

Differential capacity of CD8 α ⁺ or CD8 α [−] dendritic cell subsets to prime for eosinophilic airway inflammation in the T-helper type 2-prone milieu of the lung

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Summary

Background Different subsets of dendritic cells (DCs), identified in mouse spleen by their differential expression of CD8 α , can induce different T-helper (Th) responses after systemic administration. CD8 α [−] DCs have been shown to preferentially induce Th type 2 (Th2) responses whereas CD8 α ⁺ DCs induce Th1 responses.

Objective To study if these DC subsets can still induce different Th responses in the Th2-prone milieu of the lung and differentially prime for eosinophilic airway inflammation, typical of asthma.

Methods Donor mice first received daily Flt3L injections to expand DC numbers. Purified CD8 α ⁺ or CD8 α [−] splenic DCs were pulsed with ovalbumin (OVA) or phosphate-buffered saline and injected intratracheally into recipient mice in which carboxyfluorescein diacetate succinimidyl ester-labelled OVA-specific T cell receptor transgenic T cells had been injected intravenously 2 days earlier. T cell proliferation and cytokine production of Ag-specific T cells were evaluated in the mediastinal lymph nodes (MLNs) 4 days later. The capacity of both subsets of DCs, to prime for eosinophilic airway inflammation was determined by challenging the mice with OVA aerosol 10 days later.

Results CD8 α [−] DCs migrated to the MLN and induced a vigorous proliferative T cell response accompanied by high-level production of IL-4, IL-5, IL-10 and also IFN- γ during the primary response and during challenge with aerosol, leading to eosinophilic airway inflammation. In the absence of migration to the MLN, CD8 α ⁺ DCs still induced a proliferative response with identical levels of IFN- γ but reduced Th2 cytokines compared with CD8 α [−] DCs, which led to weak eosinophilic airway inflammation upon OVA aerosol challenge. Unpulsed DCs did not induce proliferation or cytokine production in Ag-specific T cells.

Conclusion CD8 α [−] DCs are superior compared with CD8 α ⁺ DCs in inducing Th2 responses and eosinophilic airway inflammation in the Th2-prone environment of the lung.

Keywords asthma, CD4, dendritic cells, eosinophils, lung, mouse model, sensitization, T cell activation, Th1/Th2

Submitted 3 October 2003; revised 23 April 2004; accepted 4 June 2004

Introduction

Allergic asthma is a chronic inflammatory disease of the airways. Upon contact with specific allergen in the context of antigen-presenting cells (APCs), T cells release T-helper type 2 (Th2) cytokines such as IL-4, IL-5 and IL-13 that control eosinophilic airway inflammation, goblet cell hyperplasia and bronchial hyper-reactivity [1]. We and others have previously shown that airway dendritic cells (DCs) are held responsible for the sensitization to inhaled antigen that leads to Th2-type CD4-mediated immunopathology [2–5]. This preferential priming of Th2 responses by respiratory tract DCs could be because of the presence of subsets of DCs with inherent Th2-

promoting potential in the respiratory tract, or could be because of the inherent Th2-prone milieu of the lung [6–8].

Recently, different subsets of DCs, identified by the differential expression of CD8 α in the mouse, have been described to induce different Th responses [9]. The CD8 α [−] cells in mouse spleen preferentially induce Th2 responses following footpad injection *in vivo* or following their expansion by injection of polyethylene glycol modified-granulocyte macrophage-colony stimulating factor (GM-CSF) [10, 11]. On the contrary CD8 α ⁺ DCs in the spleen induce a preferential Th1 response when injected into the footpad or when expanded *in vivo* by Flt3L treatment [10–12]. These studies have generated the idea that Th cell differentiation can be determined by the subset of DC interacting with naïve T cells. Subsequently, however, it was shown that CD8 α ⁺ or CD8 α [−] DC subsets can induce different types of Th response *in vitro* and *in vivo* depending on the type of stimulus used to induce maturation in these cells, the dose

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of antigen and the ratio of APC to responding T cell, overriding the effect of DC subsets on Th differentiation [13, 14]. Likewise, in humans, DC subsets have been described that preferentially lead to either Th1 or Th2 responses depending upon the dose of antigen, the conditions of culture, the time-point of analysis after activation or the nature of the activating stimulus [15–18].

One factor that has been ignored in most of these studies is the influence of the micro-environment in which the DCs encounter antigen. It is currently unknown if the inherent capacity of DC subsets to polarize either Th1 or Th2 responses is overruled by the route of administration and thus the environment where antigen is encountered.

