have prompted research with promising results using monoclonal antibodies (mAbs) against allergen-specific IgE.

However, mAbs are inherently monospecific, and this may limit their capacity to neutralize complex antigens consisting of multiple epitopes. Studies have demonstrated that oligoclonal antibody therapy (combinations of a small number of closely related antibodies that bind different epitopes) offers synergistic efficacy in neutralizing cytokines [11] and bacterial toxins [12] compared with single mAb. There have also been reports of synergistic effects of polyclonal antibodies (pAb) in neutralizing viruses [13, 14]. In one study, the authors identified four viral epitopes each of which bound a different mAb [15].

Although many studies have administered antibodies and/or allergen by subcutaneous or intraperitoneal (i.p.) injection, prophylactic intranasal (i.n.) administration of specific pAb has shown efficacy in the prevention of respiratory virus infections [16].

Mice rendered allergic to ovalbumin (OVA) represent a suitable animal model for human allergen vaccination because they have a comparable response to specific immune therapy [17]. Here we used mice that were allergic to OVA to investigate the feasibility of reducing airway inflamma-

tion and AHR by reducing the allergic response using i.n. administered allergen-specific recombinant pAb.

Materials and Methods

Animals

Female BALB/cJ mice aged 6–8 weeks were obtained from M&B (Ry, Denmark) and housed in Scantainer cages (Scanbur, Karlslunde, Denmark). All animal work was approved by the Danish Research Animals Committee. Mice were kept on an OVA-free diet, and given food and water ad libitum.

OVA-Specific mAb and pAb

All mice were immunized twice i.p. with 20 µg OVA (Grade V, Sigma, St. Louis, Mo., USA) in complete Freund's adjuvant. Three mice were killed and their spleens were used as a source of antibody genes. Briefly, PBMC were isolated by gradient centrifugation and total PBMC mRNA was prepared using mini-Prep kit (Qiagen) according to the manufacturer's instructions. Combinatorial phage display libraries were prepared as described [18]. Anti-OVA antibodies were isolated by panning phage libraries on OVA-coated polystyrene, cloned using standard phage display and screened as Fab fragments against OVA. CDR3 regions of OVA-specific antibodies were sequenced at Agowa (Berlin, Germany). Antibodies were selected for further study to provide maximal diversity of amino acid sequences, and clones were tested to ensure that they reacted with different epitopes on OVA.

Selected whole antibodies were expressed as murine IgG2b from stably transfected CHO cells in serum-free medium, purified on protein G (Amersham Biosciences, Uppsala, Sweden), freeze-dried, and dissolved in endotoxin-free phosphate-buffered saline (PBS). Purity was estimated to be >95% by SDS-PAGE. Specificity and binding characteristics were verified by ELISA and Western blot as described below.

Determination of Anti-OVA Specificity

Subtilisin cleaves 33 amino acids from the C-terminus of OVA. For subtilisin digestion, 1 mg/ml OVA (Grade V, Sigma) was incubated with 400 ng/ml subtilisin (Subtilisin Carlsberg, *Bacillus licheniformis*, Type VIII, Sigma) in PBS, pH 7.4, for 40 min at 37°C. Digestion by subtilisin was confirmed by SDS-PAGE seen as a shift of the OVA band from approximately 43 to 40 kDa. Pepsin cleaves 22 amino acids from the N-terminus of OVA. For digestion by pepsin, 1 mg/ml OVA was incubated with 200 µg/ml pepsin (Sigma) in 100 mM acetate, pH 4.0, overnight at room temperature (RT). Digests were subsequently desalted using PD10 columns (Amersham) according to the manufacturer's instructions. Pepsin digest was confirmed by SDS-PAGE, seen as a shift of the OVA band from approximately 43 kDa to a slightly faster migrating band. To generate the soluble OVA isoform, S-OVA, OVA (Sigma Grade VII, Sigma), 10 mg/ml, was generated by incubation overnight at 55°C in 100 mM phosphate, pH 10.0 [19]. OVA was heat denatured as described by Takahashi et al. [20]. For reduction 2 µl DTT or 10 µl 0.5 M TCEP (BondBreaker, Pierce) was added to 100 µl OVA, 1 mg/ml in PBS, followed by incubation at 80°C for 1 h. For alkylation, 10 µl 10 × PBS (Gibco) and 100 µl 200 mM N-ethyl maleimide in water was added, followed by a further 1-hour incubation at RT [21].

