J Mol Med (2006) 84: 595–603
DOI 10.1007/s00109-006-0047-3

ORIGINAL ARTICLE

Ya-Hui Chuang · Jau-Ling Suen · Bor-Luen Chiang

Fas-ligand-expressing adenovirus-transfected dendritic cells decrease allergen-specific T cells and airway inflammation in a murine model of asthma

Received: 24 April 2005 / Revised: 8 November 2005 / Accepted: 10 January 2006 / Published online: 25 March 2006
© Springer-Verlag 2006

Abstract T cells expressing a type-2 T helper profile of cytokines (Th2 cells) have been demonstrated to play an important role in the initiation and progression of allergic asthma, and it is well known that Fas ligand (FasL) induces apoptosis when bound to its receptor, Fas. In the present study, we examined the possibility of modulating asthma manifestations by dendritic cells (DCs) genetically engineered to express FasL (DC-FasL), which could deliver a death signal to T cells in an antigen-specific manner. The delivery of DC-FasL into ovalbumin (OVA)-immunized allergic mice decreased the airway hyper-responsiveness (AHR). Moreover, we established a mouse model of airway inflammation by using an adoptive transfer of Th2 cells derived from ovalbumin T cell receptor transgenic mice to study the effect of DC-FasL on airway reactivity. The administration of DC-FasL in Th2-cell-induced allergic mice had significantly decreased AHR, airway inflammation, and IL-4, IL-5 and IL-13 production. Furthermore, the numbers of OVA-specific T cells were decreased in the lung of mice receiving DC-FasL. These results demonstrate that FasL-expressing dendritic cells might be applied for the modulation of allergic responses.

Keywords OVA-specific T cells · Adenoviral vector · CD95 ligand · Apoptosis · Gene therapy

Y.-H. Chuang · B.-L. Chiang
Graduate Institute of Immunology,
National Taiwan University,
Taiwan, Republic of China

B.-L. Chiang (✉)
Department of Pediatrics, College of Medicine,
National Taiwan University Hospital,
No. 7, Chung-Shan South Road,
Taipei, Taiwan, Republic of China
e-mail: gicmbor@ha.mc.ntu.edu.tw
Tel.: +886-2-23123456
Fax: +886-2-23972031

J.-L. Suen
Department of Microbiology, Kaoshiung Medical University,
Taiwan, Republic of China

YA-HUI CHUANG
received her Ph.D. in Immunology from the National Taiwan University, Taiwan. She is presently a postdoc of University of California at Davis, USA. Her research interests include cellular immunology and immunotherapy.

BOR-LUEN CHIANG
received his M.D. from National Taiwan University, Taiwan and Ph.D in Immunology from the University of California at Davis, USA. He is presently a professor of Graduate Institute of Clinical Medicine, also professor and chairman of Department of Pediatrics in National Taiwan University, Taiwan. His research interests include immunotherapy and gene therapy for immunological diseases, immune tolerance, and autoimmune diseases.

Abbreviations FasL: Fas ligand · OVA: Ovalbumin · AHR: Airway hypersensitivity · DC: Dendritic cell · BMDC: Bone-marrow-derived dendritic cell · BALF: Bronchoalveolar lavage fluid · Penh: Enhanced pulse

Introduction

Allergic asthma is characterized by airway hypersensitivity (AHR) and cellular infiltration of the airway with predominantly eosinophils and Th2 cells. Allergen-specific Th2 cells secret IL-4, IL-5, and IL-13, which promote IgE

production, eosinophil development, release, and survival, and airway remodeling [1, 2]. Regulation of this effector Th2 asthmatic response by counterbalancing Th1 cells has been the aim of several forms of therapy such as administering anti-Th2 cytokine antibodies, providing Th1 cytokines, and employing DNA vaccines along with bacterial CpG adjuvant sequences to promote Th1 response [3–6]. However, other studies have demonstrated that Th1 cells fail to counterbalance Th2-cell-induced airway hyperreactivity but have rather caused severe neutrophil infiltration [7–10]. Based on these previous results, the development of novel therapeutic strategies for asthma should, therefore, be focused on the selective inactivation of the Th2 cells and not on the stimulation of Th1 cells.

Fas/Fas ligand (FasL)-mediated apoptosis is an important mechanism for the downregulation of T-cell-mediated immune responses [11, 12]. Several studies have used FasL to confer protection to allogeneic grafts, induce regression of tumors, and abrogate autoimmune responses [13–17]. In our previous study, the administration of FasL to ovalbumin (OVA)-sensitized mice significantly decreases asthmatic manifestations including AHR, the infiltration of eosinophils and lymphocytes, and levels of cytokines and chemokines such as IL-5, IL-13, eotaxin, KC, and tumor necrosis factor-α [18].

Dendritic cells (DCs) are a specialized population of professional antigen-presenting cells. There are several DC subsets that can either induce antigen-specific immunity or tolerance [19, 20]. The capacity of DCs to induce tolerance generates considerable interest in the potential use of these cells as therapeutic agents in transplantation, autoimmune disease, and allergy [21]. Genetic modification of DC with FasL is an ideal approach for the generation of tolerogenic DC because FasL-expressing DCs could deliver a death signal to activate T cells in an antigen-specific manner. Moreover, T cells upregulate Fas expression upon interaction with DCs and, thus, become sensitive to Fas/FasL-induced apoptosis [21]. In the present study, we attempted to eliminate allergen-specific T cells by FasL-expressing DCs for the treatment of asthma. To this end, we administered bone-marrow-derived dendritic cells (BMDCs), genetically modified to express FasL by adenoviral-mediated gene transfer, to OVA-immunized and -challenged mice and Th2-cell-induced allergic mice. The results demonstrate that the dendritic cells genetically engineered to express FasL (DC-FasL) could be used to eliminate or reduce the number of allergen-specific T cells responsible for the progression of allergic asthma. This approach could be an alternative therapy for allergic asthma.

Materials and methods

Mice

BALB/c mice and $OVA_{323-339}$-specific I-A^d-restricted TCR-transgenic mice (DO11.10) on a BALB/c background were obtained from and maintained in the Animal Center of the College of Medicine, National Taiwan University. All mice were female and were used at 6–8 weeks of age. Animal care and handling protocol was approved by the Animal Committee of College of Medicine National Taiwan University.