In this paper, we studied whether CD8 α^+ and CD8 α^- DC subsets have differential capacity to induce Th2 immune responses to inhaled antigen and differential capacity to prime for eosinophilic airway inflammation in the Th2-prone milieu of the lung.

Materials and methods

Reagents

Ovalbumin (OVA) was from Worthington Biochemical Corp (Lakewood, NJ, USA) and was screened for low contamination with endotoxin (<29 EU/mg OVA in *Limulus* assay). Flt3L was provided by Amgen Corp. (Seattle, WA, USA). Carboxyfluorescein diacetate succinimidyl ester (CFSE) and CellTracker™ Green 5-chloromethylfluorescein diacetate (CMFDA) were from Molecular Probes (Eugene, OR, USA). Collagenases type 2 and type 3 were from Worthington Biochemical Corp. (Lakewood). DNase I was from Sigma (Zwijndrecht, the Netherlands). Microbeads coupled with anti-CD8 α antibodies were from Miltenyi Biotec (Bergisch-Gladbach, Germany). The PE-conjugated anti-I-Ad/I-Ed (M5/114.5.2) was from Pharmingen (Heidelberg, Germany). The PE-conjugated anti-CCR3 and KJ1-26 (clonotypic OVA-T cell receptor (TCR)) were from R&D (Abingdon, UK) and Caltag Laboratories (Burlingame, CA, USA), respectively. The Cy-chrome-labelled anti-CD3 (145.2c11) and anti-CD45R (RA3-6B2), as well as the APC-labelled anti-CD4 (RM4-5) and anti-CD11c (HL3) were from Pharmingen.

Mice

Balb/c mice (6–8-week-old) were purchased from Harlan (Zeist, the Netherlands). OVA-TCR transgenic mice (DO11.10) on a Balb/c background were bred at the Erasmus University (Rotterdam, the Netherlands).

Preparation of spleen dendritic cells

Mice were injected intraperitoneally with 10 μ g of recombinant human Flt3L for 9 consecutive days. Splenic DC were purified as already described [11, 12]. Briefly, spleen cells were digested with collagenase III and cultured in RPMI-1640 containing 2% Ultrosor HY (Life Technologies, Paisley, Scotland, UK) for 2 h at 37°C. Non-adherent cells were removed. Adherent cells were cultured overnight in the presence or absence of 100 μ g/mL of OVA. The enriched DCs were separated according to CD8 α expression by incubation with anti-CD8 α^- coupled microbeads

followed by a passage over a MACS column. CD8 α^+ or CD8 α^- cells pulsed or not with OVA were enumerated and resuspended in phosphate-buffered saline (PBS) at a concentration of 12.5×10^6 /mL.

Intratracheal injection of dendritic cells

Mice were anaesthetized with avertin (2% v/v in PBS) and 80 μ L of the cell suspensions (1×10^6 DCs) were injected intratracheally.

Protocol I: migration of dendritic cells

Following overnight culture in the presence or absence of OVA, DCs were collected and labelled with CMFDA (5 μ M) for 30 min at 37°C. Cells were extensively washed and resuspended in PBS. 1×10^6 DCs were instilled into the trachea of naïve Balb/c mice. Twenty-four hours later, the optimal time-point to detect DC in the draining nodes following an intratracheal (i.t.) injection, mediastinal lymph nodes (MLNs) were collected and minced using iris scissors. The MLNs were then incubated for 1 h at 37°C in RPMI-1640 containing 5% fetal calf serum (FCS), 1 mg/mL collagenase type II and 0.02 mg/mL DNase I, according to a modified protocol [19]. Adding PBS containing 10 mM EDTA stopped the reaction. The cells were centrifuged and CMFDA $^+$ MHC II $^+$ DCs were detected by flow cytometry on a FACScalibur. Cell viability was determined by propidium iodide exclusion and was >95%.

Protocol II: effect of CD8 α^+ and CD8 α^- dendritic cells on the priming of ovalbumin-specific T cells

Because the frequency of OVA-specific T cells is very low in immunized animal, the primary activation of a naïve T cell is hard to detect. To avoid this problem, a detectable number of naïve T cells purified from DO11.10 mice were adoptively transferred into Balb/c mice. Briefly, MLNs and spleen were collected from DO11.10 mice and homogenized. After red blood cell lysis, cells were labelled with CFSE. Cells were enumerated and dead cells, stained for trypan blue, were excluded. 10×10^6 cells were injected intravenously in the lateral tail vein of Balb/c mice (day 2). On day 0, the mice received an i.t. injection of CD8 α^+ DCs or CD8 α^- DCs pulsed with OVA, or control unpulsed DCs. On day 4, the optimal time-point to detect cytokine production by T cells *in vivo* in mice injected i.t. with DCs, MLNs were collected, homogenized and stained for the presence of KJ1-26 $^+$ CD4 $^+$ reactive OVA-specific T cells. Some of the LN cells (200 000 cells/well in triplicates) were resuspended in RPMI-1640 containing 5% FCS and antibiotics (gentamycin, 50 μ g/mL) and placed in 96-well plates. Four days later, supernatants were harvested and analysed for the presence of Th1 (IFN- γ) and Th2 (IL-4, IL-5, and IL-10) cytokines by ELISA.