For Western blot analysis of anti-OVA Fab specificity, native and subtilisin- and pepsin-digested OVA was subjected to SDS-PAGE and transferred to PVDF membranes by standard protocols. Nonreducing SDS-PAGE was done by omitting reducing agent (5 vol% 2-mercaptoethanol) during heat denaturation of samples. Fab fragments (crude periplasmic extract desalted on PD10 columns, Amersham) were used at 1:2 dilution in 5% BLOTTO (dry skimmed milk) 0.05% Tween in PBS. Bands were visualized using an HRP-conjugated anti-mouse Fab secondary antibody (Sigma), UVP West chemiluminescent substrate and a CCD camera.

For ELISA analysis of anti-OVA Fab specificity, native, subtilisin-digested, pepsin-digested, S-OVA, heat-denatured as well as reduced and alkylated OVA was coated on ELISA plates (Costar) at 10 µg/ml in 50 mM carbonate, pH 9.6 overnight at 4°C. After incubation the plates were washed and blocked in 5% BLOTTO. Phage Fab fragments were diluted from 1:3 to 1:81 in 5% BLOTTO, PBS, 0.05% Tween and incubated in the plates for 2 h at RT. After washing, an HRP-conjugated anti-M13 mAb secondary antibody (Pharmacia), diluted 1:5,000 in 2% BLOTTO, PBS, 0.05% Tween was added to the plates and incubated for 1 h at RT. Plates were developed with TMB substrate (Dako), and stopped with 1 M sulfuric acid. The optical density at 450 nm was determined in a standard microplate reader.

Quantification of Nebulized OVA and i.n. Administered Antibody

To quantify the amount of antibody reaching the lungs by i.n. administration, 75 µg rabbit pAb (AB1225, Chemicon) in 50 µl PBS was administered i.n. before bronchoalveolar lavage (BAL) was obtained from both lungs 0, 1, 2 and 3 h postadministration. The amount of antibody recovered was determined using the OVA ELISA as described above using HRP-conjugated anti-rabbit antibody (BD Pharmingen, Mountain View, Calif., USA) as secondary detection agent. To measure the amount of OVA instilled by nebulization, mice were exposed to nebulized OVA for 10 or 20 min. The recovery of OVA at 3 h postadministration was done using OVA-specific ELISA as described above.

Induction of Allergy

Mice were sensitized by two i.p. injections of 20 µg OVA in 2.25 mg alum (Imject, Pierce, Rockford, Ill., USA) in 100 µl PBS on days 0 and 14, and challenged on day 28, and on each of the following 4 days with either nebulized PBS or 1% OVA in PBS for 20 min (~10 ml) using a jet nebulizer. i.n. administration of 75 µg of monoclonal or polyclonal anti-OVA murine IgG2b or an isotype control polyclonal murine IgG2b (Zymed, San Francisco, Calif., USA) was done 3 h prior to all challenges in 50 µl PBS under light anesthesia (methohexitone, Brierley Sodium, Eli Lilly, Indianapolis, Ind., USA) [22]. The control antibody did not react with OVA as assessed by ELISA. Dexamethasone, which is a widely used treatment for asthma (Intervet, Boxmeer, The Netherlands), 60 µg/mouse, was administered by i.p. injection as a positive control.

Airway Hyperresponsiveness

AHR was assessed 24 h after the last OVA challenge using single-chamber whole-body plethysmography from Buxco (Wilmington, N.C., USA), following the method described by Hamelmann et al. [23]. We measured enhanced pause (Penh), a dimensionless value closely correlated to bronchoconstriction [23] in response to increasing doses of methacholine chloride (Sigma). First, baseline

Penh was recorded for 3 min; next, PBS was nebulized for 3 min followed by recording the response to PBS for 3 min. There was a 1-min pause followed by nebulization of 3.125 mg/ml methacholine for 3 min, recording for 3 min, and 1 min pause. This procedure was continued with 2-fold dose increments up to a dose of 50 mg/ml.

Bronchoalveolar Lavage

The right lung only was lavaged by 500 µl PBS 3 times before pooling the fractions from individual mice. BAL assessments were performed immediately following nebulization. Total cell counts were determined on a FACScalibur (Becton Dickinson, San Diego, Calif., USA) using the LeucoCOUNT system (Becton Dickinson) and differential cell counts were determined using May-Grünwald Giemsa staining.