Preparation and infection of BMDCs

The construction and purification of adenovirus-expressing FasL (Ad-FasL) were performed as previously described [18]. BMDCs were prepared as described previously [22]. OVA (500 µg/ml, Sigma, MO, USA) was added to the BMDC cultures on day 5. On day 6 of the culture, nonadherent cells (BMDCs) were infected with Ad-FasL or Ad-eGFP (control virus) [multiplicity of infection (MOI) = 250] and incubated for one more day. The purity of the BMDCs was analyzed by flow cytometry, examining the expression of MHC class II, B7-1, B7-2, and CD11c. The optimal MOI of Ad-FasL infection was chosen by the evaluations of viability, eGFP expression, and killing function in vitro of infected DC. To prevent the proliferation of DCs and nonspecific responses induced by DCs, DCs were irradiated (3,000 rad) in the present study.

^{3}H-thymidine release assay of Jurkat cells and OVA-specific T cells

Jurkat cells (1×10^4) were labeled with 5 µCi/ml ^{3}H-thymidine for 4 h at 37°C as target cells. They were incubated with an indicated ratio of Ad-FasL- or Ad-eGFP-infected DCs in a total volume of 200 µl/well for 18 h. Unfragmented high molecular weight DNA was harvested onto glass fiber filters and counted in a scintillation counter. Data are expressed as a percentage of ^{3}H-thymidine release: $100\times[1-(cpm_{experimental}/cpm_{spontaneous})]$. ^{3}H-Thymidine-labeled CD4+ T cells (10^5 cells/well isolated from DO11.10 mice) were incubated for 5 h with γ-irradiated OVA-pulsed DC, DC-eGFP, or DC-FasL (10^4 cells/well). The intact cells were harvested and intracellular radioactivity quantified. Data are expressed as a percentage of ^{3}H-thymidine release: $100\times(cpm_{DC\text{-}eGFP\ or\ DC\text{-}FasL}/cpm\ DC)$.

Generation of Th2 cells

To generate OVA-specific Th2 cells, 5×10^5 cells/well of CD4+ T cells isolated from the spleen of DO11.10 mice and 4×10^6 cells/well of antigen presenting cells (APCs) from BALB/c mice were placed in 48-well plates in 1 ml of RPMI-1640/10% FCS supplemented with OVA, recombinant murine IL-4 (1,000 U/ml), and IL-2 (20 ng/ml). After 10 days, the cells were restimulated with APCs and OVA under the same conditions used for the initial stimulation. Th2 cells were confirmed by intracellular staining and enzyme-linked immunosorbent assay (ELISA) for IL-4 and IFN-γ production of those cells.

Murine model of asthma

In the OVA-sensitized and -challenged model (Fig. 2a), the mice were sensitized intraperitoneally by injection of 20 μg of OVA emulsified in 2 mg of aluminum hydroxide (AlumImuject, Pierce Chemical, Rockford, IL, USA) on day 0. At 10 and 20 days later, the mice were boosted with 50 μg of OVA with adjuvant. On days 53 and 54 after the first immunization, the mice were challenged with OVA by intranasal administration on consecutive days. Airway hypersensitivity to inhaled methacholine was measured 1 day after the last challenge of OVA (day 55).

In the Th2-cell-induced murine model of asthma (Fig. 3a), cultured Th2 cells were injected intravenously into BALB/c mice (5×10^6/mouse). Eight days after the transfer of cells, the mice were boosted by intraperitoneal injection of 10 μg of OVA without adjuvant. Sixteen days after an OVA boost, the mice were exposed to two OVA challenges by intranasal administration on consecutive days. Airway hypersensitivity to inhaled methacholine was measured 1 day after the last challenge of OVA (day 25). Bronchoalveolar lavage fluid (BALF) and lung fixation were performed the following day (day 26).

Measurement of AHR

Airway responsiveness was measured in conscious, unrestrained mice using barometric whole-body plethysmography (Buxco Electronics, Sharon, CT, USA) by recording respiratory pressure curves in response to inhaled methacholine (acetyl-β-methylcholine chloride, Sigma), as described in detail previously [18, 23]. Although there has been a huge controversy over whether unrestrained plethysmography and enhanced pause (Penh) is a reliable measurement of airway responsiveness recently, it is a good measurement for some strains of mice such as BALB/c [24, 25]. Penh=pause×(peak expiratory box flow/peak inspiratory box flow).

Analysis of the cellular composition in BALF

The mice were killed and the tracheas were lavaged three times via a trachea cannula with 1 ml of Hank's balanced salt solution. The total numbers of the cells in the BALF were counted with a standard hemocytometer. Differential cell counts were performed by counting at least 200 cells on cytocentrifuged preparation (cytospin, Shandon, Runcorn, Cheshire, UK), stained with Liu's stain, and differentiated by standard morphological criteria.

OVA-specific T cell and in situ terminal deoxynucleotidyltransferase-mediated UTP end labeling apoptosis staining

Lungs embedded in Tissue-Tek oxacalcitriol compound (Sakura Finetek, Torrance, CA, USA) and 5-μm-thick cryostat sections were prepared. The tissue sections were stained with KJ1-26 monoclonal antibody (a clonotypic monoclonal antibody for the OVA-specific T cell receptor) and terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL). The TUNEL apoptosis staining kit was purchased from Roche (Roche Molecular Biochemicals, Mannheim, Germany) and the samples were stained according to the manufacturer's instructions.

Histopathological study

The lungs fixed in 10% buffered formaline, embedded in paraffin, and cut into 5-μm-think sections were stained with hematoxylin and eosin.

Measurement of cytokine production

Quantifications of IL-4, IL-5, and IL-13 in the BALF supernatants were evaluated by commercially available ELISA (Duoset, R&D, Minneapolis, MN, USA).

Statistical analysis

The results were expressed as the mean±SD or the mean±SEM. An unpaired two-tailed Student's t test was used to compare pairs of groups. The significance levels were set at a p value of 0.05.

