



Therapeutic effects of anti-B7-1 antibody in an ovalbumin-induced mouse asthma model

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Abstract

Allergic asthma is a chronic inflammatory disorder of airways, which is characterized by attacks provoked by exposure to so-called asthma triggers, such as pet dander, second-hand tobacco smoke, dust mites, and mold spores. B7-1 (CD80), perhaps one of the most studied co-stimulatory molecules involved in asthma, plays a key role in regulating allergen-induced T cell activation in asthma, probably through T cell recruitment and Th cell differentiation upon allergen provocation. The present study was designed to test the hypothesis that anti-B7-1 antibody has therapeutic effects in asthma by blocking B7-1/CD28 pathway. The asthma model was established by ovalbumin (OVA) sensitization and challenging in female Balb/c mice. One hour after the last induction, mice were sacrificed and whole lung lavage was conducted. Cell numbers in bronchoalveolar lavage fluid (BALF) were determined and the expression levels of IFN- γ and IL-4 in supernatant were measured by an enzyme-linked immunosorbent assay method. Sedimental cells smears were stained with Wright's–Gimsa mixed coloring method. The B7-1 expression was detected by immunohistochemistry method with frozen tissue sections. The anti-B7-1 antibody treatment could alleviate asthmatic syndromes induced by OVA. The number of recoverable eosinophils in BALF in the anti-B7-1 antibody treated group was significantly lower than that in the control group ($P < 0.01$) and the eosinophils peribronchial infiltration was remarkably reduced in anti-B7-1 treated asthmatic mice, based on histological evaluation. The treatment with the anti-B7-1 antibody induced IFN- γ expression and decreased IL-4 expression, compared with the asthmatic control group ($P < 0.01$). In conclusion, the anti-B7-1 antibody approach may provide a novel therapy for allergic asthma.

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1. Introduction

Asthma is a complex and heterogeneous disease characterized by spontaneous airflow limitation, lung tissue remodeling, increased serum allergen-specific IgE concentration, mast cell activation and airway hyper-responsiveness, with the infiltration of lymphocytes and eosinophils into the

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airway submucosa [1,2]. The incidence and severity of asthma have risen dramatically in the past few decades, with the greatest increases being observed in the developed nations of North America and Europe [3].

Inhalation of aerosolized allergens can induce inflammation of the major airways of the lung, which is mediated by cytokines and chemokines of the innate and Th2-type adaptive immune responses [4,5]. The engagement of B7 on antigen presenting cells (APC) with CD28/CTLA-4 on T cells is a well-characterized co-stimulatory pathway for T and B cell activations [6]. B7-1 (CD80), one of the most studied members of the B7 family, is often expressed at low levels in "professional" antigen presenting cells (APC), such as dendritic cells [7], macrophages [8], and up-regulated in these APCs as well as B cells following activation by cytokines and ligation of cell surface molecules [9]. Expression of B7-1 is mainly activation-induced [10].

We hypothesize that anti-B7-1 antibody may have therapeutic effects in asthma. In the present study, the protective effects of an anti-B7-1 antibody against asthma were investigated in a murine model induced by ovalbumin. The OVA sensitization and challenge procedure is a reliable and widely used method to induce asthma [11–13]. The inhibitory effects of the antibody on B7-1 levels in mouse lungs were detected by immunohistochemistry; its effects on expressions of interferon- γ (IFN- γ), interleukin-4 (IL-4), and peribronchial eosinophils were measured by ELISA or immunohistochemistry method. The results from the present may provide a basis for future development of B7-1 antibodies for the treatment of asthma.

2. Materials and methods

2.1. Anti B7-1 antibody

Anti-B7-1 antibody was kindly provided by the Department of Immunology, Soochow University, Suzhou, China. The hybridoma cell line was obtained by using the B lymphoma hybridoma technique after immunization of Balb/c mice with XG7-B7 cells. Ascites were induced to produce the monoclonal anti-B7-1 antibody (4E5). Subclass of the anti-B7-1 antibody was IgG1. The potency of mAb was more than 1:1000. The mAb could completely block the reaction between standard anti-B7-1 antibody and antigen by competitive inhibition test. The results indicated 4E5 and standard anti-B7-1 antibody recognized the same or semblable antigen site. In vitro, 4E5 can obviously inhibit the growth in Raji and Daudi cells with high expression of B7-1 and block the co-stimulatory signals of B7/CD28 when the concentration was at 2.5 μ g/ml.