Protocol III: effect of CD8 α^+ and CD8 α^- dendritic cells on the pulmonary inflammatory reaction following a secondary exposure to ovalbumin aerosols

On day 0, Balb/c mice were immunized by i.t. instillation of CD8 α^+ or CD8 α^- DCs pulsed with OVA or with PBS. From

days 10–13, mice were exposed to 30 min-OVA aerosols (grade III, Sigma). Mice were killed 24 h following the last aerosol.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed with 3×1 mL of Ca^{2+} and Mg^{2+} -free HBSS (Invitrogen, Breda, The Netherlands) supplemented with 0.1 mM sodium EDTA. The BAL fluid was centrifuged; the cells were resuspended in HBSS and enumerated in a haemocytometer. The cellular composition of BAL fluid was determined using flow cytometric analysis in a four-colour experiment as described in detail [20]. In brief, after washing, cells were stained for 30 min with anti-I-Ad/I-Ed FITC (macrophages), anti-CCR3 PE (eosinophils), anti-CD3 cy-chrome and anti-B220 cy-chrome (lymphocytes), and anti-CD11c APC (macrophages) in PBS containing 0.5% bovine serum albumin and 0.01% sodium azide. Cells were washed and analysed by flow cytometry based on the criteria published [20].

Cytokine production in mediastinal lymph nodes

MLNs were removed, homogenized, resuspended in RPMI-1640 containing 5% FCS and antibiotics before enumeration. Cells collected from the MLN were resuspended at 2×10^6 cells/mL and restimulated *in vitro* for 4 days with $10 \mu\text{g}/\text{mL}$ of OVA. Supernatants were then tested for the presence of IL-4, IL-5, IL-10 and IFN- γ .

Statistical analysis

For all experiments, the difference between groups was calculated using the Mann–Whitney *U*-test for unpaired data. Differences were considered significant if $P < 0.05$.

Results

CD8 α^- dendritic cells but not CD8 α^+ dendritic cells migrate to the draining lymph nodes

One day following instillation, CD8 α^- DCs pulsed with OVA could be detected in the MLN of the mice. However, no OVA-pulsed CD8 α^+ DCs were present in the MLN, indicating that CD8 α^+ DCs have very low capacity to

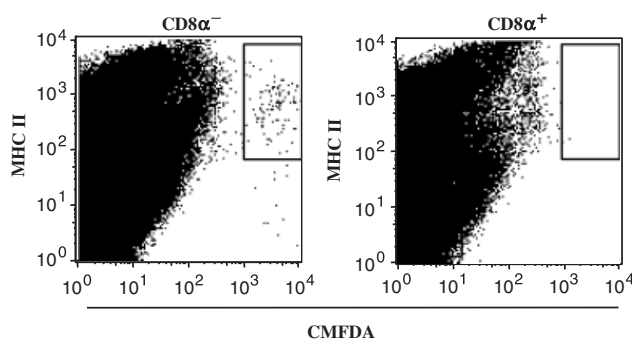


Fig. 1. Localization of 5-chloromethylfluorescein diacetate (CFMFA)-labelled CD8 α^- and CD8 α^+ dendritic cells (DCs). 1×10^6 CD8 α^- or CD8 α^+ DCs were injected in the trachea of Balb/c mice. Twenty-four hours later, mediastinal lymph nodes were enzymatically digested and analysed by flow cytometry after a staining for MHCII and CD11c. Injected DCs can be discriminated from endogenous DCs by the green fluorescence of CFMFA. Dot plots are representative of three to five mice per group.

migrate from peripheral tissues to draining lymph nodes (Fig. 1).

