

# Interleukin-10-Treated Dendritic Cells Do Not Inhibit Th2 Immune Responses in Ovalbumin/Alum-Sensitized Mice

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## Key Words

Dendritic cells · Interleukin 10 · Immunoglobulin E ·  
Ovalbumin · T helper cell 2 · Type I allergy

## Abstract

**Background:** It is well known that the immunoregulatory cytokine interleukin (IL)-10 inhibits the accessory function of human dendritic cells (DC) in vitro. Recently, we have shown that these IL-10 DC inhibit the production of T helper cell 1 (Th1) and T helper cell 2 (Th2) cytokines by T cells from atopic individuals in vitro. The current study was set out to analyze whether IL-10 DC also exert inhibitory effects in vivo in a murine model of allergy to ovalbumin adsorbed to the adjuvant aluminium hydroxide (OVA/alum). **Methods:** OVA-pulsed or unpulsed bone marrow-derived DC, treated with IL-10 or left untreated during generation, were injected intravenously into BALB/c mice prior to and during OVA/alum sensitization, and sera and immune responses of mesenteric lymph node cells were analyzed. Additionally, bronchoalveolar lavage was performed after intranasal challenge with OVA. **Results:** Treatment of BALB/c mice with OVA-pulsed DC led to a significantly enhanced proliferation as well as Th2 (IL-4, IL-5), Th1 (interferon- $\gamma$ ) and IL-10 cytokine production after restimulation of lymph node cells with OVA

in vitro compared with OVA immunization alone. In contrast, using OVA-pulsed IL-10 DC for transfer, proliferation and cytokine production by lymph node cells were not enhanced. OVA-specific immunoglobulin G1 (IgG1) and IgG2a production were significantly increased after transfer of OVA-pulsed DC and OVA-pulsed IL-10 DC, respectively, whereas anti-OVA IgE production and airway eosinophilia remained unchanged. **Conclusions:** Our data indicate that IL-10 treatment of DC decreases the Th1 and Th2 stimulatory capacity of DC but does not actually inhibit systemic (IgE) and local (airway inflammation) allergen-specific immune responses in a murine model of allergy.

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## Introduction

It is well established that dendritic cells (DC) are the most potent antigen-presenting cells able to initiate T helper cell 1 (Th1) and T helper cell 2 (Th2) immune responses [1]. In addition, they play an important role in the regulation of the immune response by inducing peripheral T-cell tolerance through the differentiation of T regulatory (Tr) 1 cells [2, 3]. Tolerance-inducing DC can be generated by maturation of DC in the presence of the immunoregulatory cytokine interleukin (IL)-10, which has been shown to induce Tr1 cells and to inhibit the terminal differentiation of human and mouse DC in vitro,

I.B. and S.S. contributed equally to this paper.

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characterized by a decreased expression of major histocompatibility complex (MHC) class II molecules and several costimulatory and adhesion molecules [4–7]. Additionally, IL-10 treatment of DC strongly inhibits IL-12 production [8–10]. In humans, we and others have demonstrated that IL-10-treated DC are not only able to inhibit allergen-specific Th1 cells, due to their reduced production of IL-12, but also to inhibit allergen-specific Th2 cells [7, 8]. These anergic T cells produce IL-10 themselves and behave as regulatory T cells, as they suppress activation and function of peripheral T cells in a cell-to-cell contact-dependent manner [11].

In mice, the effects of IL-10 on the accessory function of DC are less clear. Whereas splenic DC treated with IL-10 induce a Th2 response when injected into mice, antigen presentation is generally impaired by IL-10-treated bone marrow-derived dendritic cells (BMDC) [6, 9, 12–14]. In an ovalbumin (OVA) T-cell receptor transgenic mouse model, IL-10-treated BMDC also inhibit delayed type hypersensitivity reactions in the footpads of prophylactically or therapeutically vaccinated animals [15]. However, in all experiments described so far, the influence of IL-10-treated BMDC on Th2 immune responses has not been investigated [6, 9, 15]. In the present study, we analyzed the effect of IL-10-treated BMDC in a Th2 model of allergy using OVA adsorbed to aluminium hydroxide (OVA/alum) for sensitization of BALB/c mice. We found that transfer of IL-10-treated OVA-pulsed BMDC prior to and during sensitization led to a lack of stimulation of Th1 and Th2 cytokine production when compared with OVA-pulsed DC that were not treated with IL-10, but immunoglobulin E (IgE) responses and airway inflammation were not changed compared with OVA/alum-sensitized mice.