Results

Biological activity of FasL-expressing DC

To determine whether the exogenous FasL expressed in the adenovirally infected DCs were biologically active, a ^{3}H-thymidine release assay was performed. An observation of 87–91% lysis of Jurkat cell was made with DC-FasL treatment at an effector/target ratio of 10:1 compared with 10–14% killing activity for the non-infected DCs and DC-eGFP controls (Fig. 1a). Moreover, the addition of blocking Fas:Fc effectively inhibited release of ^{3}H-thymidine. This inhibition was dose dependent and specific as it was unaffected by the addition of control IgG1 (Fig. 1b). To test the ability of DC-FasL in inducing activated T-cell lysis, CD4+ T cells were activated in the presence of ^{3}H-thymidine and the resulting primed CD4+ T cells used as a target. OVA-pulsed DC-FasL induced death of activated T cells (Fig. 1c). These results demonstrated that FasL-expressing DCs were able to induce Fas/FasL-mediated T-cell lysis.

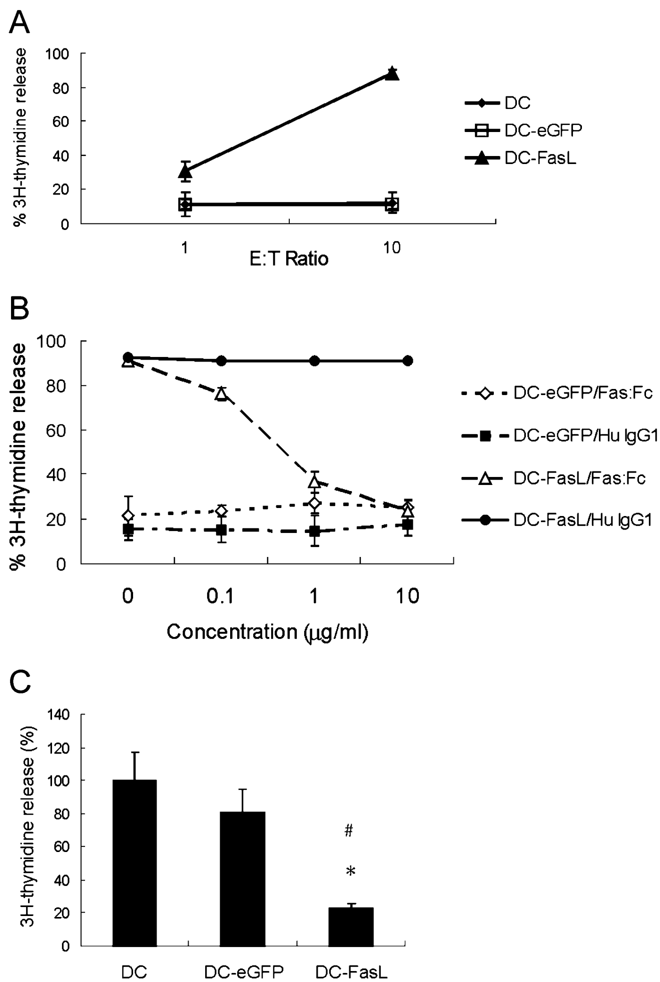

Fig. 1 In vitro functional properties of DC-FasL. **a** ^{3}H-thymidine release assay. Jurkat cells were labeled with ^{3}H-thymidine and incubated with graded numbers of Ad-FasL or Ad-eGFP-infected DC for 18 h. **b** Inhibition of ^{3}H-thymidine release by soluble Fas:Fc, ^{3}H-thymidine-labeled Jurkat cells were mixed with Ad-FasL or Ad-eGFP-infected DC (E:T ratio 10:1), and human Fas:Fc protein or control human IgG1 was added at the beginning of the cultures. Data are expressed as a percentage of ^{3}H-thymidine release: $100\times[1-(\text{cpm}_{\text{experimental}}/\text{cpm}_{\text{spontaneous}})]$. **c** γ-irradiated and OVA-pulsed DC, DC-eGFP or DC-FasL (10^4 cells/well) was incubated with ^{3}H-thymidine-labeled pre-activated T cells for 5 h. Data are expressed as a percentage of ^{3}H-thymidine release: $100\times(\text{cpm}_{\text{DC-eGFP or DC-FasL}}/\text{cpm}_{\text{DC}})$. Results were expressed as the mean±SD of three independent experiments. *Asterisk* means $p<0.0005$ as compared with the DC group. *Number sign* means $p<0.0005$ as compared with the DC-eGFP group

DC-FasL decreased AHR in OVA-immunized and -challenged mice

To determine whether FasL-expressing DCs were able to be immunoregulatory cells for the treatment of asthma, we administered irradiated DC-FasL to the OVA-immunized and -challenged allergic mice (Fig. 2a). BALB/c mice that had been sensitized and challenged with OVA revealed an increase in airway responsiveness to the methacholine inhalation over the normal saline-sensitized and -challenged mice (Fig. 2b, OVA+HBSS group vs normal saline-immunized and -challenged group). The administration of

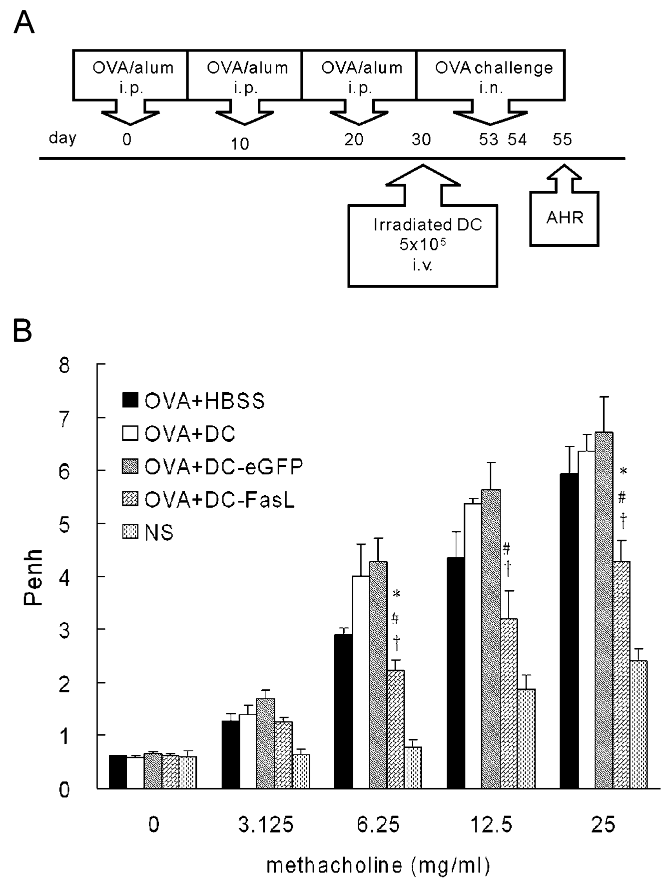

Fig. 2 DC-FasL decreased AHR in OVA-immunized and -challenged mice. **a** Time line representation. *i.p.* Intraperitoneal, *i.n.* intranasal, *i.v.* intravenous. **b** Airway responsiveness was assessed. *NS* Normal saline-immunized and -challenged. Results were expressed as the mean±SEM with four to five mice in each group. The experiments were repeated in variation for two times but with similar results. *Asterisk* means $p<0.05$ as compared with the sham injection (*HBSS*) group. *Number sign* means $p<0.05$ as compared with the DC group. *Dagger symbol* means $p<0.05$ as compared with the DC-eGFP group

