

Sensitization by intratracheally injected dendritic cells is independent of antigen presentation by host antigen-presenting cells

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Abstract: Adoptive transfer of antigen-pulsed dendritic cells (DC) in the airways of mice has been used as a model system for eosinophilic airway inflammation, which allows studying the DC-specific contribution of genes of interest or reagents to induced inflammation by genetically modifying DC or exposure of DC to compounds prior to injection in the airways. Antigen transfer and CD4⁺ T cell priming by endogenous APCs may interfere with the correct interpretation of the data obtained in this model, however. We therefore examined antigen transfer and indirect CD4⁺ T cell priming by host APCs in this model system. Transfer of antigen between injected DC and host cells appeared to be minimal but could not be totally excluded. However, only direct antigen presentation by injected DC resulted in robust CD4⁺ T cell priming and eosinophilic airway inflammation. Thus, this adoptive transfer model is well suited to study the role of DC in eosinophilic airway inflammation. *J. Leukoc. Biol.* 85: 000–000; 2009.

Key Words: eosinophilic airway inflammation · CIITA · antigen transfer · CD4⁺ T cells

INTRODUCTION

Adoptive transfer of phenotypically (e.g., exposed to certain cytokines) or genotypically (overexpression or deletion of genes) modified dendritic cells (DC) is a powerful approach to study the role of genes of interest in DC function in vivo. This strategy can also be applied to tailor an adaptive immune response in a desired way for immunotherapeutic purposes. Adoptive transfer of DC in the airways has been used to establish a murine model of eosinophilic airway inflammation, as the DC are responsible for inducing Th2 sensitization to inhaled antigen by priming Th2 responses [1, 2]. This model system was subsequently used to study CD4⁺ T cell division and migration in the lung-draining lymph nodes and airways [3, 4], to analyze the effect of modulating compound exposure of DC on eosinophilic airway inflammation [5–7], and to study if CCR7 is required for DC migration from the lung to the lymph nodes [8]. In addition, intratracheal (i.t.) injection of DC overexpressing IL-12 has been shown to be able to prevent eosinophilic airway inflammation [9]. Some investigators have

also used adoptive intrapulmonary transfer of wild-type (WT) antigen-pulsed DC to restore a lung phenotype of gene-deficient animals to prove that a particular gene of interest exerts its function through modulation of lung DC function [10].

However, to correctly interpret the data from the studies mentioned above and to be able to use DC for immunotherapeutic purposes, it is important that antigen is not transferred between injected DC and endogenous host DC in the lung. Several studies have shown that transfer of cellular material and peptide-MHC class II (MHCII) complexes between transferred DC and host DC occurred in vivo in other model systems [11–15], but in these experimental setups, the natural barriers were breached. Harris et al. [4] have briefly looked upon the requirement for direct antigen presentation by i.t.-injected DC for CD4⁺ T cell priming and eosinophilic airway inflammation; however, in their model system, transfer of cellular material between DC and antigen transfer in the lymph nodes could not be examined as a result of the use of peptide-pulsed, nonviable DC.

In this study, we investigated the occurrence of antigen transfer from i.t.-injected, exogenous DC to resident host APC in more detail and the consequences of this phenomenon on CD4⁺ T cell priming and development of eosinophilic airway inflammation in a DC-mediated mouse model of eosinophilic airway inflammation.

MATERIALS AND METHODS

Mice

B6.SJL-*Ptprca*^a *Pep3*^b/BoyJ mice congenic for the *Ptprca*^a allele (CD45.1/Ly5.1) and *c2ta*-deficient mice [class II transactivator knockout (CIITA KO)] were obtained from Jackson Laboratories (Bar Harbor, ME, USA). TCR transgenic mice harboring CD4⁺ T cells restricted specifically for OVA_{323–339} presented by I-A^b on a C57Bl/6 background (OT-2) were bred in-house. C57Bl/6 mice (WT) were purchased from Harlan (Horst, The Netherlands). Mice were housed

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in microisolators under specified pathogen-free conditions, and experiments were performed under approval of the Erasmus MC Committee for Animal Ethics (Rotterdam, The Netherlands).