## Material and Methods

### *Mice*

Female BALB/c mice 8–10 weeks of age were used in all experiments. Mice were bred under specific pathogen-free conditions in the Central Animal Facility of the University of Mainz according to German guidelines for animal care and received an OVA-free diet.

### *Cell Culture Medium*

Culture medium consisted of RPMI 1640 (Gibco BRL) supplemented with L-glutamine (2 mM), streptomycin (100 µg/ml), penicillin (100 U/ml), 2-mercaptoethanol (5 mM), Hepes buffer (20 mM), Na-pyruvate (1 mM), Na-hydrogencarbonate (24 mM), and 5% heat-inactivated FCS (PAA Laboratories GmbH, Pasching, Austria) for culture of lymph node cells (lymph node culture

medium) or 2% human heat-inactivated AB serum (Blood Transfusion Center, Mainz, Germany) for DC culture (DC culture medium).

### *Generation of BMDC*

BMDC were prepared according to published protocols. Briefly, femurs and tibias of BALB/c mice were removed and the marrow was flushed out with a syringe. The tissue was suspended in DC culture medium, passed through nylon mesh, and erythrocytes were lysed with 0.15 M NH<sub>4</sub>Cl. After washing,  $1 \times 10^6$  cells/ml were placed in 24-well plates in 1 ml of DC culture medium supplemented with 10 ng/ml granulocyte/macrophage colony-stimulating factor (GM-CSF) and 10 ng/ml IL-4 (Tebu-Bio GmbH, Offenbach, Germany). On day 2, nonadherent cells were washed out and fresh medium containing GM-CSF and IL-4 was added. At the same time point, cells were treated with 30 ng/ml IL-10 (Tebu-Bio GmbH) or left untreated. On day 6 of culture, nonadherent cells were transferred to 6-well plates and pulsed with 100 µg/ml OVA grade VI (Sigma-Aldrich, Deisenhofen, Germany) or left unpulsed. The endotoxin content of OVA was lower than 10 ng/ml as measured by QCL-1000 chromogenic LAL (Bio-Whittaker, Walkersville, Md., USA) according to the instructions of the manufacturer. After 24 h, DC were harvested and used for in vivo transfer into BALB/c mice. In all experiments, expression of CD11c, MHC class II and costimulatory molecules (CD80, CD86) was controlled by flow cytometry.

### *Model of Type I Allergy*

For the model of type I allergy, mice (n = 4 per group) were injected intravenously with  $5 \times 10^5$  DC on days –9, –2 and 12, followed by immunization by intraperitoneal injection of 100 µl of OVA grade VI diluted in PBS (20 µg; Sigma-Aldrich) adsorbed to an equal volume of aluminium hydroxide (Pierce Biotechnology, Inc., Rockford, Ill., USA) on days 0 and 14. Sera were collected 4 days after the last immunization and stored at –20°C until determination of OVA-specific IgE, IgG1 and IgG2a titers.

### *Model of Allergic Airway Inflammation*

For the model of allergic airway inflammation, mice were treated with DC and sensitized with OVA/alum as described above. On days 25, 26 and 27, mice were challenged intranasally with 100 µg OVA in PBS or PBS alone. Twenty-four hours after the last challenge, mice were sacrificed by an overdose of dimethylether. Then, a 24-gauge cannula was introduced into the trachea and the lungs were lavaged with 1 ml PBS. Bronchoalveolar lavage (BAL) cells were counted, spun onto glass slides using a cytospin (Shandon, Asmoor, UK) and stained with Diff-Quick (Dade Behring AG, Düringen, Switzerland) according to the manufacturer's instructions. Eosinophils, neutrophils, lymphocytes and monocytes were enumerated microscopically using standard histological criteria (400 cells for each sample were counted).

### *Determination of OVA-Specific Antibody Titers in the Serum*

Anti-OVA IgE, IgG1 and IgG2a antibody concentrations in the serum from each mouse were measured by ELISA as described, with the modification that OVA (25 µg/ml) was used as antigen [16]. The antibody titer was defined as the reciprocal se-

rum dilution, yielding an absorbance reading of an optical density of 0.2 after linear regression analysis.

#### Flow Cytometry

Surface phenotyping of DC was performed by staining  $5 \times 10^4$  DC with specific monoclonal antibodies for 20 min at 4°C. Antibodies used were fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse MHC class II I-A/I-E (clone 2G9), FITC-conjugated hamster anti-mouse CD11c (clone HL3), R-phycoerythrin (R-PE)-conjugated hamster anti-mouse CD80 (clone 16-10A1), R-PE-conjugated rat anti-mouse CD86 (clone GL1) and FITC- or R-PE-conjugated hamster or rat isotype controls (all from BD Biosciences, San Diego, Calif., USA). After being washed with PBS containing 0.1% BSA, the cells were analyzed in a FACSCalibur (BD Biosciences) equipped with CELLQuest Software.