2.2. Animals

Female, specific-pathogens free, Balb/c mice, aged 6–8 weeks and weighed 18–20 g, were purchased from the Animal Use Center of Soochow University, Suzhou, China. They were housed under constant conditions at a temperature of 23 ± 1 °C, a humidity of $40 \pm 5\%$, and on a 12-h light/ 12-h dark cycle. They had free access to pellet food and tap water. The animal experiments were performed according to the European Community guidelines for care and use of animals, and approved by the Ethic committee for Animal Use of Soochow University.

2.3. Study design

Thirty mice were randomly assigned into three groups (10/group): the normal control group, the asthmatic control group, and the anti-

B7-1 antibody group. For the asthmatic control group and the anti-B7-1 antibody group, 1 ml of 10% OVA (Sigma, Los Angeles, USA) in saline was intraperitoneally administered. From the 15th day post-injection, they were challenged with aerosolized 5% OVA daily for 7 consecutive days. For the anti-B7-1 antibody treated group, 1 ml of 50 μ g/ml anti-B7-1 antibody was intraperitoneally administered on days 2, 3, 4, 15, and 17 post the first OVA injection. The normal and asthmatic control mice were intraperitoneally administered with saline on the same days as above.

One hour after the last OVA injection, the mice were sacrificed and whole lung lavage was conducted with saline. The bronchoalveolar lavage fluid (BALF) was collected, the cells concentrations of BALF were determined by cytometry, and the total number of cells in BALF was then calculated. BALF was centrifuged at 1200 rpm for 10 min and the supernatant was measured and then stored at -20 °C for further use. The expression levels of IFN- γ and IL-4 in supernatant were measured by using the enzyme-linked immunosorbent assay (ELISA) kits (Jingmei, Shenzhen, China) according to the instructions of the manufacturer. Sedimental cells smears were stained with Wright's–Gimsa mixed coloring method.

2.4. Immunohistochemistry detection

The mouse left lungs were first perfused with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), fixed with formalin, and embedded with paraffin. Paraffin sections (5- μ m-thick) were stained with hematoxylin and eosin (H&E). The right lungs were immediately frozen by liquid nitrogen and sectioned (8- μ m-thick) and the frozen sections were incubated with the rat anti-mouse CD80 antibody (Serotech, Oxford, England) at -4 °C overnight. After incubation with the antibody against rat IgG conjugated with horseradish peroxidase (KPL, London, England), the specimens was developed with the DAB method. The sections were then counterstained with hematoxylin and mounted with Canada balsam.

2.5. Statistical analysis

The data were expressed as the mean $\pm$ SEM and analyzed by using SPSS 11.5 software (SPSS Inc., Chicago, IL). The statistical differences were analyzed by one-way ANOVA and the significance level was set at $P < 0.05$.

3. Results

3.1. The anti-B7-1 antibody treatment alleviates asthmatic syndromes induced by OVA

On days 15 to 21, syndromes of all mice were observed after challenge by OVA aerosol challenge for 30 min. The normal control mice were healthy. Many asthmatic syndromes were observed in the asthmatic control mice, such as cough, tachypnea, dysphoria, abdominal muscle spasm and stool and urine incontinence, after 10 min aerosol challenge. The severity of these syndromes was much less in asthmatic mice treated with the anti-B7-1 antibody and the syndrome showed up more than 10 min for challenge. And compared to the asthmatic control mice group, the syndromes of the anti-B7-1 antibody group disappeared more quickly after stopping aerosol challenge.

3.2. The anti-B7-1 antibody decreases the levels of B7-1 in the lung tissues

As shown in Fig. 1, the numbers of B7-1 positive cells were remarkably increased by OVA sensitization and challenge. The anti-B7-1 antibody treatment significantly decreased the levels of B7-1