Both CD8 α^- dendritic cells and CD8 α^+ dendritic cells can prime ovalbumin-specific T cells

As CD8 α^- DCs pulsed with OVA can reach the draining lymph nodes after instillation in the trachea, we next investigated whether these DCs could prime OVA-specific naïve T cells in the MLNs. Figure 2 shows that 4 days

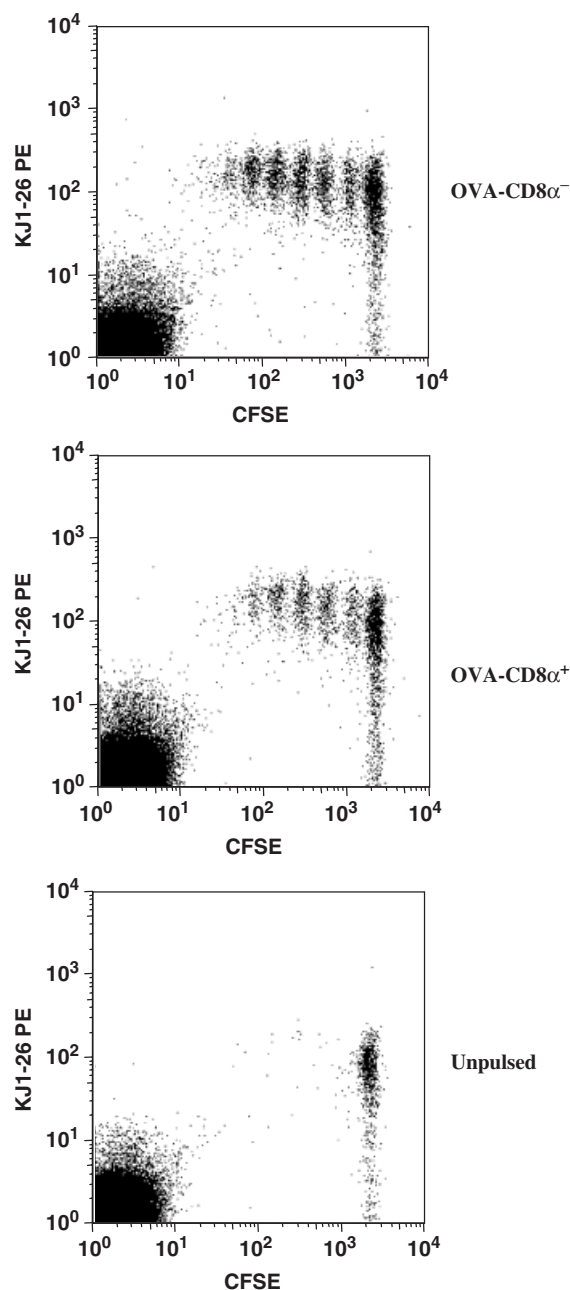


Fig. 2. Local response of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labelled T cells after intratracheal injection of CD8 α^+ or CD8 α^- dendritic cells (DCs) pulsed with ovalbumin or control unpulsed splenic DCs. Lymph node T cells were gated for scatter characteristics, CD4 expression and PI exclusion. The proliferation of KJ1-26 $^+$ CFSE $^+$ T cells was analysed by flow cytometry. Dot plots are representative from 10 mice per group.

following instillation of CD8 α^- DCs, T cells in the MLNs proliferated and had undergone six divisions. Surprisingly, while not detectable in the draining lymph nodes, CD8 α^+ DCs administered intratracheally induced T cells to proliferate (Fig. 2). However, in mice injected with CD8 α^+ DCs, fewer cells were recruited into divisions as compared with mice instilled with CD8 α^- DCs. In the MLNs, $1.3 \pm 0.2\%$ of total lymph node cells demonstrated a CFSE profile consistent with at least one division vs. $2.7 \pm 0.6\%$, respectively. Within the OVA-reactive population of OVA-TCR transgenic T cells, $53.9 \pm 3.9\%$ of cells demonstrated a CFSE profile consistent with at least one division in CD8 α^+ DC immunized mice vs. $67.7 \pm 3.0\%$ of cells in the CD8 α^- DC immunized group. No T cell proliferation was observed in the control mice injected with unpulsed DCs.

As both CD8 α^- and CD8 α^+ DCs can induce the proliferation of T cells, we next looked at the profile of cytokines produced by T cells from MLN of mice instilled with one or the other subset of DCs. In mice instilled with CD8 α^- DCs, T cells obtained from MLNs produced higher levels of the Th2 cytokines IL-4, IL-5 and IL-10 compared with mice receiving CD8 α^+ DCs (Fig. 3). The levels of Th2 cytokines induced by OVA-pulsed CD8 α^+ were, however, higher than those induced by unpulsed DCs, illustrating some degree of Th2 cytokine priming by this subset. The amounts of IFN- γ were not significantly different in both groups, but still higher compared with unpulsed DC immunized mice. These data suggest that CD8 α^- DCs are able to prime T cells for a both Th1 and Th2 cytokine production, with a predominance of Th2 cytokines being induced, whereas CD8 α^+ DCs primed predominantly for Th1 cytokine production with a minor Th2 component.