#### Cell Preparation and Antigen Restimulation in vitro

Mesenterial lymph node cells were prepared from DC-injected and OVA/alum-primed mice or control animals 4 days after the last immunization. Lymph nodes from each group were pooled and the tissue was dispersed through stainless steel mesh. Then, lymph node single cell suspensions were washed twice with PBS.

For cytokine production assays,  $2.5 \times 10^6$  lymph node cells were cultured in 24-well tissue-culture plates in lymph node culture medium in a final volume of 1 ml per well. OVA was added at a final concentration of 100 µg/ml. After 72 h, supernatants were harvested and stored at -20°C until analysis. IL-4, IL-5, IL-10 and interferon-γ were measured by ELISA according to instructions of the manufacturer of the employed pairs of antibodies (R&D Systems, Wiesbaden, Germany). The limit of detection was 15 pg/ml for all cytokines.

For proliferation assays,  $2 \times 10^5$  lymph node cells were incubated in lymph node culture medium in 96-well flat-bottom tissue-culture plates in 200 µl per well in triplicates with or without the addition of 100 µg/ml OVA. After 4 days, the cells were pulsed with 37 kBq/well of <sup>3</sup>H-TdR (methyl-<sup>3</sup>H-thymidine, ICN, Irvine, Calif., USA) for 6 h, and <sup>3</sup>H-TdR incorporation was evaluated in a beta counter (1205 Betaplate, LKB Wallac, Turku, Finland).

#### Statistics

Student's unpaired t test was employed to test statistical significance of results. A p value <0.05 was considered statistically significant.

## Results

#### *Transfer of OVA-Pulsed IL-10-Treated DC into BALB/c Mice prior to and during OVA Sensitization Leads to Reduced T Cell Proliferation Compared with OVA-Pulsed DC But Does Not Inhibit the OVA-Specific Proliferative Response Compared with No Transfer of DC*

To analyze the influence of IL-10-treated DC in a murine model of type I allergy, BALB/c mice were injected intravenously with  $5 \times 10^5$  OVA-pulsed or unpulsed

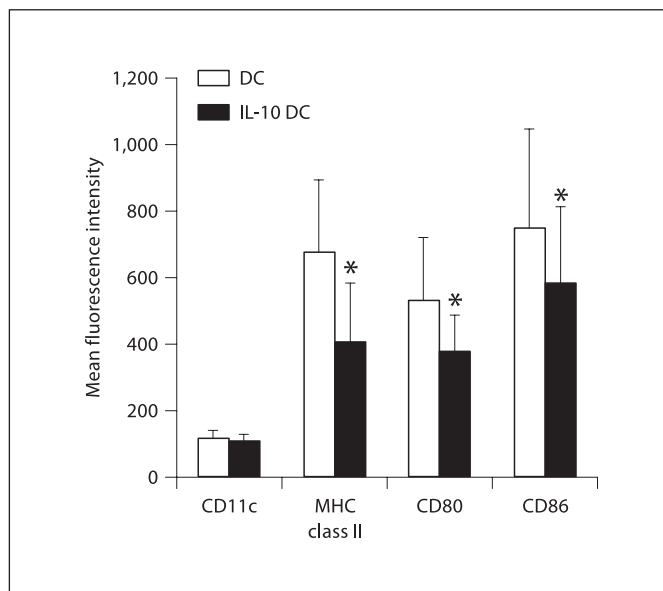


**Fig. 1.** IL-10 treatment of BMDC reduces expression of MHC class II and costimulatory molecules: bone marrow cells were cultured in the presence of GM-CSF and IL-4 with or without the addition of 30 ng/ml IL-10 from day 2 onwards. After 7 days of culture, nonadherent DC were harvested and analyzed for expression of CD11c, MHC class II, CD80 and CD86 by flow cytometry. Data represent the means  $\pm$  SD of the mean fluorescence intensity from 6 independent experiments. \* p < 0.05 comparing DC with IL-10 DC.

BMDC on days -9, -2 and 12. DC culture had been performed with or without addition of IL-10 from day 2 onwards. Flow cytometric analysis revealed that expression of the DC-specific marker CD11c was not affected by IL-10 treatment, while expression of MHC class II and the costimulatory molecules CD80 and CD86 was significantly decreased (fig. 1). There was no difference in surface marker expression between OVA-pulsed or unpulsed control DC (data not shown).