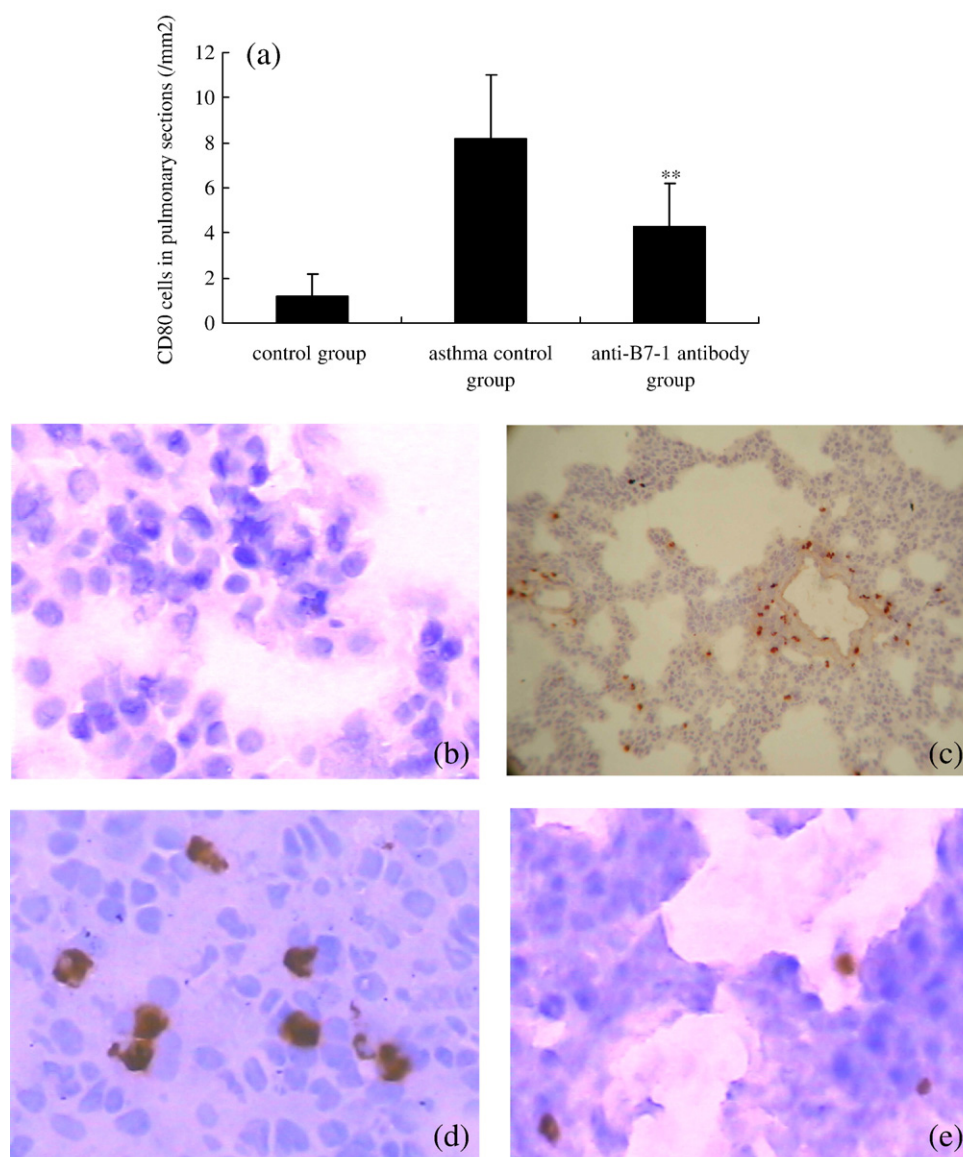


Figure 1 CD80 Positive cells in various groups. Cells were counted and statistically analyzed (400×) (a); CD 80 expression in murine of control group (400×) (b), asthmatic control group (100×, c; 400×, d), and anti-B7-1 antibody group (400×) (e).

in lung tissues compared with the asthmatic control group ($P < 0.01$).

3.3. The anti-B7-1 antibody abrogates the hypercellularity and eosinophils in BALF

As shown in Table 1, the total number of cells in BALF of mice of asthmatic control group was $(54.28 \pm 6.84) \times 10^4$ cells/ml, which

was more than two folds as high as that of the control group. The total number of cells was significantly decreased in mice treated with anti-B7-1 antibody $(38.05 \pm 4.19) \times 10^4$ cells/ml, significantly lower than that of asthmatic controls ($P < 0.01$). In the asthmatic control mice, the number of eosinophils was $(18.88 \pm 2.94) \times 10^4$ cells/ml in BALF, significantly higher than that in the normal control group $((0.26 \pm 0.27) \times 10^4$ cells/ml, $P < 0.01$). The number of recoverable eosinophils in BALF in the anti-B7-1

Table 1 The numbers of cells in bronchoalveolar lavage fluid in different groups ($n=10$)

Groups	Cells (10^4 /ml)	EOS%	N%	L%	M%
Control group	21.36 ± 4.02	0.26 ± 0.27	0.88 ± 0.31	1.77 ± 0.32	97.10 ± 0.54
Asthmatic control group	54.28 ± 6.84	18.88 ± 2.94	3.94 ± 0.82	11.75 ± 1.23	65.46 ± 3.41
Anti-B7-1 antibody group	$38.05 \pm 4.19^{**}$	$4.30 \pm 0.80^{**}$	$2.09 \pm 0.55^{**}$	$3.97 \pm 0.87^{**}$	$89.64 \pm 1.40^{**}$

EOS: eosinophils; N: neutrophil; L: lymphocyte; M: macrophagocyte.