Lung Histology

The left lung was tied off at the bronchi before the trachea was opened and cannulated for lavage of the right lung. The left lung was then excised, fixed in formaldehyde and stained with Giemsa. An observer blinded to the samples evaluated lung inflammation. The degree of inflammation was scored at three different views of each mouse lung on a scale from 0 (no inflammation) to 4 (severe inflammation) and averaged as described by Tumas et al. [24].

Serum Anti-OVA IgE Levels

ELISA wells were coated overnight with 5 µg/ml OVA to measure serum levels of total and OVA-specific IgE. A biotin-conjugated IgE-specific antibody (1 µg/ml) followed by HRP-conjugated avidin (1:1,000) was used as detection reagent (both from BD Pharmingen). IgE levels (in arbitrary units) were calculated from an internal standard consisting of serum from OVA-immunized mice. Mouse sera were tested at a 100-fold dilution. The procedure for OVA coating and ELISA are as described above, except that all incubation times were 1 h.

Statistics

The groups were compared in a paired Student t test (two-sided) and expressed as means $\pm$ SEM except where noted. A two-way ANOVA test was used to evaluate the therapeutic effect over the entire methacholine dose range. Probability values <0.05 were considered statistically significant.

Results

Isolation of OVA-Specific Antibodies

A total of 33 OVA-specific mAb were isolated from mice immunized with OVA, using conventional combinatorial phage display. Sequencing showed that these were divided into three groups: I–III based on their heavy chain CDR3 region sequences. Table 1 shows the binding characteristics to OVA of the different antibody types. As can be seen the three antibody types covered at least three distinct epitopes on OVA, as determined by ELISA and Western blotting (table 1). The antibody with the highest binding activity to OVA in ELISA, gc32 belonging to type I, was selected for further studies. Based on variable domain

Table 1. Reactivity of OVA-specific pAb

OVA	Digestion	Detection	gc32	Type I	Type II	Type III
Native	non	WB	+	+	+	+
Native	subtilisin	WB	+	+	+	+
Reduced	non	WB	–	–	–	+
Reduced	subtilisin	WB	–	–	–	+
Native	non	ELISA	+	+	+	–
S-OVA	non	ELISA	+	+	+	–
Native	pepsin	ELISA	+	+	–	+
Native	subtilisin	ELISA	+	+	+	–
Reduced	non	ELISA	+	+	–	–
Heat, reduced, alkylated	non	ELISA	–	–	–	+

non = No enzymatic digestion; WB = Western blotting.

Table 2. Recovery of antibody (Ab) and OVA in BAL after 75- μ g i.n. administration

i.n. administration	Amount administered	Time after administration, h			
		0	1	2	3
Ab, μ g	75	28.7 $\pm$ 4.3	23.6 $\pm$ 13.6	17.6 $\pm$ 6.1	9.0 $\pm$ 7.4
OVA, μ g	75	ND	ND	ND	10.8 $\pm$ 6.2

Data from one of two experiments representing the mean of 3 mice $\pm$ SD is shown. ND = Not done.

sequences and epitope mapping studies (table 1), a further six anti-OVA antibodies were selected to provide a diverse pAb composition. Thus, the anti-OVA pAb comprises equal amounts of gc32, one additional type I antibody, two type II antibodies and three type III antibodies.

Bioavailability of Antibodies Administered i.n.

We assessed the bioavailability of rabbit pAb administered i.n. (to facilitate identification of exogenous antibody) by measuring the fractions of administered antibody or OVA in BAL fluid. The clearance from the airways of antibodies administered i.n. during the 3-hour period following administration was also measured. Of the administered antibody, 28.7 $\pm$ 4.3 μ g (38%) could be recovered from BAL immediately after administration. At 1, 2, and 3 h after exposure 23.6 $\pm$ 13.6, 17.6 $\pm$ 6.1, and 9.0 $\pm$ 7.4 μ g was recovered, respectively, resulting in a calculated pulmonary half-life of approximately 2.5 h. The amount of OVA recovered from BAL after 20 min exposure to aerosolized 1% OVA solution was 44.1 $\pm$ 3.1 ng (table 2). These results suggest that there was approximately a 100-fold molar excess of antibodies over OVA (0.05 nM vs. 0.65 pM) at the mucosal surface.