CD8 α^- dendritic cells induce a stronger eosinophilia in the bronchoalveolar lavage compared with CD8 α^+ dendritic cells

As CD8 α^+ and CD8 α^- DCs can prime T cells for predominantly Th1- and Th2-like responses, respectively,

we next looked at the effect of the two subsets of DCs on the development of eosinophilic airway inflammation. In mice immunized with CD8 α^- DCs pulsed with OVA, and exposed to OVA aerosols (CD8 α^- /OVA), an inflammatory response was observed as confirmed by the higher number of lymphocytes and eosinophils in the BAL as compared with mice immunized with unpulsed DCs and exposed to OVA aerosols (Fig. 4). On the contrary, in mice injected with OVA-pulsed CD8 α^+ DCs and exposed to OVA aerosols, the number of inflammatory cells, namely eosinophils and lymphocytes, was much lower compared with mice injected with OVA-pulsed CD8 α^- DCs. However, the number of airway eosinophils was increased as compared with mice immunized with unpulsed DCs.

We next looked at the production of Th1/Th2 cytokines by T cells obtained from MLNs of mice immunized with CD8 α^- or CD8 α^+ DCs and challenged with OVA aerosols. In mice immunized with OVA-pulsed CD8 α^- DCs, the levels of IL-4, IL-5, IL-10 and IFN- γ were increased as compared with mice receiving unpulsed DCs. In mice immunized with OVA-pulsed CD8 α^+ DCs, the levels of IL-4 and IL-5 were significantly lower compared with mice that received CD8 α^- DCs, but still higher than mice immunized with unpulsed DCs (Fig. 5). The levels of IFN- γ in mice that received OVA-pulsed CD8 α^- or CD8 α^+ DCs were not significantly different, but higher than mice receiving unpulsed DCs of either subset.

Discussion

In this paper, we demonstrate that DC subsets in the mouse, identified by their respective absence or presence of the marker CD8 α , have differential capacity to induce Th2 immune responses to inhaled antigen and differential capacity to prime for eosinophilic airway inflammation in the Th2-prone milieu of the lung.

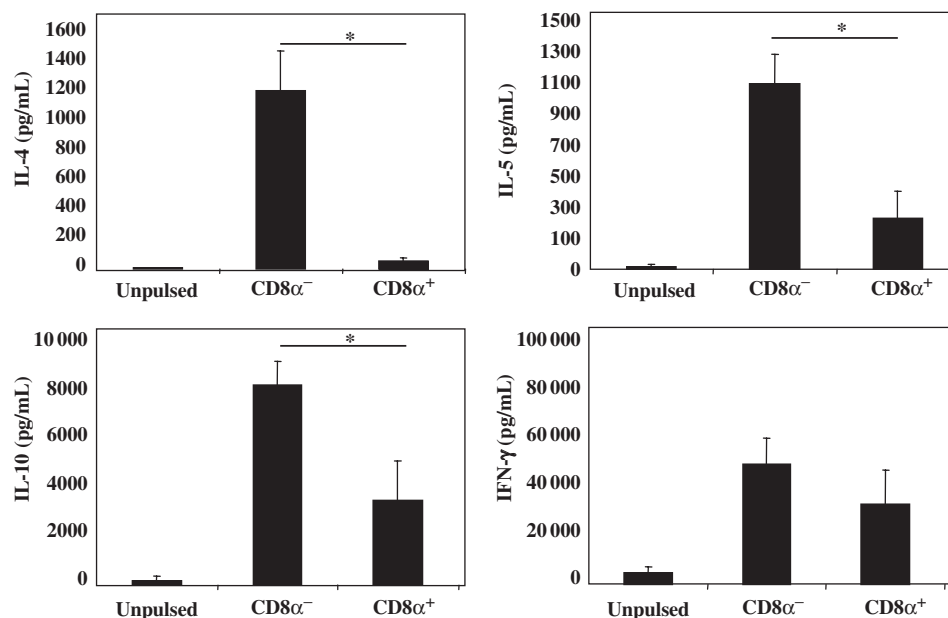


Fig. 3. CD8 α^- dendritic cells (DCs) and CD8 α^+ DCs induce a different cytokine response in primed T cells. On day 2, Balb/c mice received a cohort of carboxyfluorescein diacetate succinimide ester-labelled ovalbumin (OVA)-specific naïve T cells. On day 0, mice were administered intratracheally with CD8 α^- DCs and CD8 α^+ DCs pulsed with OVA or with unpulsed DCs. Four days later, T cells from mediastinal lymph nodes were collected and incubated for another 4-day period at 37 °C in the absence of OVA. Supernatants were harvested and assayed for the presence of IL-4, IL-5, IL-10 and IFN- γ . Results are represented as mean SEM from 10 mice per group.