Generation and i.t. injection of bone marrow-derived DC (BM-DC)

BM-DC were generated as described [16]. Briefly, after RBC lysis, BM cells were resuspended at 2×10^5 /ml in DC culture medium (DC-CM), RPMI 1640 containing glutamax-I (Invitrogen, Carlsbad, CA, USA), supplemented with 5% (v/v) FCS (Biocell, Rancho Dominguez, CA, USA), 50 μ M β -ME (Sigma, Zwijndrecht, The Netherlands), 50 μ g/ml gentamycin (Invitrogen), and 20 ng/ml recombinant murine (rm)GM-CSF (a kind gift from Prof. Kris Thielemans, The Vrije Universiteit, Brussels, Belgium). Cells (2×10^6) were seeded in tissue-culture-grade, 100 mm Petri dishes (Day 0). At Day 3, 10 ml fresh DC-CM was added. On Days 6 and 8, 10 ml each plate was centrifuged and resuspended in 10 ml fresh DC-CM. At Day 9, cells were pulsed with 100 μ g/ml OVA (Worthington Biochemical Corp., Lakewood, NJ, USA). Twenty-four hours later, mature DC were harvested by gentle pipetting. For injection, harvested DC were washed twice with PBS, and up to 2×10^6 DC were injected i.t. in anesthetized mice in a volume of 80 μ l PBS using the technique of Ho and Furst [17].

DC tracking experiments

BM-DC were generated from BM of CD45.1 mice, labeled with CFSE as described [5], and injected i.t. Thirty-six hours later, mediastinal and axillary lymph nodes were isolated and digested for 60 min at 37°C in digestion mixture (Collagenase type II, 1 mg/ml, Worthington Biochemical Corp.; DNase-I, 2 U/ml, Sigma). After staining with appropriate antibodies, the transfer of the CFSE signal from the CD45.1 donor DC to CD45.1-negative endogenous cells was analyzed using flow cytometry. To analyze transfer of MHCII molecules in vivo, BM-DC were generated from I-A^b-enhanced green fluorescent protein (EGFP) mice BM [18] (crossed back on a CD45.2⁺ C57Bl/6 background). BM was a gift from Ron Germain and Anne-Marie Lennon. BM-DC (2×10^6) were i.t.-injected in CD45.1 mice, and 36 h after injection, the mediastinal and axillary lymph nodes were isolated. I-A^b-EGFP transfer was assessed by confocal microscopy and flow cytometry.

T cell activation in vivo

DC were generated from CIITA KO mice and WT mice BM, pulsed with OVA protein, and injected i.t. into WT or CIITA KO mice as described above. For experiments examining direct antigen presentation by injected DC, 12×10^6 pooled lymphocytes and splenocytes isolated from OT-2 mice were labeled with CFSE as described [5] and adoptively transferred i.v. into these mice 48 h before DC injection. To exclude antigen presentation by MHCII⁺ cells present in the transferred population in the experiments analyzing the contribution of endogenous DC, CD4⁺ T cells were isolated by negative magnetic selection using the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany; purity routinely >95%). Next, the CD4⁺ T cells were CFSE-labeled, and 3×10^6 cells were injected i.v. 48 h before DC injection. Four days later, mediastinal lymph nodes were dissected, and single-cell suspension was analyzed for OT-2 T cell division by flow cytometry.

Confocal microscopy

Lymph nodes were isolated and snap-frozen in liquid nitrogen, and 6 μ m cryosections were prepared. Sections were fixed in acetone (10 min, room temperature), blocked with 10% FCS, and incubated with biotinylated anti-CD45.2 (eBioscience, San Diego, CA, USA) for 1 h, followed by a 1-h incubation with Streptavidin-quantum dot 655 (Qdot655; Invitrogen). Image acquisition was carried out with a LSM 510 confocal microscope (Zeiss, Göttingen, Germany) equipped with 488 nm and 633 nm lasers. Images were collected and analyzed using Imaris software (Bitplane, Zurich, Switzerland).

Flow cytometry and cell division analysis

Anti-Fc γ RII/III antibody (2.4G2, American Type Culture Collection, Manassas, VA, USA) was included in all cell-surface stainings to reduce nonspecific antibody binding. Dead cells were excluded by labeling with 7-amino-actinomycin D (7-AAD; BD Biosciences, Erembodegem, Belgium), propidium iodide (PI; Sigma), or 4',6-diamidino-2-phenylindole (DAPI; Invitrogen). To detect CFSE- and EGFP-labeled I-A^b transfer from exogenous DC to endogenous DC,

lymph node cell suspensions were labeled with anti-CD45.1-PE and anti-CD11c-allophycocyanin. For I-A^b EGFP transfer experiments, anti-CD45.2-allophycocyanin-Cy7 was included in the staining panel.