the DC and DC-eGFP resulted in an increase in Penh to the lower concentration such as 6.25–12.5 mg/ml of methacholine inhalation. In contrast, the OVA-immunized and -challenged mice that were treated with DC-FasL had a significant decrease in airway hyperreactivity (Fig. 2b). The data suggested that the delivery of DC-FasL alleviated AHR in OVA-immunized and -challenged mice.

Th2-cell-induced murine model of asthma

To determine whether DC-FasL reduced AHR due to suppression of Th2 cells, a Th2-cell-induced asthma model was set up by using OVA-specific Th2 cell transferred to naïve BALB/c mice (Fig. 3a). The purity of cultured OVA-specific T cells was more than 95%. After two cycles of culture, Th2 cells secreted high levels of IL-4 and very low levels of IFN-γ by 2 days of anti-CD3 and anti-CD28 antibodies stimulation (data not shown). BALB/c mice that had been adoptively transferred with OVA-specific Th2

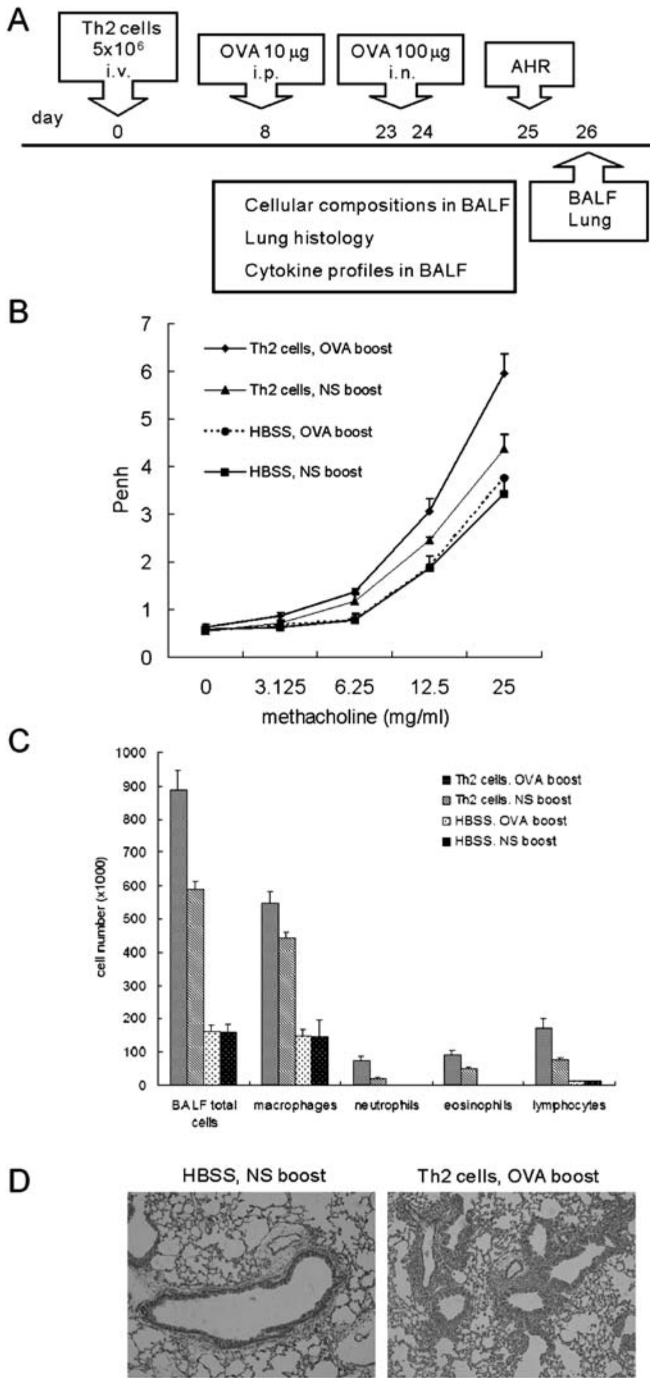

Fig. 3 Th2-cell-induced murine model of asthma. **a** Time line representation. **b** Airway responsiveness was assessed. **c** The cell compositions in BALF were analyzed. Results were expressed as the mean±SEM with three to seven mice in each group. **d** Representative histological examination of lung section was stained with hematoxylin and eosin (×100 magnification). *Th2 cells, OVA boost* means mice were adoptively transferred with Th2 cells and boosted with OVA. *NS* Normal saline. The experiments were repeated in variation for two times but with similar results