Two days after the second and third DC transfer, mice were intraperitoneally sensitized with low doses of OVA/alum, a protocol leading to enhanced Th2 immune responses including IgG1 and IgE production. As shown in figure 2, lymph node cells from OVA-sensitized mice showed increased proliferation after restimulation with OVA in vitro. While transfer of OVA-pulsed DC prior to and during OVA-sensitization significantly enhanced proliferation in response to OVA, no enhancement was observed after transfer of OVA-pulsed IL-10-treated DC. Despite the use of different sera for DC generation and for culture of lymph node cells (human AB serum vs.

FCS) to minimize serum-specific background proliferation levels, we nevertheless observed unspecific proliferation after transfer of OVA-pulsed or unpulsed DC. One explanation of this finding might be that T cells activated by transferred DC presenting peptides from human serum proteins cross-recognized peptides from bovine serum proteins presented by DC during the proliferation assay. Another explanation might be the enhanced activation of DC (e.g. augmented IL-6 production after pulsing with OVA; data not shown) as has been reported before with allergen-pulsed human DC [17]. DC were not generated in mouse serum due to reduced cell numbers (only 10% recovery compared with culture in FCS or AB serum).

*Treatment of BALB/c Mice with OVA-Pulsed IL-10-DC prior to and during OVA Sensitization Leads to Reduced Th1 and Th2 Priming Compared with OVA-Pulsed DC But Does Not Inhibit the OVA-Specific Cytokine Production Compared with No Transfer of DC*

Next, we investigated the cytokine expression levels of lymph node cells from DC-treated and OVA-sensitized mice after restimulation with OVA in vitro. Production of the Th2 cytokines IL-4 and IL-5 and of IL-10 was significantly increased in lymph node cells from mice which had been treated with OVA-pulsed DC prior to and during OVA sensitization compared with OVA sensitization alone, whereas transfer of OVA-pulsed IL-10 DC resulted in no significant changes in cytokine production (fig. 3). Additionally, an enhanced production of the Th1 cytokine interferon- $\gamma$  was observed after transfer of OVA-pulsed DC but not if OVA-pulsed IL-10 DC were used. However, a suppression of OVA-induced cytokine production compared with no DC priming (sensitization of OVA/alum alone) was not observed.

*Transfer of IL-10-Treated DC into BALB/c Mice prior to and during OVA Sensitization Does Not Inhibit OVA-Specific Immunoglobulin Production or Airway Inflammation*

The humoral immune response after OVA sensitization was characterized by the production of OVA-specific IgE as well as by high OVA-specific IgG1 antibody titers and low IgG2a antibody levels, indicating a Th2 immune response (fig. 4). Treatment of mice with OVA-pulsed DC significantly enhanced OVA-specific IgG1 and IgG2a antibody concentrations. However, in contrast to proliferation and cytokine production, which were lower using OVA-pulsed IL-10 DC for transfer,