****:** $P < 0.01$ compared with asthmatic control group.

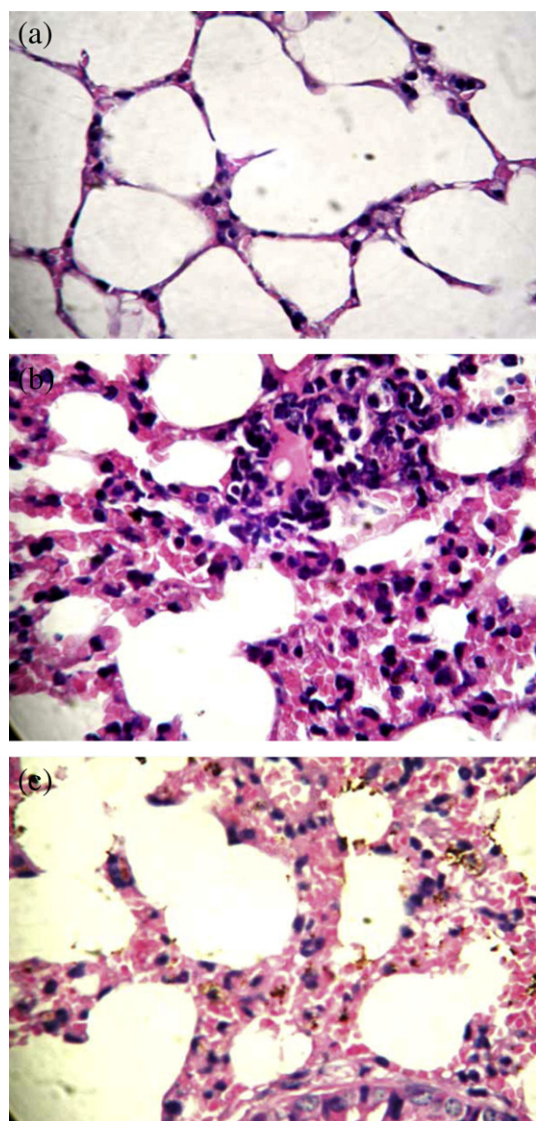


Figure 2 H&E stained pulmonary sections of control group (a), asthmatic control group (b), and anti-B7-1 antibody group (c) (×400).

antibody treated group was $(4.30 \pm 0.80) \times 10^4$ cells/ml, which was significantly lower than that of the asthmatic control group ($P < 0.01$).

Histological evaluation of the lungs of the sensitized mice revealed that eosinophils had infiltrated into the tissues surrounding the bronchial airways (Fig. 2b). In the anti-B7-1 antibody treated asthmatic mice, histological evaluation revealed a remarkable suppression of eosinophils peribronchial infiltration (Fig. 2c).

3.4. The anti-B7-1 antibody increases the expression level of IFN- γ and decreases the expression level of IL-4

IFN- γ concentrations in BALF in the normal control group and the asthmatic control group were 75.35 ± 18.38 ng/L, and 40.15 ± 11.94 ng/L, respectively ($P < 0.01$, Fig. 3). The treatment with the anti-B7-1 antibody significantly increased the IFN- γ con-

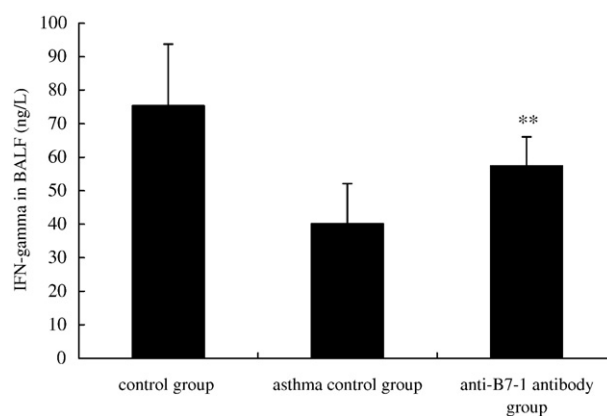


Figure 3 IFN- γ expression levels in BALF as measured by ELISA. **: $P < 0.01$ compared with the asthmatic control group.

centrations in BALF (57.40 ± 8.69 ng/L, $P < 0.01$ compared with the asthmatic control group).