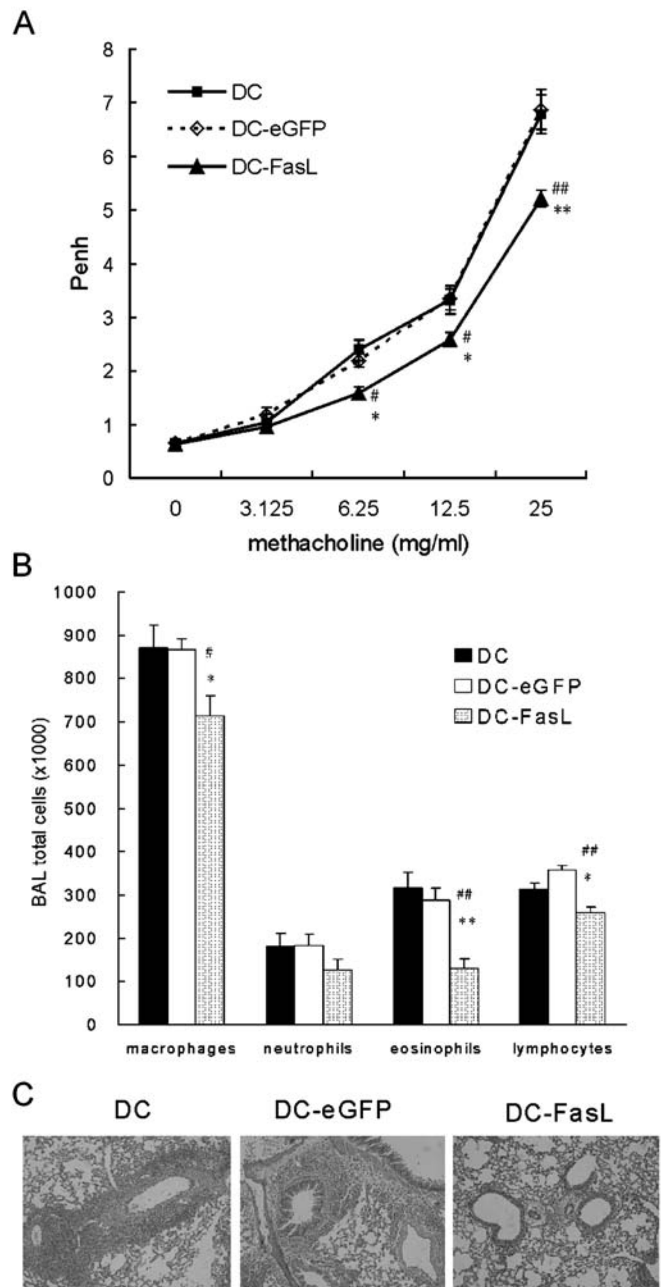

Fig. 4 DC-FasL-attenuated AHR and inflammation. Mice received DCs after 8 days of Th2 cells transfer. Airway responsiveness (**a**) and cell compositions in BALF (**b**) were analyzed. Results were expressed as the mean±SEM with six mice in each group. The experiments were repeated in variation for two times with similar results. *One asterisk* means $p<0.05$ and *two asterisks* mean $p<0.005$ as compared with DC group. *One number sign* means $p<0.05$ and *two number signs* mean $p<0.005$ as compared with DC-eGFP group. **c** Representative histological examination of lung section stained with hematoxylin and eosin (×100 magnification)

cells, boosted with OVA without adjuvant, and challenged with OVA intranasally revealed a marked increase in airway responsiveness over other control mice (Fig. 3b). The inflammatory cells including macrophages, neutrophils, eosinophils, and lymphocytes increased in Th2-cells-transferred mice (Fig. 3c). Moreover, the pathological examination of pulmonary tissues showed peribronchial and perivascular inflammation in Th2-cells-transferred mice (Fig. 3d). The mice that were adoptively transferred with Th2 cells not boosted with OVA revealed moderate

airway responses and airway inflammation. However, in mice that were only immunized with OVA without adjuvant, airway hypersensitivity and airway inflammation cannot be induced (Fig. 3).

DC-FasL attenuated the AHR and airway inflammation

Th2-cells-transferred mice treated with DC-FasL had a significant decrease in AHR. In contrast, mice treated with DC-eGFP had a similar airway hyperreactivity as that of the DC-receiving group (Fig. 4a). The administration of DC-FasL decreased the number of cells that infiltrated the lungs, especially eosinophils and lymphocytes. However, DC-eGFP did not decrease the recruitment of inflammatory cells in the airway (Fig. 4b). A histological analysis of the lungs showed a reduction in the number of inflammatory cells in the DC-FasL-treated mice compared with that of the DC-eGFP- or DC-treated control mice (Fig. 4c). Taken together, these results demonstrated that an intravenous injection of DC-FasL was able to reduce AHR and airway inflammation.

Reduction of OVA-reactive T cells by the delivery of DC-FasL

To examine whether clonal deletion of allergen-specific T cells by DC-FasL resulted in decreased pulmonary allergic responses, we tested the ability of DC-FasL in inducing apoptosis of allergen-specific T cells. BALB/c mice were adoptively transferred with OVA-specific Th2 cells and subsequently injected with DC-eGFP or DC-FasL as described above, and the numbers of OVA-specific T cells in the BALF were determined by staining with an anti-TCRαβ clonotypic antibody (KJ1-26) and an anti-CD4 antibody. The number of OVA-specific T cells (KJ1-26^{+}CD4^{+}) was lower in mice receiving DC-FasL than in mice receiving DC-eGFP (Fig. 5a,b). To understand whether transferred Th2 cells were dead by apoptosis in mice receiving DC-FasL, we analyzed OVA-specific T cells and apoptotic cells in the lung section. The OVA-specific T cells were found to be apoptotic in the lung section in mice receiving DC-FasL but not that in mice receiving control DC-eGFP (Fig. 5c).