Polyclonal, but Not Monoclonal OVA-Specific Antibodies Reduce AHR

We investigated whether i.n. administration of OVA-specific antibodies can reduce OVA-mediated AHR in vivo. OVA-sensitized mice were challenged with aerosolized OVA after i.n. administration of antibodies; subsequently AHR was induced by metacholine. This protocol significantly increased AHR in the absence of treatment, but had minimal effect in dexamethasone-treated mice (fig. 1).

i.n. administration of OVA-specific pAb (IgG2b) significantly reduced AHR in OVA-sensitized mice challenged with OVA and methacholine (fig. 1). In contrast, neither the OVA-specific mAb nor the control antibody had any effect on AHR. In addition, the protective effect of anti-OVA pAb was significant over the entire methacholine dose range as analyzed by two-way ANOVA ($p < 0.05$ vs. controls; data not shown). Thus, i.n. administration of anti-OVA pAb effectively reduced AHR in this mouse model of allergic asthma.

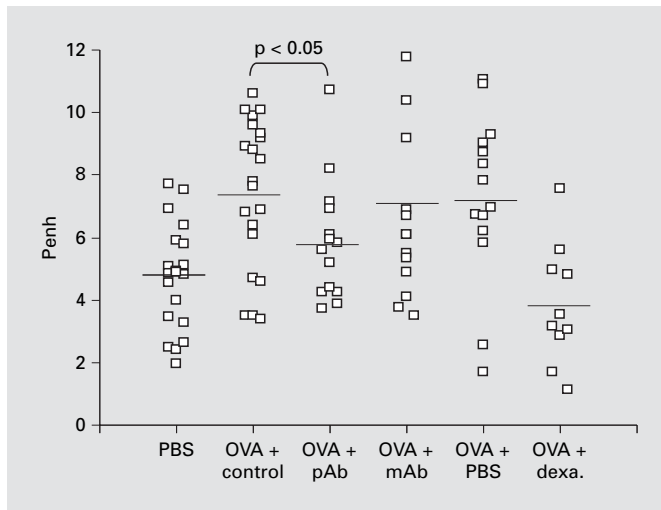


Fig. 1. Anti-OVA pAb reduce AHR to methacholine. BALB/cJ mice were sensitized and challenged with OVA on days 0 and 14. On day 28 and the following 4 days mice were challenged with OVA 3 h after i.n. antibody administration. Study groups included PBS challenge (n = 21), OVA challenge (n = 21), OVA + anti-OVA pAb treatment (n = 14), OVA + anti-OVA mAb treatment (n = 13), OVA + control pAb treatment (n = 14), and OVA + dexamethasone (dexa.) treatment (n = 10). Penh measurements were taken following administration of 50 mg/ml methacholine. Pooled data of individual mice from three separate experiments are shown; bars represent the mean value of each group. Statistical significant differences between individual groups are indicated.

Topical Administration of IgG2b Reduces Leukocyte Influx, Particularly Eosinophilia, into the Airway Lumen

We evaluated the effect of antibody administration on airway inflammation following OVA challenge in sensitized mice. To assess leukocyte influx into the airway lumen, we measured leukocyte levels in BAL, with differential counts of eosinophils and other leukocytes, mainly alveolar macrophages and lymphocytes. As expected, OVA-sensitized mice showed a marked increase in leukocyte influx following challenge, compared with negative controls. In these studies total leukocyte influx was reduced significantly by treatment with anti-OVA-pAb, anti-OVA-mAb and dexamethasone (fig. 2a). A reduction was also seen with control pAb, but the difference did not reach statistical significance (fig. 2a). The influx of mononuclear cells was reduced significantly by anti-OVA-mAb and dexamethasone, whereas anti-OVA-pAb and dexamethasone decreased eosinophilia in the BAL (fig. 2b). To assess whether airway tissue inflammation was affected by antibody treatment peribronchial and perivascular inflammation was scored in mice. Using this approach we found inflammation to be reduced slightly, but significantly in anti-OVA pAb- and anti-OVA mAb-treated mice as compared to OVA-challenged nontreated mice (fig. 3).