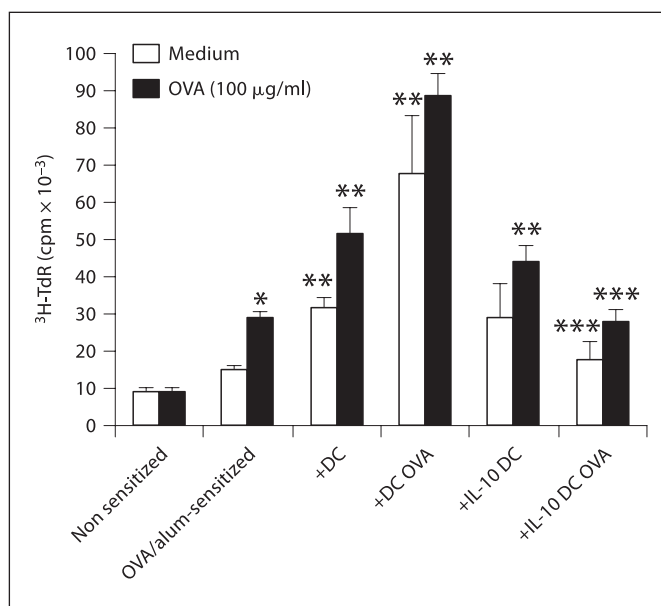


**Fig. 2.** Reduced proliferation of lymph node cells from OVA-sensitized mice after transfer of OVA-pulsed IL-10 DC compared with OVA-pulsed DC: OVA-pulsed or unpulsed BMDC, which had additionally been treated with IL-10 or left untreated during DC culture, were injected intravenously into BALB/c mice (n = 4 per group) on days -9, -2 and 12. Mice were immunized with OVA/alum by means of intraperitoneal injection on days 0 and 14. Additional mice received no treatment at all (nonsensitized control) or were only immunized with OVA/alum. On day 18, lymph node cells from all mice per group were pooled and cultured with 100 µg/ml OVA or medium for 4 days and proliferation was measured in triplicates by <sup>3</sup>H-TdR incorporation during the last 6 h of culture. Data show the means ± SD from 1 representative of 3 independent experiments. \* p < 0.05 compared with non-sensitized control; \*\* p < 0.05 compared with OVA/alum; \*\*\* p < 0.05 compared with DC OVA.

OVA-specific IgG1 and IgG2a production was stimulated by OVA-pulsed IL-10 DC in a similar way to OVA-pulsed DC. OVA-specific IgE production was neither increased by OVA-pulsed DC nor by OVA-pulsed IL-10 DC (fig. 4).

Additionally, using a model of allergic airway inflammation, OVA-pulsed IL-10 DC significantly inhibited the numbers of monocytes in the BAL, but not airway eosinophilia, after OVA/alum sensitization and subsequent intranasal challenge with OVA (fig. 5). Lymphocyte numbers were significantly enhanced after transfer of OVA-pulsed DC and OVA-pulsed IL-10 DC, respectively, which is consistent with earlier reports showing that lymphocyte recruitment into the lung oc-

