



## CORRESPONDENCE

## Notch ligand-expressing adenovirus infection enhances the efficacy of dendritic cell-based immunotherapy for allergic asthma in mice

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According to the World Health Organization, the prevalence of asthma is rising worldwide, and asthma causes 250,000 deaths annually.<sup>1</sup> Allergic asthma is a type of chronic airway inflammation caused by dysregulated T helper type 2 (Th2)-type immune responses. Patients with allergic asthma are characterized by elevated serum immunoglobulin E (IgE) antibody levels, mucus production, and pulmonary inflammation as well as airway hyperresponsiveness (AHR).<sup>2</sup> Current therapeutic methods for asthma include inhaled corticosteroids and  $\beta_2$  agonists to relieve asthma syndromes, but these treatments do not change the chronic disease course in patients. Some patients need high doses of inhaled corticosteroids or require long-term daily treatment with oral steroids.<sup>3</sup> Therefore, efforts should be targeted towards developing innovative therapeutic strategies to improve patient quality of life and reduce unwanted deaths. One of the advances in the development of future therapies is the use of genetically modified dendritic cells (DCs) to decrease pathological Th2-mediated responses and maintain a disease-modifying effect long term. DC activation is crucial for inducing T cell immunity as well as determining T cell differentiation.<sup>4</sup> Importantly, DCs can be cultured in vitro, and large amounts of generated clinical-grade DCs have become available. Additionally, DCs has been shown to be permissive of adenoviral (Ad) vector infection in vitro, and Ad vectors are a promising gene delivery platform for a variety of therapeutic and vaccine applications.<sup>5</sup> In this regard, overexpressing immunoregulatory proteins in Ad-modified DCs would be an effective way to suppress Th2 cell immunity and establish an antiallergic response.

The Notch signaling network is a highly conserved signaling system that is an important regulator of T cell development and differentiation. In mammals, there are five Notch ligands (Delta-like 1 (DLL1), DLL3, DLL4, Jagged1, and Jagged2) and four Notch receptors (Notch1–4). The Notch pathway can be activated by Notch receptor-ligand interactions between DCs and T cells. Several studies have revealed that DLL ligands are closely associated with the development of Th1 cells, while Jagged ligands favor the induction of Th2 and regulatory T (Treg) cells. Notably, recent studies have addressed the possibility of targeting Notch in T cells to treat diseases such as autoimmune diseases, cancer, and graft transplantation. Targeting DLL1 and DLL4 via blocking antibodies ameliorates the severity of experimental

autoimmune encephalomyelitis, a disease caused by an auto-reactive Th1 response.<sup>6</sup> Another study showed that Notch signaling in lymphoid cells mediated by DLL1 enhances antitumor T cell responses.<sup>7</sup> Moreover, Lin et al. reported that the overexpression of Jagged1 prolongs allograft survival via the induction of Treg cells.<sup>6</sup> Thus, based on this evidence, we propose that Notch may suppress excessive Th2 responses arising in an allergic state by regulating the balance of Th1/Th2 responses or inducing Treg cells. In this study, we evaluated whether bone marrow-derived DCs (BMDCs) genetically engineered to over-express DLL4 (DLL4-DCs) can exert the therapeutic effect of inhibiting aberrant Th2-mediated allergic asthma by altering the Th1/Th2 cell ratio.

First, we constructed a DLL4-expressing adenovirus (Ad-DLL4) as well as a control adenovirus (Ad-mock) that did not express the DLL4 gene. Mouse DCs were prepared using tibial and femoral bone marrow from BALB/c mice. Subsequently, immature DCs were infected with Ad-mock or Ad-DLL4 using a multiplicity of infection (MOI) of 20, 100, 200, or 500. Infected and uninfected immature DCs were further stimulated with ovalbumin (OVA) to induce DC activation. As shown in Fig. 1a, the immature DCs, Ad-uninfected DCs (control-DC), and Ad-mock-infected DCs (mock-DC) included very low numbers of DLL4<sup>+</sup> DCs. In contrast, Ad-DLL4 infection produced increased percentages of DLL4<sup>+</sup> DCs in a dose-dependent manner. Furthermore, an allogeneic mixed lymphocyte reaction was performed to clarify whether the DLL4-DCs exhibited immunomodulatory effects on T cells in vitro. Different Ad-transduced BMDCs were irradiated and cocultured with allogeneic CD4<sup>+</sup> T cells. The proliferative ability and interferon (IFN)- $\gamma$  production capacity of the activated T cells were determined. Compared to control-DCs and mock-DCs, DLL4-DCs infected with Ad-DLL4 at an MOI of 200 significantly enhanced T cell proliferation at a DC/T cell ratio of 1:10 (Fig. 1b). Additionally, the production of IFN- $\gamma$  (a Th1-type cytokine) by T cells was obviously enhanced by the DLL4-DCs compared to the control-DCs or mock-DCs. Collectively, these results demonstrated that the overexpression of DLL4 by DCs enhanced the immunomodulatory ability to promote T cell activation and Th1 cell polarization.