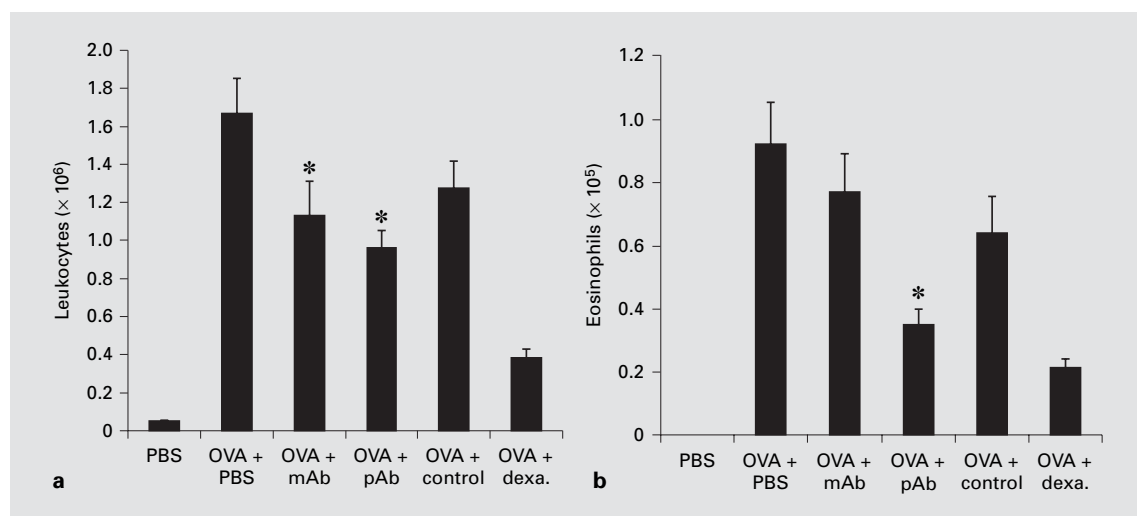


Fig. 2. Reduced levels of leukocytes (a) and eosinophil granulocytes (b) in BAL after administration of anti-OVA pAb. BALB/cJ mice were sensitized and challenged with OVA on days 0 and 14. On day 28 and the following 4 days mice were challenged with OVA 3 h after i.n. antibody administration. Study groups included PBS challenge (n = 21), OVA challenge (n = 21), OVA + anti-OVA pAb treatment (n = 14), OVA + anti-OVA mAb treatment (n = 13), OVA + control pAb treatment (n = 14), and OVA + dexamethasone (dexa.) treatment (n = 10). The BAL was harvested subsequent to Penh measurements as described in Materials and Methods. Means + SEM are shown. * p < 0.05 compared to nontreated OVA-challenged mice.

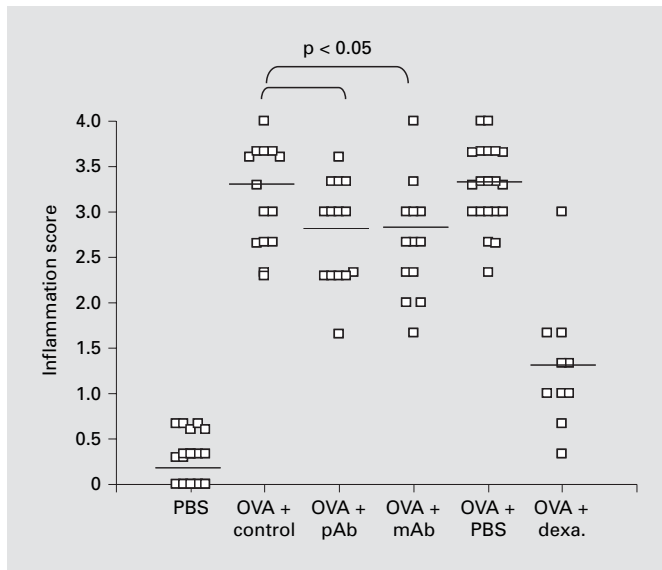


Fig. 3. Reduced airway inflammation after administration of anti-OVA pAb. BALB/cJ mice were sensitized and challenged with OVA on days 0 and 14. On day 28 and the following 4 days mice were challenged with OVA 3 h after i.n. antibody administration. Study groups included PBS challenge (n = 16), OVA challenge (n = 13), OVA + anti-OVA pAb treatment (n = 14), OVA + anti-OVA mAb treatment (n = 10), OVA + control pAb treatment (n = 8) and OVA + dexamethasone (dexa.) treatment (n = 10). Inflammation was scored by a blinded observer on a scale of 0–4. Statistical significant differences between individual groups are indicated.