OT-2 CD4⁺ T cells were detected by staining with anti-V α 2-biotin, followed by streptavidin-allophycocyanin-Cy7 or streptavidin-allophycocyanin, in combination with anti-V β 5-PE staining. When streptavidin-allophycocyanin was not used, anti-CD4-allophycocyanin was included in the staining. For quantification of T cell division, algorithms provided by FlowJo software (Treestar, Ashland, OR, USA) were applied to CFSE dilution profiles as described [5]. The resulting proliferation index is the average number of cell divisions of the total dividing CFSE-positive cell population. To determine the cellular composition in bronchoalveolar lavage fluid (BALF), cells were stained with anti-MHCII-FITC, anti-CCR3-PE [19], anti-CD3-PE, and anti-CD19-PE. All fluorochrome-conjugated antibodies were purchased from BD Biosciences or eBioscience, except anti-CCR3-PE, which was from R&D Systems (Minneapolis, MN, USA). Events were acquired on a FACSCalibur or FACSARIA flow cytometer (BD Biosciences) and analyzed with FlowJo.

Eosinophilic airway inflammation

Groups of mice ($n=5-8$ per group) were immunized i.t. on Day 0 with 1×10^6 WT DC or CIITA KO DC, pulsed overnight with OVA (Worthington Biochemical Corp.). From Day 10 onward, mice were exposed to OVA aerosols [1% (w/v) in PBS generated through jet nebulizers] for 3-4 consecutive days, 30 min daily, as described previously [1]. Twenty-four hours after the last aerosol exposure, mice were killed, BAL was performed, and mediastinal lymph nodes were isolated.

Lymph node cultures

Single-cell suspensions of lymph nodes were prepared by mechanical disruption, and cell suspensions (2×10^6 cells/ml) were restimulated with 10 μ g/ml OVA protein (Worthington Biochemical Corp.) for 96 h, after which, supernatants were harvested and assayed for cytokine content. Levels of IL-4, IL-5, IL-10, and IFN- γ were measured using an OptEIA kit (BD Biosciences) according to the manufacturer's instructions. IL-13 levels were measured using a commercially available kit from R&D Systems.

Statistical analysis

Reported values are expressed as mean $\pm$ SEM, unless indicated otherwise. Statistical analyses were performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA) using one-way ANOVA followed by Tukey-Kramer post-testing. *P* values less than 0.05 were considered significant.

RESULTS

Minimal transfer of cellular material from injected DC to host cells

To determine whether cellular material derived from DC injected in the airways is present in endogenous cells of the draining lymph nodes of the lung, we labeled OVA-pulsed BM-DC with CFSE, injected these into the airways of naïve mice, and studied the distribution of the CFSE label in the mediastinal lymph node. To discriminate between i.t.-injected, exogenous DC and host cells (expressing CD45.2), the exogenous DC were derived from BM of CD45.1 congenic mice. As shown in **Figure 1A**, after 36 h, the CFSE label could be detected merely in injected DC (CD45.1-positive) in draining lymph nodes of the lung ("mediastinal LN" dot plot) but not in peripheral lymph nodes ("axillary LN" dot plot). We could not detect any endogenous cells (CD45.1-negative) with similar CFSE-staining intensity. However, some CD45.1-negative cells exhibited a small increase in CFSE fluorescence (Fig. 1A, arrow). To determine whether these host cells were DC, we next analyzed CFSE dye transfer between exogenous DC and host DC, through inclusion of the DC marker CD11c in the analysis