Based on the above in vitro findings, we hypothesized that DLL4-DCs might produce an antiallergic effect by inducing Th1 immune responses in Th2-mediated allergic asthma. Thus, we

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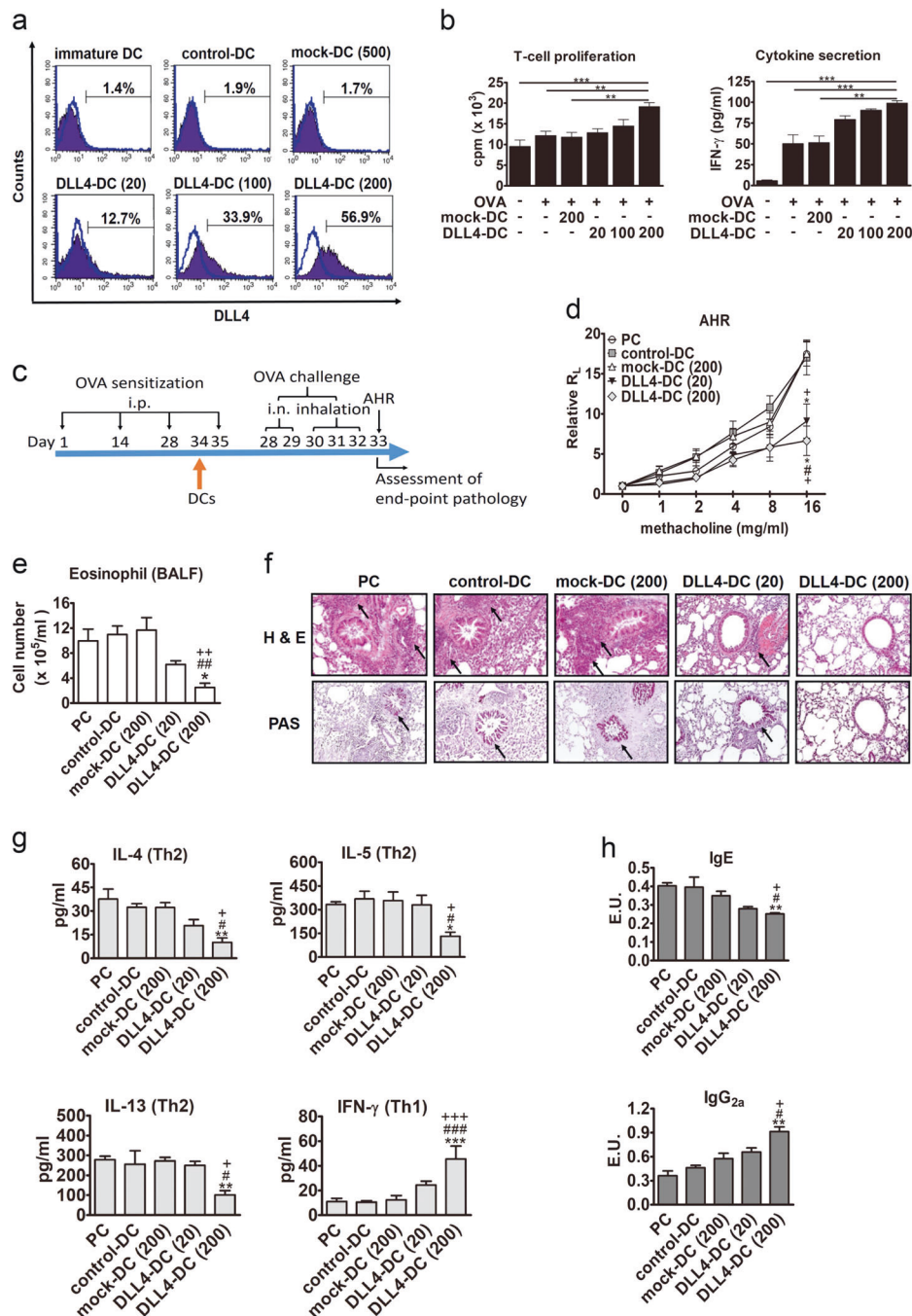