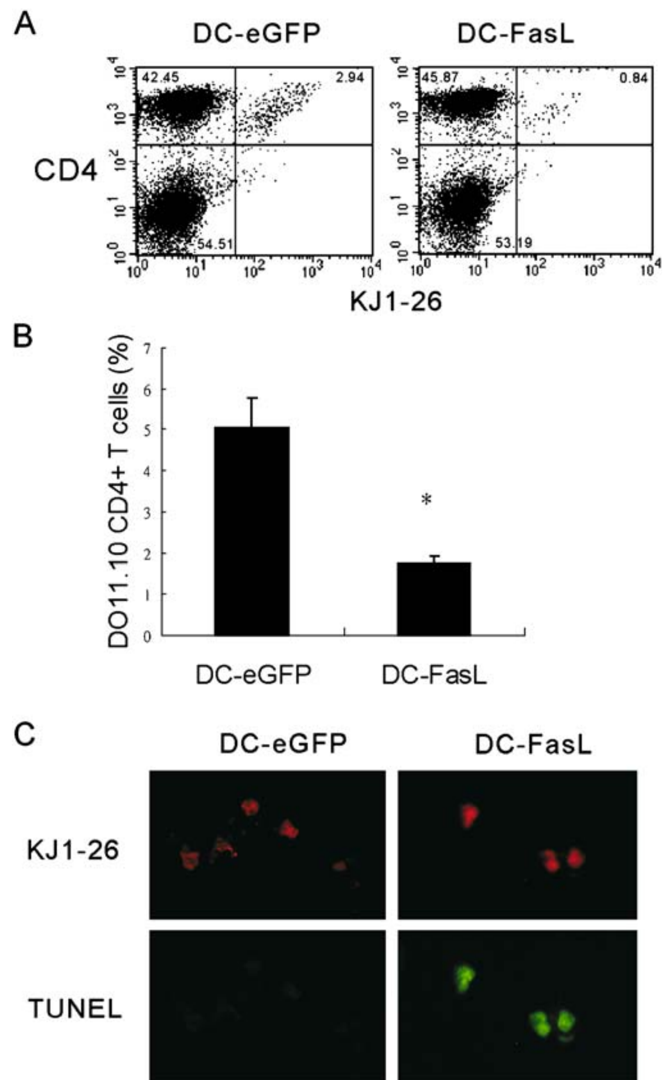

Fig. 5 Decrease of OVA-reactive T cells by the delivery of DC-FasL. OVA-specific T cells in the BALF of Th2-cells-transferred mice treated with DC-eGFP or DC-FasL were stained with surface marker and analyzed by flow cytometry. **a** Representative flow cytometry profile of expression of KJ1-26 and CD4 on the gated BALF cells. **b** The mean±SEM of the percentage of KJ1-26^{+} CD4^{+} T cells in various groups (four mice per group). **c** OVA-specific T cells were found to be apoptotic in the lung section of mice receiving DC-FasL but not that in mice receiving control DC-eGFP. The lung tissues of mice receiving DC-eGFP or DC-FasL were stained with KJ1-26 antibodies (labeled *red* with phycoerythrin) and TUNEL (labeled *green* with fluorescein isothiocyanate) (×400 magnification). *Asterisk* means $p<0.005$

Cytokine levels in the BALF reduced in mice receiving DC-FasL

The Th2-cells-transferred mice, which had been administered with DC or DC-eGFP, revealed a marked expression of IL-4, IL-5, and IL-13. However, the levels of IL-4, IL-5, and IL-13 were decreased in the BALF of mice receiving DC-FasL (Fig. 6). Taken together, these results indicated that DC-FasL administration was able to reduce Th2 cytokine levels, and the reduction of cytokine levels might be due to the decrease in the number of OVA-specific T cells.

Discussion

Allergic inflammation is orchestrated by Th2 cells, leading to IgE production and eosinophil activation. The important role of the allergen-specific Th2 cells in the immunologic and pathologic process of allergic asthma makes them an interesting target cell for therapy [1, 2]. In our previous

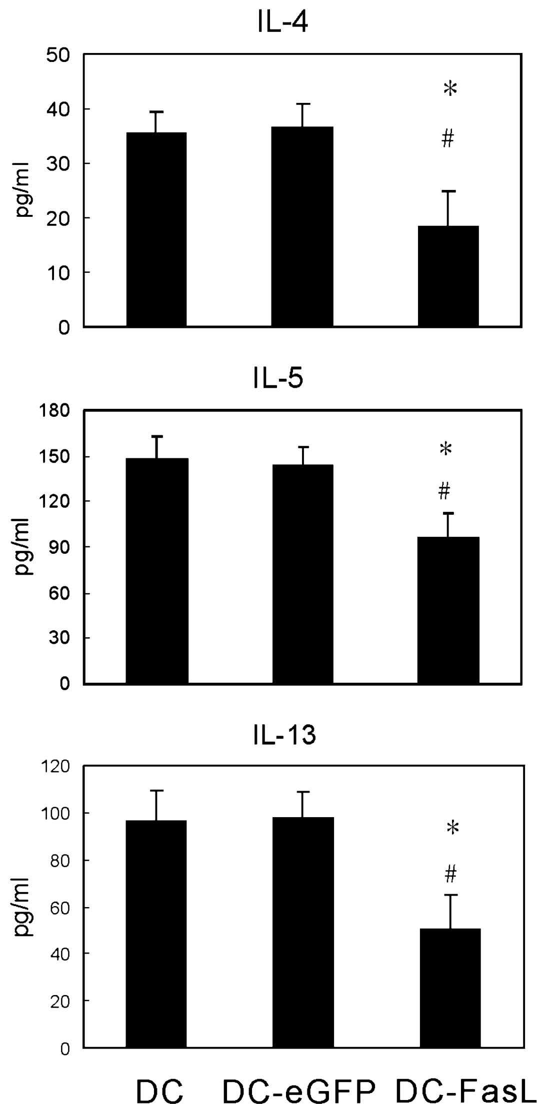

Fig. 6 Levels of IL-4, IL-5, and IL-13 were decreased in the BALF of mice receiving DC-FasL. Results were expressed as the mean± SEM for six mice in each group. The experiments were repeated in variation for two times with similar results. *Asterisk* means $p<0.05$ as compared with the DC group. *Number sign* means $p<0.05$ as compared with the DC-eGFP group

study, we demonstrated that the administration of recombinant adenovirus expressing murine FasL (Ad-FasL) in OVA-immunized mice significantly suppressed pulmonary allergic responses [18]. To specifically eliminate allergen-specific T cells in allergic asthma, we use DCs genetically engineered to express FasL to perform this study. Genetic modification of DC with FasL is an ideal approach for the generation of tolerogenic DC. Several studies have shown the therapeutic potential of FasL-expressing DCs as immunoregulatory cells for the treatment of allograft rejection [26–29], autoimmune disease [30–35], and chronic infection [36, 37]. Our results demonstrated that a single intravenous administration of DC-FasL to allergic mice significantly attenuated the development of AHR, inhibited airway inflammation, and decreased Th2 cytokines, such as IL-4, IL-5 and IL-13 production.

Although the mechanism of disease attenuation by DC-FasL has not been clearly elucidated, our results suggested that administration of DC-FasL was able to eliminate OVA-specific T cells. The OVA-specific T cells were found to be apoptotic in the lung sections of mice receiving DC-FasL but not that in mice receiving control DC-eGFP. Moreover, Th2 cytokines including IL-4, IL-5, and IL-13 in the BALF were decreased in mice receiving DC-FasL. Taken together, DC-FasL appears to eliminate OVA-specific Th2 cells and might subsequently suppress allergic asthma. These results are similar to earlier studies in which intravenous injection of FasL-expressing DC inhibits T-cell responses through the induction of T-cell apoptosis [26, 28, 35]. However, others have reported that intravenous injection of DC-FasL to naïve mice causes severe lung granulomatous vasculitis [38]. There were no significant pathological changes in the liver or lung in our experiments and in earlier studies [26, 28, 35]. It is possible that administration of DC-FasL induces neutrophil-mediated tissue destruction in naïve mice while it deletes reactive T cells in antigen-immunized mice or allogeneic mice.