It has been shown by us and others that splenic DC subsets in the mouse have an inherent capacity to promote either Th1 or Th2 responses following footpad injection because of the selective production of polarizing cytokines (IFN- γ , IL-12 favouring Th1 and IL-10 favouring Th2) by DC subsets [10, 12, 19, 21, 22]. However, the route of footpad injection is rather non-physiological, as the skin barrier is breached by the injection with an intradermal needle. In the present study, DCs were instilled into the lung of anaesthetized animals through a minimally invasive procedure, avoiding the breaching of any barriers. Bone-marrow-derived DCs instilled into the airways have been shown to reach the draining MLNs where they interact with naïve T cells and prime for the development of an effector T cell response with

inflammatory potential [2, 23, 24]. This same model was used here to address the question whether DC subsets have the potential to migrate to the draining nodes across the pulmonary epithelial or alveolar barrier. To our surprise, we observed that CD8 α ⁻ DCs were able to reach the draining MLNs by 24 h after injection, whereas CD8 α ⁺ DCs were clearly inactive in this respect. Even after prolonged observation times (72 h), CD8 α ⁺ DCs did not reach the draining lymph nodes (data not shown). One possible explanation for the failure of CD8 α ⁺ DCs to migrate to the lymph nodes might be the presence of a signal that leads to retention of these cells in the alveolar compartment. We noticed that CD8 α ⁺ DCs expressed higher levels of the α E β 7 integrin CD103, known as a retention signal for T cells in the alveolar compartment by interaction with E-cadherin (data not shown and [25]). We have not addressed the expression of other factors known to be involved in DC migration to lymph nodes such as the expression of the chemokine receptor CCR7 by subsets of DCs to offer an explanation for the differential migratory capacity [26]. Future studies will address whether migration of CD8 α ⁺ DCs can be stimulated by stimulating DC maturation with bacterial 'danger' motifs such as LPS or BCG.

We next studied direct T cell priming by OVA-pulsed DC subsets in mice adoptively transferred with CFSE-labelled, OVA-specific T cells. Despite the lack of migratory capacity of CD8 α ⁺ DCs, we noticed that OVA-specific T cells were induced to proliferate by both subsets of DCs and that the overall difference in primary T cell activation by DC subsets was rather quantitative than qualitative. Indeed, individual T cells had undergone up to six divisions in the draining nodes of mice immunized with both DC subsets, but the overall size of the pool of responding T cells (i.e. the number of cells induced to proliferate) was smaller in CD8 α ⁺ DC immunized mice. The most striking difference, however, was in the type of cytokine response induced in the MLNs in OVA-specific T cells. Although CD8 α ⁻ DCs primed for both enhanced IL-4, IL-5, IL-10 and IFN- γ production, the CD8 α ⁺ DCs primed predominantly for IFN- γ production and weaker levels of

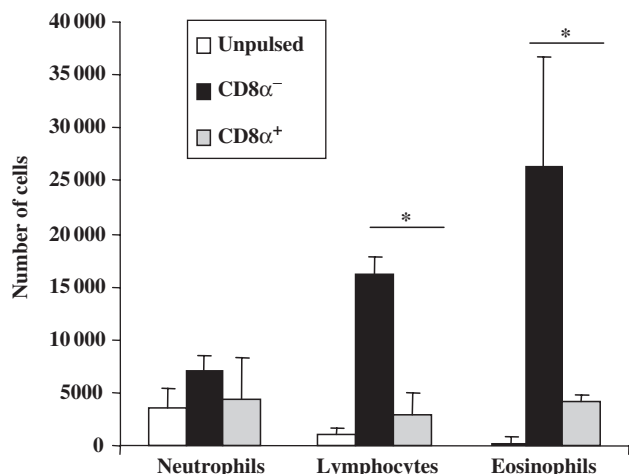


Fig. 4. Effect of CD8 α ⁻ dendritic cells (DCs) and CD8 α ⁺ DCs on the cellular composition of the bronchoalveolar lavage (BAL). On day 0, mice received an intratracheal injection of CD8 α ⁻ DCs and CD8 α ⁺ DCs pulsed with ovalbumin (OVA) or unpulsed DCs. From days 10–13, mice were exposed to OVA aerosols. Twenty-four hours after the last aerosol, BAL was performed. Cells were stained and analysed by flow cytometry for the presence of macrophages (MHCII⁺ CD11c⁺), eosinophils (CCR3⁺), neutrophils (CCR3⁻) and lymphocytes (CD3⁺ B220⁺). Results are expressed as mean $\pm$ SEM from 8–10 mice per group.