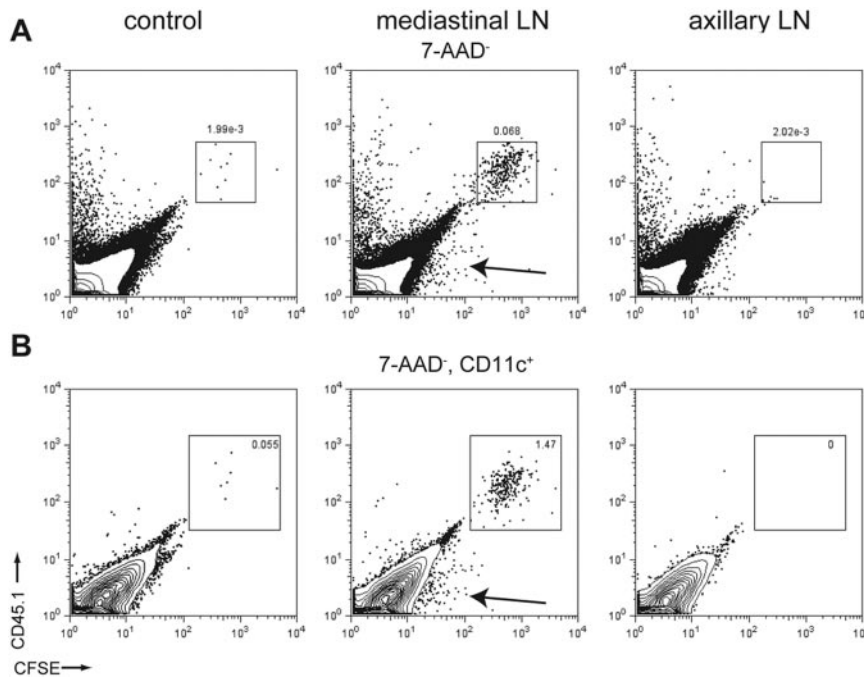


Fig. 1. Transfer of cell fragments from exogenous DC to host cells is minimal. BM-DC (2×10^6) derived from CD45.1 donor mice were OVA-pulsed, CFSE-labeled, and i.t.-injected into the airways of C57Bl/6 (CD45.2) recipient mice ($n=8$). After 36 h, mediastinal lymph nodes (LN) were isolated, stained, and analyzed by flow cytometry. (A) CFSE-labeled cell population gated on 7-AAD⁻ cells. (B) CFSE-labeled cell population gated on 7-AAD⁻ CD11c⁺ cells. The plot labeled “control” depicts mediastinal lymph node populations from an untreated mouse. Between 350,000 and 500,000 events were acquired.

gate (Fig. 1B). We observed a similar phenomenon compared with analysis of total alive cells, and a small subset of DC appeared to have taken up CFSE-labeled cell fragments (Fig. 1B, arrow). This suggests that transfer of CFSE-labeled cell fragments from injected DC to host cells, including DC, was minimal but could not be excluded totally.

No detectable transfer of MHCII peptide complexes between injected and host cell populations

Another possibility that could lead to indirect presentation of antigen by host DC is transfer of peptide-loaded MHCII molecules from injected DC to host DC, which has been shown previously [14, 15, 20–22]. To detect a possible occurrence of this phenomenon in our model system, we transferred DC derived from CD45.2-positive I-A^b EGFP mice [18], in which EGFP is fused to the cytoplasmic tail of I-A^b into the airways of congenic CD45.1 recipient mice. Thirty-six hours later, the draining mediastinal lymph nodes were isolated and analyzed for segregation of EGFP and CD45.2 signal. Flow cytometric analysis revealed a significant proportion of cells in the GFP⁺-CD45.1⁺ gate, indicating transfer of GFP-tagged MHCII molecules from exogenous DC (CD45.2) to host cells (CD45.1; **Fig. 2A**, Gate 1, left panel). However, closer examination showed that the majority of cells in this gate simultaneously expressed high levels of CD45.2 (Fig. 2A, Gate 1, solid line, middle panel), indicating cotransfer of the CD45.2 marker or most likely, cell clusters containing CD45.1-positive host cells and significant numbers of injected DC.

To circumvent this potential FACS-associated artifact, we resorted to confocal imaging of cryosections of mediastinal lymph nodes, thereby preserving the spatial location of cells in vivo. EGFP-tagged DC could be readily observed in lymph node sections of mice injected i.t. with I-A^b EGFP-derived DC (Fig. 2B). Costaining with antibody against CD45.2 to identify

injected cells revealed transfer of I-A^b EGFP molecules to some host (CD45.2-negative) cells (Fig. 2B, arrowheads). We concluded that in our model system, transfer of cellular material and I-A^b molecules from injected DC to recipient cells appears to be restricted or below the limit of detection of the techniques applied here.

CD4⁺ T cell priming is largely dependent on i.t.-injected DC

As the results of the experiments were not entirely conclusive and still showed some transfer of cellular material and MHCII-peptide complexes to host cells, we addressed the possible occurrence of antigen transfer in an alternative manner.