Numerous studies have demonstrated that DCs are critical in developing a specific immune response in the lungs [39–42]. Intratracheal injection of allergen-pulsed DCs are potent in inducing asthma-like diseases by recruiting lymphocytes to the lung-draining lymph nodes and by stimulating Th2 responses [39–41]. However, it has been demonstrated that antigen presentation by airway DCs that express the inducible co-stimulatory molecule ligand and secrete IL-10 causes the development of regulatory T cells that can inhibit subsequent inflammatory effector responses [43, 44]. Furthermore, injection of allergen-pulsed modulated DCs, such as ribavirin, CpG DNA sequence, and Th1 cytokines, can affect the allergic immune response and prevent airway inflammation [4, 45]. In the present study, intravenous administration of OVA-pulsed DCs to both OVA-immunized and -challenged mice and OVA-specific Th2-cells-transferred mice induced T cell activation and subsequently increased AHR and airway inflammation. The T cell activation was antigen-specific because there was no such response when we administered human γ globulin-pulsed DCs to those mice (data not shown). However, an intravenous administration of OVA-pulsed FasL-expressing DC to allergic mice eliminated activated T cells and reduced AHR and airway inflammation.

To induce a long term, systemic Th2-cell-induced allergic asthma model similar to the OVA-immunized and -challenged allergic model, OVA-specific Th2-cells-transferred mice were boosted with OVA (without adjuvant) and challenged with droplets of OVA. The results showed that this protocol can induce recruitment of inflammatory cells to the lungs and an increase in AHR even 25 days after transfer of the OVA-specific Th2 cells. The levels of serum-specific IgE, IgG1, and even IgG2a in the Th2-cells-transferred mice were significantly higher than that of control mice (data not shown).

In summary, we constructed a bioactive adenoviral vector expressing murine FasL. DC-FasL induced killing of antigen-specific T cells in vitro and in vivo. The delivery

of DC-FasL into OVA-immunized and -challenged mice can decrease the AHR. We also established a murine model of OVA-specific Th2-cell-transferred asthma. In this model, the administration of DC-FasL before the airway challenge could significantly attenuate AHR, inhibit airway inflammation, and decrease IL-4, IL-5, and IL-13 production. In addition, the numbers of OVA-specific T cells in the BALF were also decreased in mice receiving DC-FasL. Moreover, the OVA-specific T cells were found to be apoptotic in the lung section of mice receiving DC-FasL but not that of mice receiving control DC-eGFP. Taken together, these results indicate that DC-FasL is able to decrease allergen-reactive T cells, resulting in efficient treatment of allergen-induced allergic airways disease.

Acknowledgement This work was supported by a grant from National Science Council of the Republic of China.