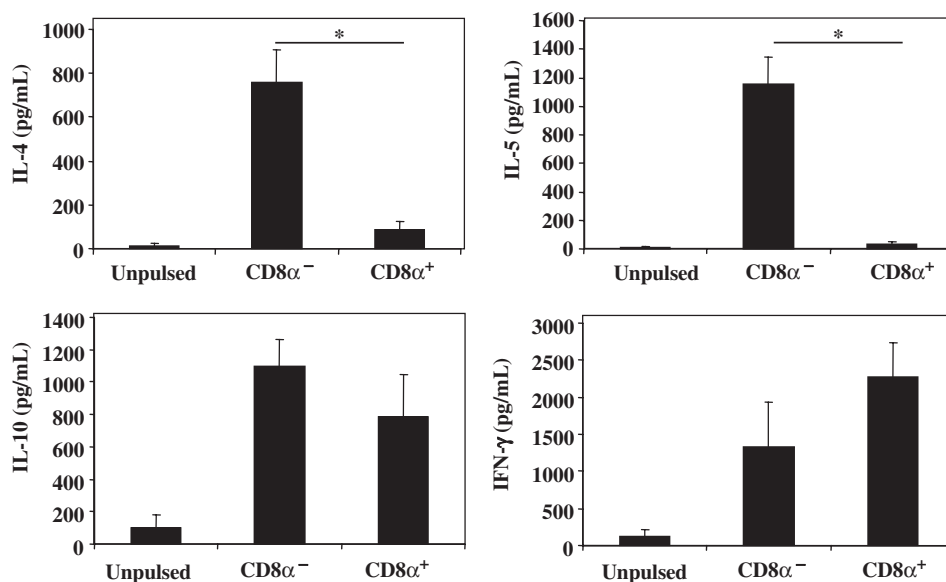


Fig. 5. CD8 α ⁻ dendritic cells (DCs) and CD8 α ⁺ DCs induce a different cytokine response in mediastinal lymph node (MLN) T cells following exposure of the mice to ovalbumin (OVA) aerosols. On day 0, mice received an intratracheal injection of CD8 α ⁻ DCs and CD8 α ⁺ DCs pulsed with OVA or unpulsed DCs. From days 10–13, mice were exposed to OVA aerosols. Twenty-four hours after the last aerosol, cells from MLNs were collected and restimulated for 4 days in the presence of OVA. The supernatants were harvested and assayed for the presence of IL-4, IL-5, IL-10 and IFN- γ . Results show the mean $\pm$ SEM from 10 mice per group.

Th2 cytokines *ex vivo*, reflecting a polarization towards enhanced Th2 responses by CD8 α^- DCs (ratio of IL-5 to IFN- γ of 0.87) and a Th1 polarization by CD8 α^+ DCs (ratio of IL-5 to IFN- γ of 0.01). The reason as to why CD8 α^+ DCs predominantly prime for Th1 responses while inducing a weak Th2 response has been the focus of intense study, and is related to the capacity of these cells to produce IL-12. Indeed, we previously reported that the capacity of CD8 α^+ DCs to direct Th1 differentiation is strictly dependent on IL-12 [27]. More recently, we have transfected myeloid DCs with a retroviral construct encoding bioactive IL-12 and observed that the Th2 response was turned into a Th1 response in the Th2-prone milieu of the lung [28]. However, we cannot exclude that the observation of reduced Th2 priming by the CD8 α^+ subset of DCs could also be because of the reduced migration of DCs to the draining nodes, and thus to an effect of antigen dose or APC/T cell ratio on Th cell proliferation. It is unlikely, however, that reduced antigen dose or APC/T cell ratio would lead to enhanced Th1 responses, as an opposite effect on Th polarization has been described *in vitro* and *in vivo* [14, 17]. It will similarly be interesting to study whether Th2 priming by CD8 α^+ DCs can be enhanced in the pulmonary milieu when the threshold for priming is lowered. This has been shown to occur when GM-CSF is over-expressed in the lungs by adenoviral transfection or when mice lungs are infected with influenza virus [29, 30].