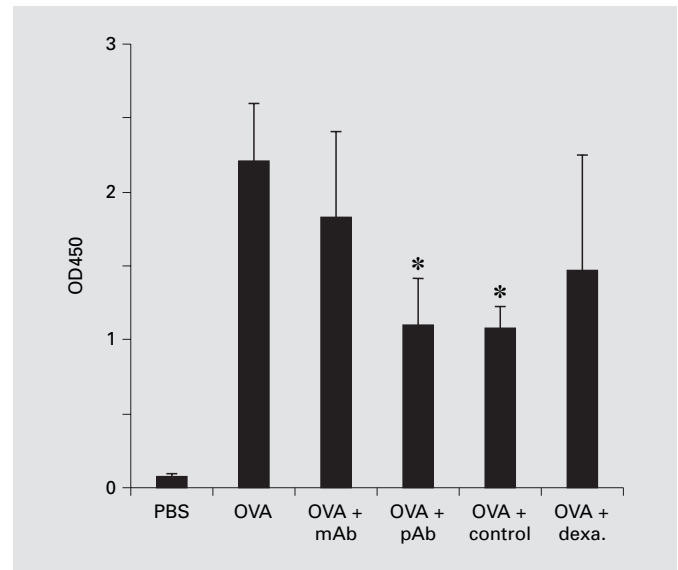


Fig. 4. Anti-OVA pAb reduce serum levels of OVA-specific IgE. BALB/cJ mice were sensitized and challenged with OVA on days 0 and 14. On day 28 and the following 4 days mice were challenged with OVA 3 h after i.n. antibody administration. Study groups included PBS challenge (n = 21), OVA challenge (n = 21), OVA + anti-OVA pAb treatment (n = 14), OVA + anti-OVA mAb treatment (n = 13), OVA + control pAb treatment (n = 14), and OVA + dexamethasone (dexa.) treatment (n = 10). Serum was harvested at the time of sacrifice following Penh measurements. OVA-specific antibodies were quantified as described in Material and Methods. ELISA. Means + SEM are shown. * p < 0.05 compared to nontreated OVA-challenged mice.

OVA-Specific pAb Reduce Specific Serum IgE Antibody Levels

To investigate the mechanisms of the anti-OVA pAb-mediated reduction in allergic inflammation and symptoms we analyzed a number of allergic mediators. Analysis of total serum IgE revealed no differences between treated and untreated mice (data not shown). However, serum levels of OVA-specific IgE were significantly lower in anti-OVA pAb-treated mice compared with controls. There was no reduction in OVA-specific IgE in anti-OVA mAb-treated mice. The control pAb also reduced specific IgE levels as part of a nonspecific effect on IgE (fig. 4).

Discussion

We cloned OVA-specific antibodies from OVA-immunized mice and tested these recombinant antibodies for prevention of OVA-induced allergy in a mouse model.

The results support that polyclonal allergen-specific IgG2b antibodies may offer better symptom control than mAb in the management of allergic asthma.

Our anti-OVA pAb significantly improved both AHR and inflammation in OVA-challenged mice. Anti-OVA mAb therapy had no effect on AHR, although there was an effect on inflammation. Interestingly, BAL analysis revealed that anti-OVA pAb had its strongest effect on the eosinophil influx, while anti-OVA mAb affected mononuclear cell influx. Dexamethasone, as a positive control, yielded predictable results: an improvement in all measures of disease activity with no effect on the IgE level. The control antibody produced an unexpected result, however, in reducing levels of specific IgE, although it had no effect on any measured disease endpoint.

These data highlight the complexity of the pathogenic mechanisms for AHR and inflammation in allergic asthma. Although both mAb and pAb reduced inflammation, they appeared to differ in their mechanism for this effect. It

is thought that allergen-specific blocking IgG acts through several mechanisms such as blocking the production of allergen-specific IgE in response to allergen challenge; interfering with IgE-mediated allergen binding to eosinophils, mast cells and basophils; and downregulation of IgE-enhanced presentation of antigen to T cells [25]. Hence, differences between the effects of individual antibodies on disease pathology may reflect varying effects on different IgE-mediated mechanisms for an allergic response.