**Fig. 1** Dendritic cells (DCs) infected with a Notch ligand DLL4-expressing adenovirus (DLL4-DCs) induced the expansion of T helper type 1 (Th1) cells and ameliorated Th2-mediated allergic asthma in mice. **a** DLL4 expression following the transduction of DCs with the DLL4-expressing adenovirus (Ad-DLL4) was assessed. DLL4 protein expression in Ad-infected DCs was measured by FACS analyses. **b** DLL4-DCs enhanced T cell proliferation and IFN- $\gamma$  production by activated T cells in vitro. The proliferative capacity of T cells is expressed as counts per minute (cpm). The levels of IFN- $\gamma$  in supernatants were analyzed by an enzyme-linked immunosorbent assay (ELISA). Bar graphs represent the mean  $\pm$  SEM values of three independent experiments. \*\* $p < 0.01$  and \*\*\* $p < 0.001$  vs. the DLL4-DC (MOI of 200) group. **c** A schematic diagram shows the curative protocol. BALB/c mice were randomized into five groups of five mice. **d** Adoptive transfer of DLL4-DCs attenuated the development of airway hyperresponsiveness (AHR) in mice with ovalbumin (OVA)-induced asthma. The results are expressed as the ratio of pulmonary resistance after methacholine nebulization ( $R_L$ ) to pulmonary resistance after PBS nebulization. **e** Cells from the bronchoalveolar lavage fluid (BALF) were stained with Liu's dye. The number of eosinophils was counted. **f** Lung sections were stained with hematoxylin/eosin (H&E) or periodic acid-Schiff (PAS). Arrowheads indicate inflammatory cell infiltration or mucus production. **g** DLL4-DCs reduced the Th2 immune response but enhanced the Th1 response. BALF samples from each group of mice were analyzed for IL-4, IL-5, IL-13, and IFN- $\gamma$  contents. **h** The levels of OVA-specific antibodies in serum samples are shown. The results are expressed as the mean  $\pm$  SEM of the five mice in each group. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  vs. the positive control (PC) group. # $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  vs. the control-DC group. + $p < 0.05$ , ++ $p < 0.01$ , and +++ $p < 0.001$  vs. the mock-DC group.

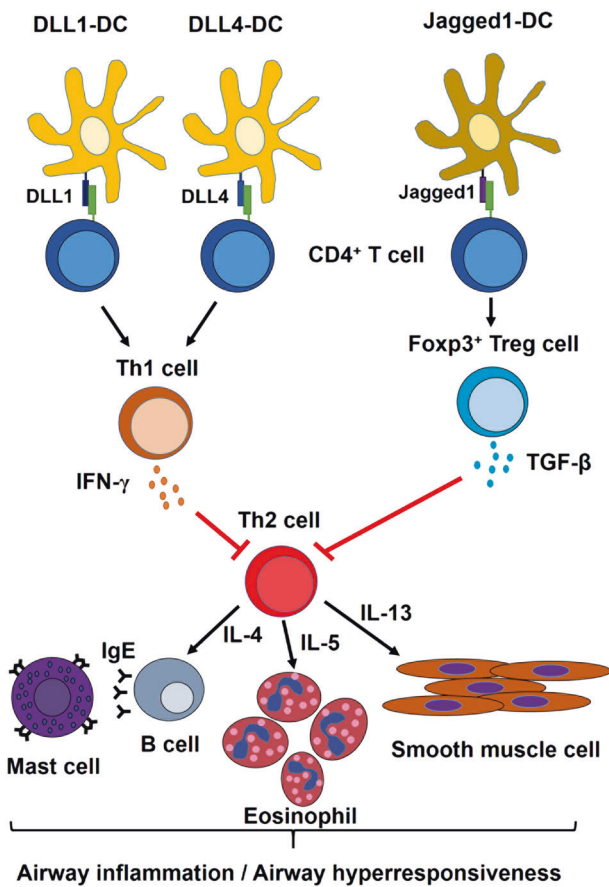


**Fig. 2** Notch ligand-modified dendritic cell (DC)-based immunotherapy for treating T helper type 2 (Th2)-mediated allergic asthma. Adenovirus (Ad)-modified DCs suppressed Th2-mediated responses by promoting Th1 or regulatory T (Treg) cell expansion