Antigen transfer between injected DC and host APC can be determined indirectly via T cell activation in a highly sensitive manner [23, 24], provided that only host APC can present antigen. To that end, we analyzed priming of OVA-specific CD4⁺ T cells after i.t.-injection of CIITA KO BM-DC, which do not express MHCII molecules on their cell surface ([25, 26], and data not shown) or C57Bl/6 (WT) BM-DC as a control. As shown in **Figure 3**, i.t. immunization with OVA-pulsed WT DC results in strong CD4⁺ T cell priming, as judged by T cell division at Day 4 in the draining lymph nodes of the lung. In contrast, immunization with CIITA KO DC resulted in a weak proliferative response (Fig. 3, lower panel). However, it could not be totally excluded that other differences in genotype of CIITA KO DC were responsible for the priming defect. In an effort to directly assess the contribution of endogenous DC to T cell priming in this model, we analyzed CD4⁺ T cell priming in the absence of host APC. We injected OVA-pulsed WT DC in the airways of WT mice or CIITA KO mice, which received a cohort of OVA-specific CD4⁺ T cells. To avoid that saturating numbers of injected DC mask any contribution of host APC to the priming of CD4⁺ T cells, we used a dose-response approach and examined T cell priming after i.t. injection of

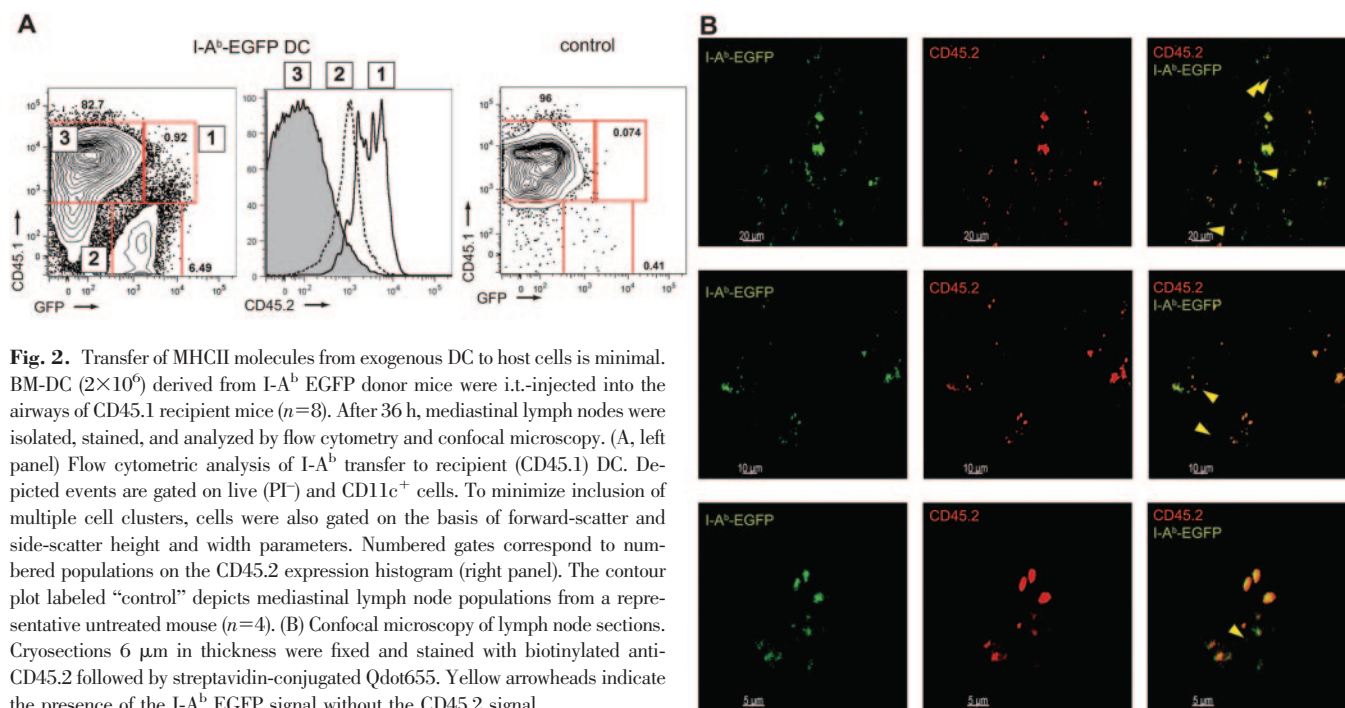


Fig. 2. Transfer of MHCII molecules from exogenous DC to host cells is minimal. BM-DC (2×10^6) derived from I-A^b EGFP donor mice were i.t.-injected into the airways of CD45.1 recipient mice ($n=8$). After 36 h, mediastinal lymph nodes were isolated, stained, and analyzed by flow cytometry and confocal microscopy. (A, left panel) Flow cytometric analysis of I-A^b transfer to recipient (CD45.1) DC. Depicted events are gated on live (PI⁻) and CD11c⁺ cells. To minimize inclusion of multiple cell clusters, cells were also gated on the basis of forward-scatter and side-scatter height and width parameters. Numbered gates correspond to numbered populations on the CD45.2 expression histogram (right panel). The contour plot labeled “control” depicts mediastinal lymph node populations from a representative untreated mouse ($n=4$). (B) Confocal microscopy of lymph node sections. Cryosections 6 μ m in thickness were fixed and stained with biotinylated anti-CD45.2 followed by streptavidin-conjugated Qdot655. Yellow arrowheads indicate the presence of the I-A^b EGFP signal without the CD45.2 signal.

