

Modulation of an Allergic Immune Response via the Mucosal Route in a Murine Model of Inhalative Type-I Allergy

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Key Words

Allergy · Aerosol · Th2 · Cholera toxin B/conjugates · Mucosal tolerance · BALB/c

Abstract

A murine model of aerosol inhalation, leading to sensitization to birch pollen (BP) and its major allergen Bet v 1, was established in order to try to influence type-I allergic immune responses via the mucosal route. We previously demonstrated that simultaneous inhalation of BP and cholera toxin, a potent mucosal adjuvant, induced a Th1-like immune response to the allergen in naive mice and modulated allergic immune responses in sensitized mice. In contrast to cholera holotoxin, mucosal application of the cholera B subunit (CTB) conjugated to antigen has been shown to induce peripheral tolerance in certain models of Th1-based autoimmune diseases. In the present study we investigated the potential of such an antigen delivery system to suppress Th2-based, allergic immune responses. Mucosal administration of CTB/Bet v 1 conjugates prior to sensitization led to significantly increased allergen-specific IgE/IgG1 and IgG2a antibody levels and cytokine production (IL-5, IFN- γ) in vitro. Thus, CTB coupled to Bet v 1 acted as an adjuvant rather than a tolerogen. On the other hand we noted that mucosal application of CTB coupled to ovalbumin led to marked suppression of antigen-specific IgE antibody levels and IL-5 production in vitro and thereby restricted allergic sensitization. These results indicated

that the effects of CTB/antigen conjugates depended on the nature of the antigen. In contrast to Bet v 1 coupled to CTB, nasal as well as oral application of low doses of unconjugated, Bet v 1 prior to aerosol sensitization inhibited allergen-specific antibody responses of all isotypes, cutaneous type-I skin tests in vivo as well as allergen-specific lymphoproliferative responses and cytokine production (IL-4, IL-5, IL-10, IFN- γ) in vitro, suggesting that both T- and B-cell tolerance to the allergen were induced. Taken together, mucosal tolerance induction as well as the use of certain transmucosal antigen delivery systems might be promising new strategies to modulate type-I allergic immune responses

Introduction

In recent years a constant increase in the prevalence of type-I allergies has been recognized [1]. The majority of type-I allergies is caused by inhalant allergens. Among the seasonal airborne allergens birch pollen (BP) and its major allergen Bet v 1 represent an important source of type-I allergens [2–4]. The treatment of choice in allergy is specific immunotherapy, i.e. injecting increasing doses of allergen to induce tolerance [5, 6]. Despite the high efficacy of this treatment in the majority of patients, there are certain disadvantages of specific immunotherapy, such as frequent injections, long duration of treatment (3 years and more) or risks of allergic side reactions. Therefore, one of the major aims

in allergy research is to develop new and safer strategies to prevent and/or treat allergic diseases.

Mucosal (oral/nasal) application of soluble antigens induces an antigen-specific, systemic immunological unresponsiveness, a phenomenon known as oral/mucosal tolerance [7–9]. On the other hand, molecules with known mucosa-binding properties can induce local and systemic immune responses when administered by the mucosal route. Among these cholera toxin (CT) is one of the most potent mucosal immunogens, which also acts as a strong adjuvant to coadministered soluble antigens [10]. In contrast to cholera holotoxin, it has recently been shown that mucosal administration of the non-toxic B subunit (CTB) conjugated to protein antigens can enhance peripheral tolerance induction [11]. Recently the effectiveness of such CTB/antigen formulation in preventing or treating certain Th1-based autoimmune diseases was demonstrated [11, 12].

We have established a murine model of aerosol inhalation leading to sensitization to BP and its major allergen Bet v 1. High allergen-specific IgE/IgG1 versus low IgG2a antibody responses as well as positive allergen-specific skin tests represent an immunological state comparable to that of human type-I allergy [13]. Using this model we previously demonstrated that simultaneous inhalation of BP and CT induced a Th1-like immune response to the allergen as well as modulated an ongoing allergic immune response towards a Th0 response [13]. In the present study we investigated whether mucosal administration of (i) CTB/Bet v 1 conjugates and (ii) unconjugated, recombinant Bet v 1 prior to aerosol sensitization have the potential to induce peripheral tolerance and thereby prevent allergic sensitization to the allergen.