evaluated the therapeutic effects of DLL4-DCs on an OVA-induced asthmatic animal model. The experimental protocol is described in Fig. 1c. All groups of mice were exposed to OVA sensitization and challenge. Four groups of mice were transferred with OVA-pulsed control-DCs, mock-DCs (MOI of 200), DLL4-DCs (MOI of 20), or DLL4-DCs (MOI of 200). OVA-sensitized and OVA-challenged mice that received a phosphate-buffered saline injection instead of an injection of DCs served as an asthmatic positive control (PC). AHR development was measured as pulmonary resistance ( $R_L$ ) values in response to increasing doses of methacholine. PC mice had high, dose-dependent  $R_L$  values, whereas the administration of DLL4-DCs reduced the severity of AHR at the maximal dose of 16 mg/ml methacholine (Fig. 1d). Furthermore, the accumulation of inflammatory cells in the bronchoalveolar lavage fluid (BALF) and lungs was detected. We observed that the numbers of eosinophils in the BALF were increased in the PC, control-DC-treated, and mock-DC-treated mice (Fig. 1e). In addition, histological examination of the lungs from the mice in these groups revealed many inflammatory cells around the bronchioles and increased mucus secretion (Fig. 1f). In contrast, DLL4-DC treatment markedly attenuated inflammatory cell recruitment and mucus production in the lungs. Moreover, the results revealed that the levels of the Th2 cytokines interleukin (IL)-4, IL-5, and IL-13 in the BALF were elevated in the PC group, whereas the administration of DLL4-DCs infected at an

MOI of 200 resulted in significant downregulation of the levels of these Th2 cytokines (Fig. 1g). In addition, the expression of IFN-γ, a Th1-type cytokine, was notably induced in the DLL4-DC (MOI of 200)-treated mice (Fig. 1g). We also assayed the titers of OVA-specific IgE and IgG<sub>2a</sub> antibodies in mouse serum. Previous studies have shown that IL-4 induces IgE production and that IFN-γ drives IgG<sub>2a</sub> secretion.<sup>8</sup> In the PC group, a high level of IgE and a low level of IgG<sub>2a</sub> production were observed (Fig. 1h). Conversely, the mice administered DLL4-DCs (MOI of 200) exhibited a reduction in the IgE level and upregulation of the IgG<sub>2a</sub> level in the serum. Collectively, these data indicated that DLL4-DCs were able to suppress Th2-induced allergic asthma through the induction of IFN-γ-producing Th1 cells.

Over the years, DCs have been used as vaccines for cancer immunotherapy and have increased overall survival in patients in phase II studies.<sup>9</sup> For immunotherapeutic treatment of allergic asthma, our previous study reported that DCs transduced with the DLL1 gene by adenoviruses possess the ability to promote Th1 cell development.<sup>8</sup> Additionally, we proved that Ad-Jagged1-infected DCs enhance the generation of Foxp3<sup>+</sup> Treg cells.<sup>10</sup> Herein, we further demonstrated that Ad-DLL4-infected DCs promoted the development of Th1 cells and exerted an immunotherapeutic effect by altering the Th1/Th2 cell ratio. Thus, we concluded that Notch ligand gene-modified DCs exhibited antiallergic effects through two regulatory pathways (Fig. 2). One is that DLL1-DCs and DLL4-DCs can induce IFN-γ-producing Th1 cells to inhibit Th2 responses. On the other hand, Jagged1-DCs can promote the differentiation of transformation growth factor (TGF)-β-producing Treg cells to ameliorate Th2-mediated allergic asthma. Overall, this evidence indicates that Notch ligand-modified DCs may be novel candidates for use as immunomodulators to treat allergic asthma.

## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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