cohorts of decreasing numbers of DC. Host APC did have a minor contribution to T cell activation, as judged from the small decrease in cell OVA-specific T cell divisions in CIITA KO mice (**Fig. 4**). In addition, cell division was quantified via modeling of the CFSE division profile, resulting in the frequency of adoptively transferred CD4⁺ T cells that divided and the average number of cell divisions of this population (proliferation index). These quantitative data revealed a small but consistent decrease in the proliferation index in CIITA KO mice compared with WT mice, indicating a minor contribution of host APC to T cell priming. In CIITA KO mice, we also observed a correlation of the proliferation index with the number of WT DC injected, confirming the dominant role of injected DC in OVA-specific T cell activation. Paradoxically, the frequency of adoptively transferred T cells that did divide was higher in CIITA KO mice compared with WT mice at lower numbers of injected DC. This is probably a result of homeostatic proliferation of transferred cells in lymphopenic CIITA KO mice, which is more pronounced when the OVA-specific CD4⁺ T cells are not activated by antigen (**Table 1**).

In summary, these results show collectively that to a large extent, T cell priming is dependent on transferred DC.

Direct antigen presentation by i.t.-injected DC is required to generate effector responses in a DC-mediated murine model of eosinophilic airway inflammation

In light of recent findings that T cell proliferation does not correlate with acquisition of bona fide T cell effector function [27], we decided to follow the fate of dividing T cells in mice immunized with WT DC and CIITA KO DC. To that end, we made use of an existing DC-mediated model of eosinophilic airway inflammation [1]. In this model, mice are immunized via i.t. injection of OVA-pulsed DC in the lower airways, followed by three consecutive local challenges with aerosolized OVA 10

days later. Airway inflammation was subsequently assessed through analysis of BAL cellular composition and cytokine production in lung-draining lymph nodes. Determination of the cellular composition of BALF revealed that OVA-pulsed WT DC induced eosinophilic airway inflammation, with increased numbers of eosinophils and lymphocytes present in the BAL. Mice immunized with OVA-pulsed CIITA KO DC did not develop inflammation, as the numbers of different cell types present in BAL were not increased compared with mice immunized with unpulsed DC (**Fig. 5A**). Inflammatory cytokine production in the mediastinal lymph nodes of immunized mice correlated with the BAL data, with significant production of IL-4, -5, -10, and -13 and IFN- γ in mice immunized with OVA-pulsed WT DC, which could not be observed in mice immunized with unpulsed DC or OVA-pulsed CIITA KO DC (**Fig. 5B**).

Altogether, in this mouse model, eosinophilic airway inflammation is decreased markedly when injected DC do not prime CD4⁺ T cells directly, indicating that antigen transfer from injected DC to host APC is not sufficient to generate effector T cell responses.

DISCUSSION

The purpose of this study was to analyze in detail the contribution of host APC to CD4⁺ T cell priming and acquisition of effector functions by ex vivo-generated DC administered in the airways of mice. Given the low number of injected DC that can be retrieved from lymph nodes compared with the relatively high numbers of DC administered and the capacity of immature DC to acquire antigen from its environment, it is highly likely that host DC take up antigen from injected DC. Direct examination of antigen transfer in our model system did not yield conclusive evidence, in contrast to several other reports that examined transfer of CFSE-labeled material to DC [11–13].