Materials and Methods

Animals, Sensitization, Tolerization

Female BALB/c mice were used in all experiments.

Sensitization was performed by one intraperitoneal injection of recombinant Bet v 1 (1 µg/mouse) in PBS adsorbed to AL(OH)₃ (2 mg, Serva, Heidelberg, Germany) and subsequent aerosol exposure to BP extract as previously described [13].

Tolerization was performed by applying, 3 times at 7-day intervals, 40 µg nasally and 120 µg orally of CTB/Bet v 1 or CTB/ovalbumin OA; a kind gift of Prof. J. Holmgren, University of Göteborg, Sweden), or 10 µg nasally or 100 µg orally of recombinant (r) Bet v 1 prior to sensitization.

Type-I Skin Tests

BP (2.5 µg/ml), rBet v 1 (2.5 µg/ml) or OA (2.5 µg/ml) were injected intradermally into the shaved, abdominal skin. 100 µl of 0.5% Evans blue was intravenously injected. After 20 min the color inten-

sity of the reaction was evaluated according to the positive control (compound 48/80) on the inverted skin of the sacrificed mice [13].

Determination of Antigen-Specific Antibodies

Microtiter plates were coated with BP (50 µg/ml), rBet v 1 (2 µg/ml) or OA (50 µg/ml). Serum samples were diluted in PBS-Tween 1:1,000 for IgG1, 1:500 for IgG2a and 1:10 for IgE. Rat anti-mouse IgG1, IgG2a and IgE (1:1,000, Pharmingen) and thereafter peroxidase-conjugated mouse anti-rat IgG antibodies (1:1,000, Jackson) were used [14].

Measurement of Lympho-Proliferative Responses and Cytokine Production

Lymphoproliferative responses of spleen and lung cells upon stimulation with BP, rBet v 1 and OA were measured after ³H-thymidine incorporation by scintillation counting [13].

Cytokine production (IL-5, IL-4, IFN-γ, IL-10) was measured in supernatants of 24- and 48- hour antigen-stimulated spleen/lung cell cultures by means of mouse ELISA kits (Endogen) [13].

Results and Discussion

Previous reports have demonstrated that mucosal administration of soluble antigen coupled to CTB led to suppression of T-cell responses in vivo and in vitro [11]. A recent study has demonstrated that CTB/antigen conjugates selectively reduced Th1 responses and therefore suggested that such an antigen-delivery system could preferentially be used for improving oral tolerance induction in models of autoimmune diseases [15]. Therefore we investigated, whether CTB conjugated to the inhalant allergen Bet v 1 would have the potential to suppress Th2-based allergic immune responses. Our data show that nasal as well oral administration of 3 mucosal applications of CTB/Bet v 1 conjugates (40 µg nasally and 120 µg orally) prior to sensitization with BP led to significantly enhanced allergen-specific IgE antibody levels compared to sensitized control animals ($p < 0.01$; fig. 1A). Similar to the IgE levels also allergen-specific IgG1 and IgG2a antibodies significantly increased ($p < 0.01$), in particular after nasal pretreatment with the conjugate. In accordance with the antibody levels allergen-specific type-I skin tests in vivo were stronger in the pretreated compared to the sensitized control animals (data not shown). Also lymphoproliferative responses and cytokine production were significantly enhanced in animals nasally or orally pretreated with CTB/rBet v 1 compared to controls (table 1). These results indicated that CTB, in conjunction with physically coupled Bet v 1, acted as an adjuvant rather than tolerogen and thus aggravated the allergic immune responses to the allergen. However, in additional experiments using OA conjugated to CTB, we noted that pretreatment with the OA/CTB conjugate led to a signifi-

Fig. 1. A IgE anti-birch pollen (BP) antibodies in serum. □ = Mean OD of sensitized control mice (1 × i.p. Bet v 1/Al(OH)₃, subsequent BP aerosol); ▨ = Mean OD values from treated mice, nasally pretreated with CTB/rBet v 1 conjugates; ■ = mean value of mice orally with CTB/rBet v 1 conjugate treated prior to sensitization. **B** IgE anti-BP antibodies in serum. □ = Mean OD values from sensitized controls (n = 6); ▨ = group of mice nasally pretreated with unconjugated recombinant Bet v 1; ■ = mean OD of mice fed with rBet v 1 prior to sensitization. Error bars = standard error of the mean. Mann-Whitney U test: *p < 0.05; **p < 0.01.