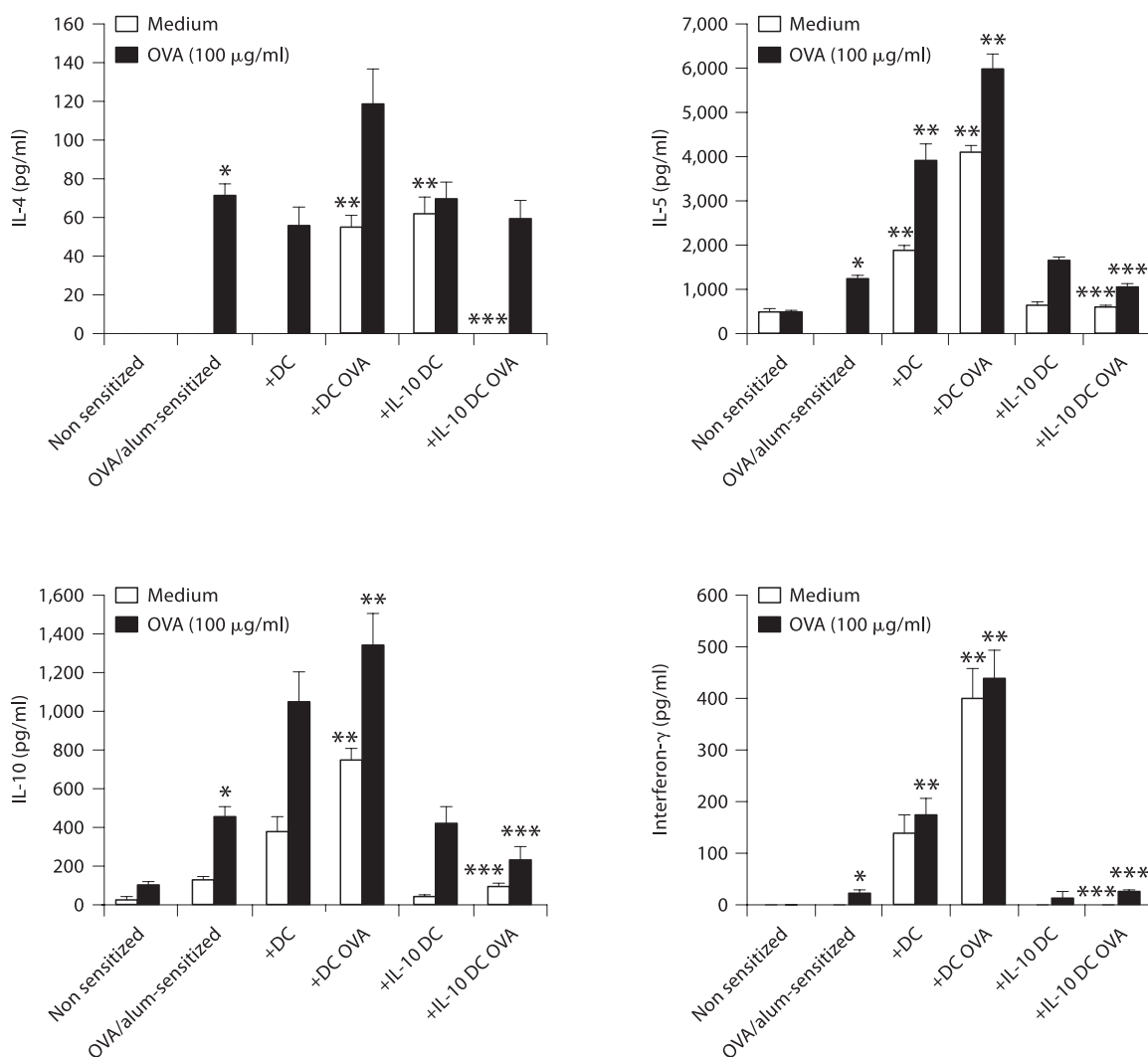


**Fig. 3.** Reduced Th1, Th2 and IL-10 cytokine production by lymph node cells from OVA-sensitized mice after transfer of OVA-pulsed IL-10 DC compared with OVA-pulsed DC: BALB/c mice were treated with DC and immunized as described in the legend to figure 2. On day 18, lymph node cells from all mice per group were pooled and cultured with 100 μg/ml OVA or medium for 72 h.

Cytokine production in supernatants was measured by ELISA in duplicates. Data show the means  $\pm$  SD from 1 representative of 3 independent experiments. \*  $p < 0.05$  compared with nonsensitized control; \*\*  $p < 0.05$  compared with OVA/alum; \*\*\*  $p < 0.05$  compared with DC OVA.

curs after systemic or local transfer of DC [18, 19]. No airway inflammation was measured in OVA-sensitized mice after intranasal challenge with PBS as control (data not shown). In all experiments performed in the model of allergic airway inflammation, the data raised with

respect to cytokine production, proliferation and OVA-specific immunoglobulin production were similar to the data obtained in the model of type I allergy, in which intranasal challenge with OVA is not performed (data not shown).

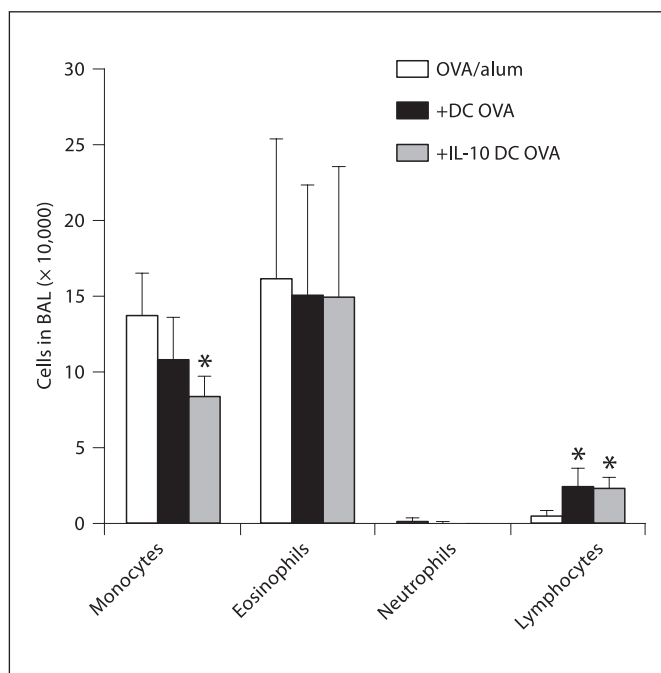
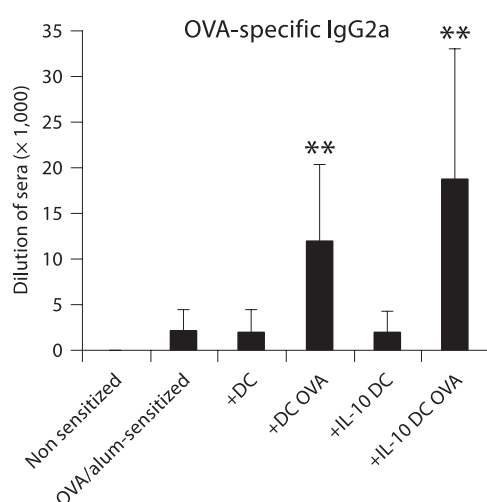
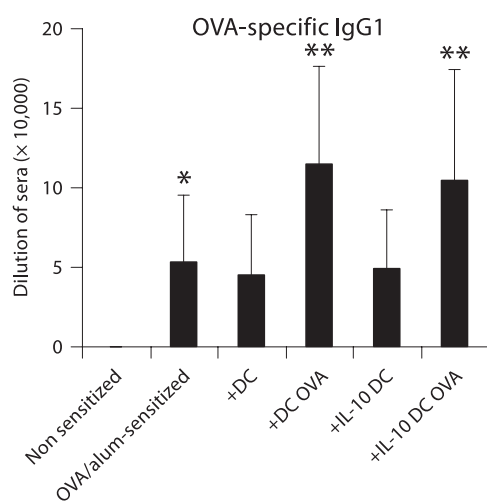
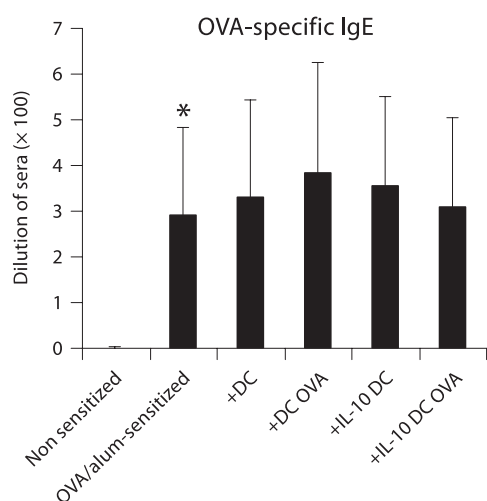