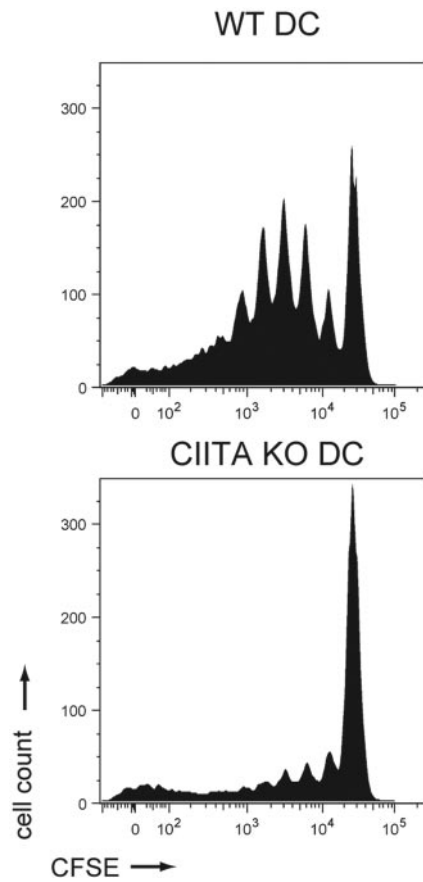


Fig. 3. Direct antigen presentation by exogenous DC is required for optimal CD4⁺ T cell activation. OVA-pulsed WT DC (1×10^6) or CIITA KO DC ($n=10$ per group) were i.t.-injected into C57BL/6 mice that had received a cohort of CFSE-labeled OT-2 T cells. Four days later, mediastinal lymph nodes were dissected, and OT-2 T cell division was analyzed by flow cytometry. To obtain sufficient events, lymph nodes of two mice were pooled. Cells were gated on DAPI⁻, CD4⁺, V α 2⁺, and V β 5⁺ cells. Priming with DC not exposed to OVA did not lead to OT-2 T cell proliferation (data not shown). The experiment shown is representative of four independent experiments.

We could also not conclusively demonstrate transfer of MHCII molecules from injected DC to host cells, a mechanism that has been detected previously in vivo [14, 15]. We did observe EGFP in the CD45.1 antibody-associated fluorescence channel, suggesting transfer of MHCII molecules to host cells, but this coassociated with the donor-derived marker (CD45.2) and therefore, was likely to represent complexes of donor and recipient cells, despite gating on single cells. Alternatively, transfer of plasma membrane fragments containing EGFP-tagged MHCII molecules and CD45.2 molecules might have occurred [22]. An important distinction between these reports and our findings that may shed light on the discrepancy is that in our model, no natural barriers are circumvented by i.p., i.v., or s.c. injection, which likely results in lower numbers of migrating DC in our model. Consequently, the chances of detection of transfer of CFSE-labeled fragments decreased.

In addition to direct analysis of antigen transfer, we investigated antigen transfer indirectly via CD4⁺ T cell activation. It has been shown previously that this particular route of DC administration results in antigen-specific CD4⁺ T cell activation in the draining lymph nodes [3, 4]. We detected limited T cell proliferation after

priming with antigen-loaded CIITA KO DC, suggesting that any antigen transferred to recipient APC is not capable of inducing efficient T cell priming or leads to deletion of reactive T cells, thereby invoking tolerance. It is likely that many administered DC die in the lung, given the low number of injected DC that can be retrieved from lymph nodes compared with the relatively high numbers of DC administered [5]. Liu and colleagues [13] have shown that injection of dying cells leads to CD8 T cell tolerance induction mediated by DC in vivo. This tolerance induction was characterized by a short burst of T cell proliferation in vivo,