The fact that T cell proliferation occurred in the absence of DC migration to the draining nodes is not without precedent. Indeed, elegant studies by Smith et al. [31] in which adoptively transferred DCs were the only cells that expressed the correct MHCII for presentation to CD4 $^+$ TCR transgenic T cells, demonstrated that antigen-peptide pulsed CD8 α^+ cells injected subcutaneously into the footpad also induced a primary T cell response without migrating to the skin draining nodes. What then might be the vehicle of immunity in the absence of DC migration to the nodes? It has been shown that preformed peptide-MHCII complexes can be transferred from one DC to another (migratory) APC, allowing the transfer of an MHC-restricted antigenic stimulus in the absence of cellular migration [32]. The vehicle of this transfer of antigenic material between non-migratory DCs and endogenous DCs is unclear at present but could involve transport by exosomes. Exosomes can be secreted by DCs and concentrate antigen-loaded peptide-MHCII complexes, with a potential to prime for CD4 T cell responses through uptake by endogenous DCs [33]. We have preliminary data to suggest that exosomes obtained from OVA-pulsed DCs and transferred into the trachea of naïve mice indeed induce primary T cell division in naïve OVA-specific T cells in the MLNs (unpublished observations). It remains to be determined, however, if the induction of T cell division by CD8 α^+ DCs in the absence of DC migration is indeed because of secretion of exosomes that are subsequently drained to the MLNs. It is less likely that intact apoptotic CD8 α^+ DCs be taken up by endogenous DCs for presentation to naïve T cells, as this would lead to fluorescein-positive endogenous DCs in the draining nodes [34].

When mice immunized with OVA-pulsed CD8 α^+ and CD8 α^- DCs were next challenged with OVA aerosol, only the CD8 α^- subset was able to prime for strong eosinophilic airway inflammation, also noticed in asthma. This was clearly

reflected by the levels of IL-4 and IL-5 in the draining nodes, which was much higher in CD8 α^- DC immunized mice compared with CD8 α^+ DCs. IL-5 is indeed essential for the growth and differentiation of tissue eosinophils, and IL-4 has been shown to have pro-inflammatory capacities by up-regulating the expression of cell adhesion molecules for inflammatory cells on the vessel wall. These data are in agreement with our previous data showing that bone-marrow-derived myeloid DCs, most closely resembling the CD8 α^- splenic subset, are able to prime for CD4 $^+$ Th2-dependent eosinophilic airway inflammation [2]. Other findings support the idea that CD8 α^+ DCs are poor in inducing sensitization to inhaled antigen and subsequent eosinophilic inflammation. These data are obtained in a mouse model of alum-driven sensitization to OVA, in which treatment of mice with Flt3L to expand endogenous CD8 α^+ DCs during sensitization was shown to inhibit all features of asthma [35]. Therefore the balance between the number of CD8 α^+ and CD8 α^- DCs within a tissue might determine the outcome of priming in that tissue. It was previously shown that the milieu of the lung is Th2 prone [7, 8]. We and others have shown that there are virtually no CD8 α^+ DCs in the periphery of the lung [5, 24, 36, 37]. Together with the present data, a possible explanation for the Th2-prone milieu of the lung could be the fact that there is a majority of CD8 α^- -like DCs in the lung that preferentially induce Th2 responses to protein antigens under baseline conditions. The fact that the CD8 α^+ DCs induced a weak Th2-skewed response with high levels of IFN- γ argues against the role for a factor within the pulmonary environment (e.g. secreted by epithelial cells) that reduces the Th1-promoting capacity of DCs.

In humans, DC subsets have also been defined *in vitro* as being monocyte derived or as being plasmacytoid, each with differential potential to prime for Th1 or Th2 responses, respectively. Here also, the conditions of stimulation, the antigen dose, the APC to T cell ratio and the time-point after stimulation seem to overrule the inherent capacity of DC lineage to determine Th polarization [15–18]. We have already demonstrated in a humanized SCID model of asthma that monocyte-derived DCs injected into the trachea induce a predominant Th2 response in the lung when DCs are derived from allergic donors, whereas mo-DCs from non-allergic donors induce Th1 responses in the lung, again demonstrating that the pulmonary environment is not pro-Th2 *per se*, but that the characteristics of the DC determines which type of response is being induced [26].

In conclusion, we have demonstrated that subsets of mouse DCs have a differential capacity to prime for Th2-dependent eosinophilic airway inflammation in the Th2 milieu of the lung.

Acknowledgements

Hamida Hammad is supported by a Marie Curie European Community grant. Victor de Vries is supported by a grant from the Dutch Asthma Foundation. Bart Lambrecht is supported by grants of the Dutch Asthma Foundation and the NWO, the Netherlands.

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