References

1. Herrick CA, Bottomly K (2003) To respond or not to respond: T cells in allergic asthma. Nat Rev Immunol 3:405–412
2. Leong KP, Huston DP (2001) Understanding the pathogenesis of allergic asthma using mouse models. Ann Allergy Asthma Immunol 87:96–109
3. Lee YL, Fu CL, Ye YL, Chiang BL (1999) Administration of interleukin-12 prevents mite Der p 1 allergen-IgE antibody production and airway eosinophil infiltration in an animal model of airway inflammation. Scand J Immunol 49:229–236
4. Chiang DJ, Ye YL, Chen WL, Lee YL, Hsu NY, Chiang BL (2003) Ribavirin or CpG DNA sequence-modulated dendritic cells decrease the IgE level and airway inflammation. Am J Respir Crit Care Med 168:575–580
5. Hamelmann E, Oshiba A, Loader J, Larsen GL, Gleich G, Lee J, Gelfand EW (1997) Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. Am J Respir Crit Care Med 155:819–825
6. Shardonofsky FR, Venzor J III, Barrios R, Leong KP, Huston DP (1999) Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. J Allergy Clin Immunol 104:215–221
7. Hansen G, Berry G, DeKruyff RH, Umetsu DT (1999) Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. J Clin Invest 103:175–183
8. Randolph DA, Stephens R, Carruthers CJ, Chaplin DD (1999) Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. J Clin Invest 104:1021–1029
9. Randolph DA, Carruthers CJ, Szabo SJ, Murphy KM, Chaplin DD (1999) Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. J Immunol 162:2375–2783
10. Takaoka A, Tanaka Y, Tsuji T, Jinushi T, Hoshino A, Asakura Y, Mita Y, Watanabe K, Nakaike S, Togashi Y, Koda T, Matsushima K, Nishimura T (2001) A critical role for mouse CXC chemokine(s) in pulmonary neutrophilia during Th type 1-dependent airway inflammation. J Immunol 167:2349–2353
11. Dhein J, Walczak H, Baumler C, Debatin KM, Krammer PH (1995) Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature 373:438–441
12. Ju ST, Panka DJ, Cui H, Ettinger R, el Khatib M, Sherr DH, Stanger BZ, Marshak-Rothstein A (1995) Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. Nature 373:444–448
13. Lau HT, Yu M, Fontana A, Stoeckert CJ Jr (1996) Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. Science 273:109–112
14. Arai H, Gordon D, Nabel EG, Nabel GJ (1997) Gene transfer of Fas ligand induces tumor regression in vivo. Proc Natl Acad Sci U S A 94:13862–13867
15. Ke B, Coito AJ, Kato H, Zhai Y, Wang T, Sawitzki B, Seu P, Busuttil RW, Kupiec-Weglinski JW (2000) Fas ligand gene transfer prolongs rat renal allograft survival and down-regulates anti-apoptotic Bag-1 in parallel with enhanced Th2-type cytokine expression. Transplantation 69:1690–1694
16. Batteux F, Lores P, Bucchini D, Chiocchia G (2000) Transgenic expression of Fas ligand on thyroid follicular cells prevents autoimmune thyroiditis. J Immunol 164:1681–1688
17. Zhu B, Luo L, Chen Y, Paty DW, Cynader MS (2002) Intrathecal Fas ligand infusion strengthens immunoprivilege of central nervous system and suppresses experimental autoimmune encephalomyelitis. J Immunol 169:1561–1569
18. Chuang YH, Fu CL, Lo YC, Chiang BL (2004) Adenovirus expressing Fas ligand gene decreases airway hyper-responsiveness and eosinophilia in a murine model of asthma. Gene Ther 11:1497–1505
19. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K (2000) Immunobiology of dendritic cells. Annu Rev Immunol 18:767–811
20. Bell D, Young JW, Banchereau J (1999) Dendritic cells. Adv Immunol 72:255–324
21. Hackstein H, Morelli AE, Thomson AW (2001) Designer dendritic cells for tolerance induction: guided not misguided missiles. Trends Immunol 22:437–442
22. Suen JL, Chuang YH, Chiang BL (2002) In vivo tolerance breakdown with dendritic cells pulsed with U1A protein in non-autoimmune mice: the induction of a high level of auto-antibodies but not renal pathological changes. Immunology 106:326–335
23. Lee YL, Ye YL, Yu CI, Wu YL, Lai YL, Ku PH, Hong RL, Chiang BL (2001) Construction of single-chain interleukin-12 DNA plasmid to treat airway hyperresponsiveness in an animal model of asthma. Hum Gene Ther 12:2065–2079
24. Adler A, Cieslewicz G, Irvin CG (2005) Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. J Appl Physiol 97:286–292
25. Schwarze J, Hamelmann E, Gelfand EW (2005) Barometric whole body plethysmography in mice. J Appl Physiol 98:1955–1957
26. Matsue H, Matsue K, Kusuhara M, Kumamoto T, Okumura K, Yagita H, Takashima A (2001) Immunosuppressive properties of CD95L-transduced “killer” hybrids created by fusing donor- and recipient-derived dendritic cells. Blood 98:3465–3472
27. Min WP, Gorczynski R, Huang XY, Kushida M, Kim P, Obataki M, Lei J, Suri RM, Cattral MS (2000) Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. J Immunol 164:161–167
28. Kusuhara M, Matsue K, Edelbaum D, Loftus J, Takashima A, Matsue H (2002) Killing of naive T cells by CD95L-transfected dendritic cells (DC): in vivo study using killer DC–DC hybrids and CD4(+) T cells from DO11.10 mice. Eur J Immunol 32:1035–1043
29. Whartenby KA, Straley EE, Kim H, Racke F, Tanavde V, Gorski KS, Cheng L, Pardoll DM, Civin CI (2002) Transduction of donor hematopoietic stem-progenitor cells with Fas ligand enhanced short-term engraftment in a murine model of allogeneic bone marrow transplantation. Blood 100:3147–3154
30. Zhan HG, Mountz JD, Fleck M, Zhou T, Hsu HC (2002) Specific deletion of autoreactive T cells by adenovirus-transfected, Fas ligand-producing antigen-presenting cells. Immunol Res 26:235–246
31. Zhang HG, Yang P, Xie J, Liu Z, Liu D, Xiu L, Zhou T, Wang Y, Hsu HC, Mountz JD (2002) Depletion of collagen II-reactive T cells and blocking of B cell activation prevents collagen II-induced arthritis in DBA/1j mice. J Immunol 168:4164–4172

32. Zhang HG, Liu D, Heike Y, Yang P, Wang Z, Wang X, Curiel DT, Zhou T, Mountz JD (1998) Induction of specific T-cell tolerance by adenovirus-transfected, Fas ligand-producing antigen presenting cells. Nat Biotechnol 16:1045–1049
33. Zhang HG, Su X, Liu D, Liu W, Yang P, Wang Z, Edwards CK, Bluethmann H, Mountz JD, Zhou T (1999) Induction of specific T cell tolerance by Fas ligand-expressing antigen-presenting cells. J Immunol 162:1423–1430
34. Matsue H, Matsue K, Walters M, Okumura K, Yagita H, Takashima A (1999) Induction of antigen-specific immuno-suppression by CD95L cDNA-transfected 'killer' dendritic cells. Nat Med 5:930–937
35. Kim SH, Kim S, Oligino TJ, Robbins PD (2002) Effective treatment of established mouse collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express FasL. Mol Ther 6:584–590
36. Zhang HG, Fleck M, Kern ER, Liu D, Wang Y, Hsu HC, Yang P, Wang Z, Curiel DT, Zhou T, Mountz JD (2000) Antigen presenting cells expressing Fas ligand down-modulate chronic inflammatory disease in Fas ligand-deficient mice. J Clin Invest 105:813–821
37. Wolfe T, Asseman C, Hughes A, Matsue H, Takashima A, von Herrath MG (2002) Reduction of antiviral CD8 lymphocytes in vivo with dendritic cells expressing Fas ligand-increased survival of viral (lymphocytic choriomeningitis virus) central nervous system infection. J Immunol 169:4867–4872
38. Buonocore S, Flamand V, Claessen N, Heeringa P, Goldman M, Floquin S (2004) Dendritic cells overexpressing Fas-ligand induce pulmonary vasculitis in mice. Clin Exp Immunol 137:74–80
39. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA (2000) Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. J Clin Invest 106:551–559
40. Lambrecht BN, Pauwels RA, Fazekas De St GB (2000) Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. J Immunol 164:2937–2946
41. van Rijt LS, Prins JB, Leenen PJ, Thielemans K, de Vries VC, Hoogsteden HC, Lambrecht BN (2002) Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C(neg) bone marrow precursors in a mouse model of asthma. Blood 100:3663–3671
42. Graffi SJ, Dekan G, Stingl G, Epstein MM (2002) Systemic administration of antigen-pulsed dendritic cells induces experimental allergic asthma in mice upon aerosol antigen rechallenge. Clin Immunol 103:176–184
43. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT (2002) Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 8:1024–1032
44. Akbari O, DeKruyff RH, Umetsu DT (2001) Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2:725–731
45. Ye YL, Lee YL, Chuang ZJ, Lai HJ, Chen CC, Tao MH, Chiang BL (2004) Dendritic cells modulated by cytokine-expressing adenoviruses alleviate eosinophilia and airway hyperresponsiveness in an animal model of asthma. J Allergy Clin Immunol 114:88–96