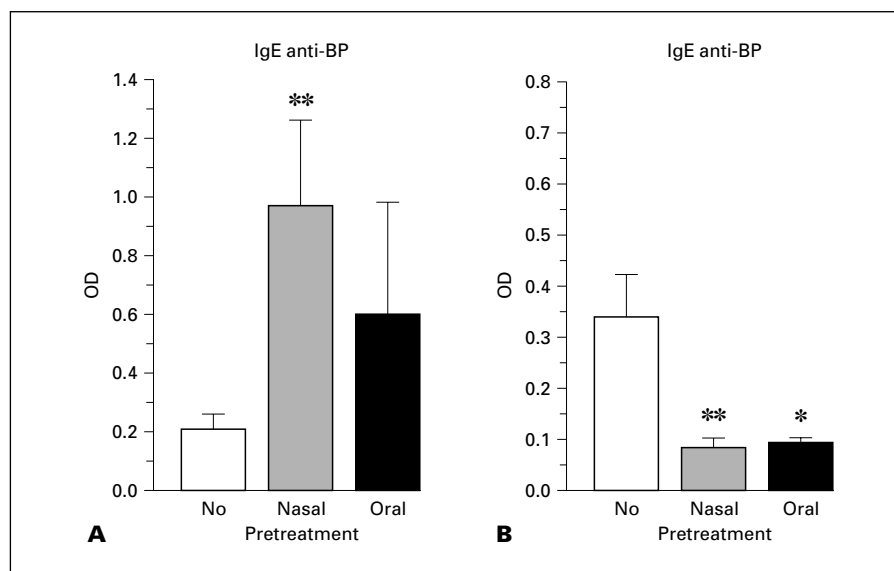


Table 1. Effects of different mucosal pretreatments on the lymphoproliferative responses and cytokine production in vitro

Pretreatment	Stimulation index	IL-5	IFN- γ
CTB/Bet	↑↑ 372	↑↑ 520	↑↑ 1,100
CTB/OA	↓ 46	↓ 12	↑ 200
rBet	↓ 56	↓ 12	↓ 2

Sensitized control mice: 1 × i.p. rBet v 1/Al(OH)₃ and BP aerosol, or 1 × i.p. OA/Al(OH)₃ and OA aerosol.

CTB/Bet v 1 = Nasally pretreated, subsequent sensitization with BP; CTB/OA = nasally pretreated, subsequent sensitization with OA; Bet v 1 = nasally pretreated, subsequent sensitization with BP. Values of the pretreated groups are expressed as relative values as compared to the values of the sensitized control group, which were put to 100%. Stimulation index = Mean proliferation/mean medium value.

cant reduction in antigen-specific IgE antibody levels and IL-5 and IL-4 production in vitro (table 1). A simultaneous increase in antigen-specific IgG2a and IFN- γ (table 1) indicated that pretreatment with the OA/CTB conjugate led to immune deviation, thereby preventing the allergic sensitization. Thus, the effects of CTB/antigen conjugates seem to closely depend on the nature of the antigen or allergen (manuscript submitted).

Apart from CTB-conjugated Bet v 1 we also used unconjugated Bet v 1 for mucosal tolerance induction. Interestingly, nasal as well as oral pretreatment with low doses of recombinant Bet v 1 (10 μ g for nasal and 100 μ g for oral

application) – i. e. lower doses than used in the conjugated form – efficiently prevented production of allergen-specific IgE, IgG1 and IgG2a antibodies (fig. 1B). Accordingly the allergen-specific type-I skin tests were negative in the pretreated mice (data not shown). Also lymphoproliferative responses as well as cytokine production (IL-5, IL-4, IFN- γ and IL-10) were markedly suppressed, in particular after intranasal pretreatment with recombinant Bet v 1 (table 1). These results show that mucosal tolerance induction with the recombinant allergen led to tolerance induction at the T- and B-cell level. Preliminary results indicate that the suppressed immune responses might be related to an enhanced production of TGF- β , a cytokine which has been shown to play a central regulatory role in mucosal tolerance induction [16, 17] (manuscript submitted).

We conclude from our data that mucosal tolerance induction as well as the use of certain mucosal adjuvants might be promising strategies for prophylaxis and perhaps also for immunotherapy of type-I allergy.

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