**Fig. 5.** Analysis of OVA-specific airway inflammation after DC transfer prior to and during OVA sensitization: BALB/c mice were treated with DC and immunized as described in the legend to figure 2. On days 25, 26 and 27, mice were challenged intranasally with 100 µg OVA in PBS. BAL was performed 24 h later and numbers of eosinophils, neutrophils, monocytes and lymphocytes were counted on cytospin preparations (400 cells per sample). Data show the means  $\pm$  SD of absolute cell numbers from 6 mice/group. The experiment was repeated once with similar results. \*  $p < 0.05$  compared with OVA/alum.

**Fig. 4.** Analysis of OVA-specific immunoglobulin production after DC transfer prior to and during OVA sensitization: BALB/c mice were treated with DC and immunized as described in the legend to figure 2. On day 18, levels of OVA-specific IgE, IgG1 and IgG2a antibodies in sera from each mouse were measured by ELISA. Data show the means  $\pm$  SD from 6 independent experiments (containing altogether 24 mice). \*  $p < 0.05$  compared with nonsensitized control; \*\*  $p < 0.05$  compared with OVA/alum.

## Discussion

The immunoregulatory cytokine IL-10 has been shown to directly inhibit T cells as well as the accessory function of antigen-presenting cells [20–22]. In addition, IL-10 affects the maturation of immature myeloid mouse and human DC, while mature DC are resistant to IL-10 [7, 23]. Recently, we have demonstrated that IL-10-treated DC are able to inhibit allergen-specific Th1 and Th2 cytokine production by T cells from atopic individuals in vitro [8]. In the present study, we used the well-established mouse models of type I allergy and of allergic inflammation [24] to analyze the effect of transfer of IL-10-treated DC in vivo on the development of Th2 responses induced by intraperitoneal injections of low doses of OVA/alum. We have demonstrated that in contrast to transfer of OVA-pulsed, but IL-10-untreated, DC prior to sensitization, which led to enhanced proliferation and cytokine production by mesenteric lymph node cells after restimulation with OVA in vitro, transfer of OVA-pulsed IL-10-treated DC did not increase CD4<sup>+</sup> T-cell proliferation nor Th1 and Th2 cytokine production. The antigen-independent proliferation and cytokine production, observed after treatment with OVA-pulsed or even unpulsed DC, might be due to cross-recognition by T cells of peptides from proteins contained in FCS and human AB serum. Still, these unspecific responses were also reduced when OVA-pulsed or unpulsed IL-10-treated DC were used for transfer. Additionally, we showed that lymphocyte numbers in the BAL as well as OVA-specific IgG1 and IgG2a production were significantly increased after transfer of OVA-pulsed DC and OVA-pulsed IL-10 DC, respectively, while IgE production and airway inflammation (eosinophilia) were not affected at all. However, transfer of OVA-pulsed IL-10 DC significantly inhibited the numbers of monocytes in the BAL compared with no DC treatment. Transfer of OVA-pulsed DC or OVA-pulsed IL-10 DC alone without further OVA/alum injections also led to enhanced proliferation and cytokine production after restimulation with OVA in vitro being significantly higher in mice transferred with OVA-treated DC compared with OVA-treated IL-10 DC. However, OVA-specific immunoglobulin production was not detectable in the sera of these mice (data not shown).