The IL-4 concentrations in BALF in the asthmatic control group was remarkably increased after OVA sensitization and challenge, compared with the normal control group ($P < 0.01$, Fig. 4). The anti-B7-1 antibody treated group had a significantly lower level of IL-4 than the asthmatic control group (15.84 ± 3.41 ng/L versus 22.60 ± 5.28 ng/L, $P < 0.01$, Fig. 4).

4. Discussion

As indicated by the Guidelines for the Diagnosis and Treatment of Asthma, the National Heart, Lung, and Blood Institute in the National Institutes of Health, asthma is a chronic inflammatory disorder of the airways and in susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyper-responsiveness to a variety of stimuli [14]. The asthma mortality has undergone a dramatic increase from 8.2 in 1978 to 17.9 per million Americans in

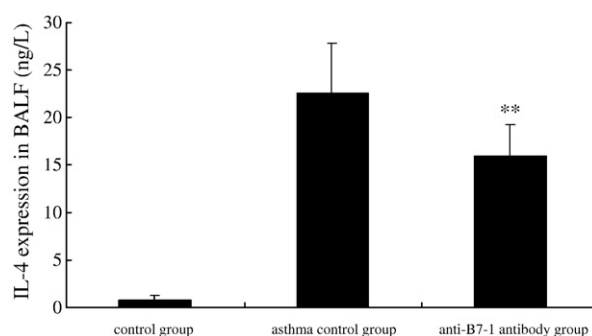


Figure 4 IL-4 expression levels in BALF as measured by ELISA. **: $P < 0.01$ compared with asthmatic control group.

1995 [15]. A number of subpopulations were especially at high risk of developing asthma and/or of suffering from asthma. They are children, the elderly, racial and ethnic minorities, the indigent, people living in urban areas, women, and adults working in certain typically blue-collar jobs [15].

Activation and differentiation of T lymphocytes have been demonstrated to play an important role in the pathogenesis of asthma [16]. The initiation of T cell activation requires a primary signal delivered by the antigen peptide presented by major histocompatibility complex (MHC) molecules, and a non-specific signal produced by the interaction of co-stimulatory molecules [17,18]. The co-stimulatory signal is produced by the interaction of CD28 on T cells with the B7 family B7-1 (CD80) and B7-2 (CD86) in antigen presenting cells (APC) [19–21]. Previous studies have demonstrated that B7-1 plays a key role in regulating allergen-induced T cell activation in asthma, probably through T cell recruitment and Th cell differentiation upon allergen provocation [22–25]. Thereafter, we hypothesized that anti-B7-1 antibody might have therapeutic effects in asthma.

In the present study, asthma was induced by OVA sensitization and challenge in female Balb/c mice. Our results indicate that the anti-B7-1 antibody treatment could alleviate the asthmatic syndromes and the number of recoverable eosinophils in BALF in the anti-B7-1 antibody treated group was significantly lower than that to the asthmatic control group. In anti-B7-1 treated asthmatic mice, histological evaluation revealed remarkable suppression of eosinophils peribronchial infiltration. The treatment with the anti-B7-1 antibody could also abrogate the development of hypercellularity and eosinophils in BALF and lung tissues. Additionally, the treatment of anti-B7-1 antibody could increase the expression levels of IFN- γ , and decrease IL-4 concentrations in BALF.

The major pathogenesis observed in allergic asthma is Th2 dominant cytokine responses [11]. In particular, overexpression of Th2 cytokines, such as IL-4, may play a central role in the development of eosinophils and inflammation. However, overproduction of IFN- γ could suppress the expression of Th2 cytokines, such as IL-4 [11]. IL-4 is significantly up-regulated in the OVA-induced murine models through NF- κ B pathway [12]. IFN- γ , a Th1 cytokine, is able to adjust the balance of Th1/Th2 and inhibit asthmatic inflammatory responses [26,27]. Gao et al. have demonstrated that allergic responsiveness is inhibited in a murine asthmatic model via IFN- γ transgene expression [11]. In the present study, inhibition of B7-1 by anti-B7-1 antibody was demonstrated to up-regulate IFN- γ expression and down-regulate IL-4 expression. The anti-B7-1 antibody was administered at both sensitization and challenge phase to alleviate asthmatic syndromes. Since there are also other stimulatory pathways such as CD40/CD40L and OX40(CD134)/OX40L(gp34) involved in inflammation process in asthma, it may be difficult to completely treat asthma by using a single antibody to stimulatory molecules [28].

In conclusion, inhibition of B7-1 by anti-B7-1 antibody can alleviate asthmatic syndromes, suppress eosinophils peribronchial infiltration, increase IFN- γ expression and decrease IL-4 expression. Our study may provide a basis for further investigation of this antibody strategy for the treatment of asthma.

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