Further mechanistic studies are required to explain the differences between the effects of monoclonal and polyclonal IgG in this mouse model. However, we conclude that the anti-OVA mAb (gc32) acted on fewer immune mechanisms than anti-OVA pAb. Both anti-OVA mAb and pAb reduced the circulating levels of specific IgE, suggesting that both antibodies inhibited IgE production in response to challenge. However, anti-OVA pAb also acted on a different mechanism to reduce AHR, while anti-OVA mAb lacked this effect.

When assessing the effect of antibody treatment we only differentiated between eosinophils and mononuclear leukocytes in this study. However, these analyses demonstrated that only the pAb reduced eosinophil influx into the airway lumen. This may hint at the mechanism by which the pAb reduced AHR in this study. Activated eosinophils release a number of cytokines including interleukin-13, which acts on smooth muscle to induce AHR [26], so reducing the number of eosinophils in the airway lumen would be predicted to reduce AHR.

Two additional findings warrant discussion: the unexpected effect of the control antibody on specific IgE levels and an apparent anomaly in the effects of the monoclonal and pAb; the mAb gc32 appeared to have a greater effect on mononuclear cell influx than the pAb, which included gc32 (fig. 2). Hence, reduced levels of OVA-specific serum IgE were seen in mice treated with OVA-specific and control antibodies. The reason for this is currently unclear. However, as the control antibody does not bind OVA this effect is not allergen-specific; instead one explanation might be the induction of FcR-mediated negative feedback resulting from polyclonal IgG2b binding to B cells in the mucosa. Reductions in allergen-specific IgE have previously been reported in patients treated with high-dose intravenous immunoglobulin [27]; despite the route of administration and the dose of immunoglobulin, this observation agrees with the present data. Given the unclear association between IgE and AHR and inflammation, the effect on OVA-specific IgE mediated by the control antibody might not contribute to the significant therapeutic effects seen on these latter parameters.

As for the discrepancy in the effect on mononuclear cells, it has previously been reported that some IgG antibodies seem to enhance, rather than block, IgE-mediated allergic responses to the birch pollen allergen Bet v 1 [28, 29]. As the individual antibodies comprised in the anti-OVA pAb were screened individually against OVA, but not against OVA-specific IgE or IgE-dependent effects, it might be possible that one of these antibodies has an opposite than gc32 on mononuclear cell-mediated inflammation.

This study provides evidence that i.n. administration is appropriate for allergic asthma. This is consistent with previous reports of i.n. administered antibody therapy for allergic asthma [30, 31]. Sehra et al. [31] demonstrated reduced lung infiltration and inflammation following i.n. administration of anti-OVA IgG in a murine model similar to the present study. However, there was no assessment of AHR in their study. Thus, the present study confirms and extends previous studies by demonstrating that mouse polyclonal IgG2b antibodies can reduce allergen-induced AHR in mice.

New biological agents targeting specific immune mechanisms underlying allergic asthma are a promising line of research, and a recombinant anti-IgE mAb (omalizumab) is now available. A recent Cochrane review of clinical trials in over 2,000 subjects concluded that although this agent significantly reduced levels of free IgE as well as inflammatory asthma symptoms, the extent of its therapeutic effect constitutes a 'debatable clinical value' [32]. Evidence suggests that omalizumab, like the anti-OVA mAb of the present study, has no effect on AHR [33]. This lack of efficacy against AHR may explain the limited effect of monoclonal anti-IgE therapy for allergic asthma, and supports the idea that pAb might offer improved efficacy by reducing both inflammation and AHR.

Another potential advantage of pAb is that they might be more effective than mAb against allergens consisting of multiple epitopes. Studies have identified multiple epitopes on grass pollen and birch allergens [34, 35]. The anti-OVA pAb used in the current study comprises seven anti-OVA antibodies falling into three types based on their heavy chain CDR3 sequences. Based on the binding characterization data, these antibodies most likely will not cover all accessible antigenic sites on the OVA molecule, which would decrease its efficacy in vivo. Increasing the number of individual antibody specificities in a therapeutic antibody composition would arguably enhance the advantages of polyclonality by way of enhanced allergen coverage and hence blocking or removal.

In conclusion, our data support the potential value of i.n. administered polyclonal recombinant antibody therapy in the treatment of allergic asthma. pAb may offer improved efficacy compared with mAb for this indication, due to pleiotropic effects on the immune mechanisms for the allergic response. This study also provides further information on the mechanisms by which monoclonal and polyclonal IgG exerts its therapeutic effects on allergic asthma.

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