Our results are supported by previous findings which demonstrate that BMDC activated by CpG oligodeoxynucleotides and pulsed with OVA were able to increase the influx of neutrophils and lymphocytes in the airways upon allergen challenge but were not able to protect from

developing allergen-induced Th2 responses [19]. In contrast, *Leishmania major*-induced Th2 responses could be inhibited by vaccination with CpG-treated BMDC pulsed with *L. major* lysate [25]. Furthermore, in an earlier work by Müller et al. [15], it was shown that IL-10-treated DC expressing lower levels of MHC class II and costimulatory molecules when compared with untreated DC are able to prevent sensitization in OVA T-cell receptor transgenic CD4<sup>+</sup> T cells and to suppress ongoing immune responses in presensitized T cells as well as delayed type hypersensitivity reactions. However, in the latter study, mice were primed with immunogenic peptide in Incomplete Freund's Adjuvant, a protocol not associated with Th2 cytokine production and high IgE levels, while in our study, a typical Th2-inducing protocol was used and Th2 cytokines and IgE were analyzed. In fact, we used an immunization protocol with multiple injections of OVA/alum, which results in strong and sustained OVA-specific IgE production and therefore resembles the situation in allergic patients. The Th2 responses induced after sensitization of mice with OVA and alum appear to be less accessible to inhibition by IL-10 DC.

Our results are also in accordance with a report from Haase et al. [6] who have demonstrated that both exogenous and endogenous IL-10 inhibits the maturation and T-cell priming capacity of Th1- and Th2-inducing BMDC in vitro and in vivo, mainly due to downregulation of IL-12 production. However, studies with splenic IL-10 DC have shown the induction, but not the inhibition, of a Th2 immune response [9, 14]. The reason for this discrepancy is probably the use of different subsets and maturation stages of DC. It has been found for example that splenic CD8 $\alpha$ <sup>+</sup> DC produce high amounts of IL-12 and are capable of activating Th1 cells, while IL-10 is required for development of Th2 cells by CD8 $\alpha$ <sup>–</sup> DC [26]. Recently, Wakkach et al. [3] have described a natural CD11c<sup>low</sup> CD45RB<sup>high</sup> DC subset in the spleen and lymph nodes of mice that secretes high levels of IL-10 after activation and induces tolerance through the differentiation of Tr1 cells. Interestingly, these cells can be generated in vitro by culture of BMDC in the presence of IL-10. These findings suggest that autocrine IL-10 production by DC plays an important role in maintaining the balance of the immune system to prevent strong immune reactions to inhaled or ingested antigens.

Besides the prevention of protein antigen-specific immune responses, IL-10 was described to inhibit induction of autoimmune diseases such as experimental autoimmune encephalomyelitis or arthritis [27, 28]. Furthermore, IL-10 induces the differentiation of Tr1 cells which

suppress immune responses including Th2-specific responses and IgE production by means of cell-to-cell interactions or the production of IL-10, transforming growth factor- $\beta$ , or both [4, 29, 30]. Thus, induction of Tr1 cells seems to be a novel promising therapeutic strategy in autoimmune and allergic diseases. In our system, OVA-specific IgE production and airway inflammation were not inhibited by IL-10-treated DC apart from a reduction in monocyte numbers in the BAL, while anti-OVA IgG1 and IgG2a production was increased compared with OVA/alum sensitization alone. Together with the observation that IL-10 production was not enhanced after restimulation of lymph node cells from IL-10 DC-treated animals, it appears that the transferred IL-10 DC in our experimental setup were not able to sufficiently induce Tr1 cells. Another explanation may be that the type of DC employed in this study (BMDC) is not ideally suited for these approaches, as it has been recently shown that plasmacytoid DC prevent asthmatic reactions to in-

haled antigen, whereas myeloid DC are important for generation of effector T cells [31].

In summary, our data provide evidence that treatment of mice with OVA-pulsed IL-10 DC decreased their T-cell priming capacity compared with regularly matured DC, as shown by reduced proliferation and Th1 and Th2 cytokine production after restimulation with OVA in vitro. However, the IgE response and local inflammation was not actively inhibited by IL-10 DC, nor by cells induced by them in this experimental setting. Additional studies are required to identify approaches to achieve long-lasting tolerance and immunoregulation of allergen-specific Th2 responses in vivo as well.

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