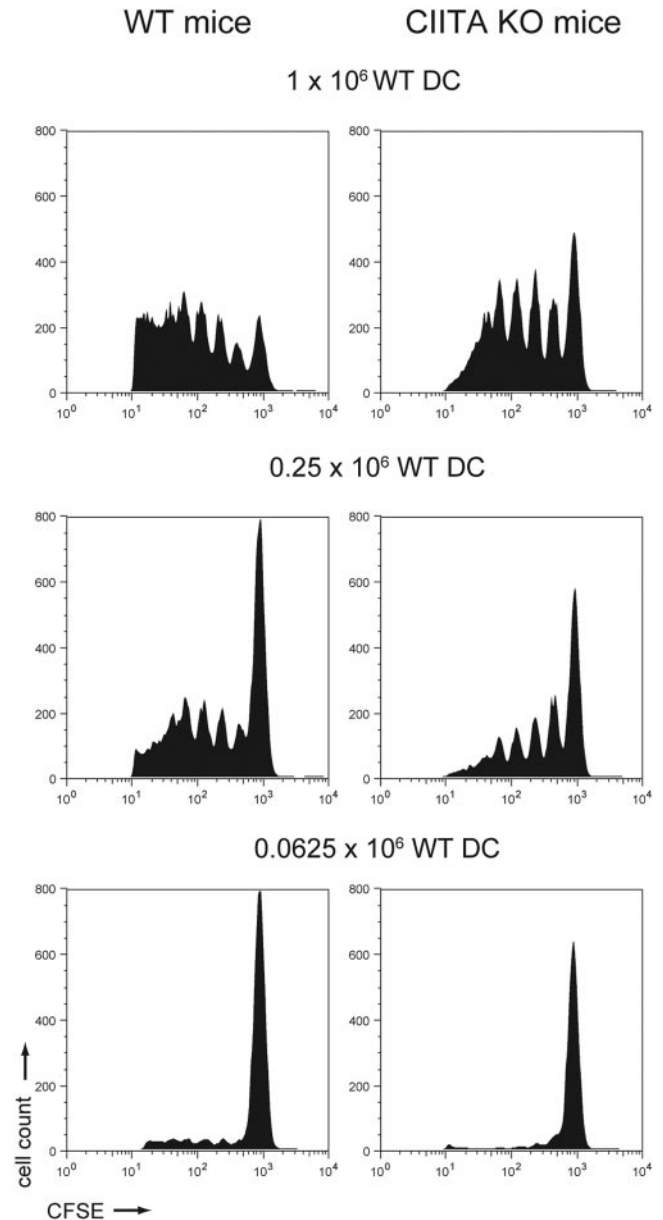


Fig. 4. Endogenous DC enhance the primary immune response but are not absolutely required for priming. Decreasing numbers of OVA-pulsed WT DC were i.t.-injected into WT or CIITA KO mice ($n=3$ per group) that had received a cohort of CFSE-labeled OT-2 T cells. Four days later, mediastinal lymph nodes were dissected, and OT-2 T cell division was analyzed by flow cytometry. Cells were gated on PI⁻, V α 2⁺, and V β 5⁺ cells. Endogenous T cells with an identical TCR repertoire but negative for CFSE staining were gated out. For display purposes, histogram plots shown are derived from electronically pooled data from mice of each group with data analysis software.

TABLE 1. Quantification of OVA-Specific CD4⁺ T Cell Proliferation in Mediastinal Lymph Nodes

WT DC-injected (cell number)	% Divided (%)		Proliferation index ^a	
	WT mice	CIITA KO mice	WT mice	CIITA KO mice
1×10^6	50.8 ± 1.2	47.6 ± 4.5	2.217 ± 0.052	1.890 ± 0.126
0.25×10^6	23.5 ± 3.6	29.7 ± 7.6	2.013 ± 0.017	1.513 ± 0.143
0.0625×10^6	4.1 ± 1.6	8.8 ± 1.1	2.037 ± 0.249	1.300 ± 0.055

^aDefined as the average number of cell divisions of the dividing V α 2⁺, VB5⁺, PI⁻ cell population. ^bValues represent the mean $\pm$ SEM of the data ($n = 3$ animals per group).

followed by deletion. This abortive T cell activation and subsequent deletion have been described for CD4⁺ T cells as well, in various model systems administering antigen under nonimmunogenic conditions, including the lungs [28–30]. However, in these reports, divided T cells were not yet deleted at the time-point examined in this study (4 days), making it unlikely that in our experimental system T cells progressed into T cell division and were deleted before Day 4 but rather represent an immunological, null event. Our findings are in agreement with previously published data, showing that s.c. injection of necrotic and apoptotic DC or transfer of ethanol-fixed DC into the airways does not lead to CD4⁺ T cell responses in vivo [4, 11]. Alternatively, if host DC acquire antigen from injected MHCII-deficient DC, it could be argued that T cell activation is delayed. In that case, we would have expected a secondary immune response in our model of allergic airway inflammation, which did not develop after immu-

nization with CIITA KO DC. The role of endogenous APC in CD4⁺ T cell priming was also addressed by injection of WT DC in CIITA-deficient mice or WT animals, showing the minor contribution of host-derived APC to CD4⁺ T cell priming in this animal model. It has been described recently that BM-derived CIITA KO DC produce more IL-10, which could inhibit T cell priming [26]. Quantification of IL-10 gene expression by quantitative PCR revealed a small, relative increase in IL-10 transcripts level compared with WT DC, but absolute expression levels of IL-10 mRNA were low in both DC types and unlikely to affect CD4⁺ T cell priming significantly (data not shown). These findings support the results of Kleindienst and Brocker [11], who also showed that immunization with antigen-loaded DC, not capable of direct antigen presentation, did not lead to efficient CD4⁺ T cell priming. However, in this study, endogenous DC did enhance the immune response when priming was induced with MHCII-positive

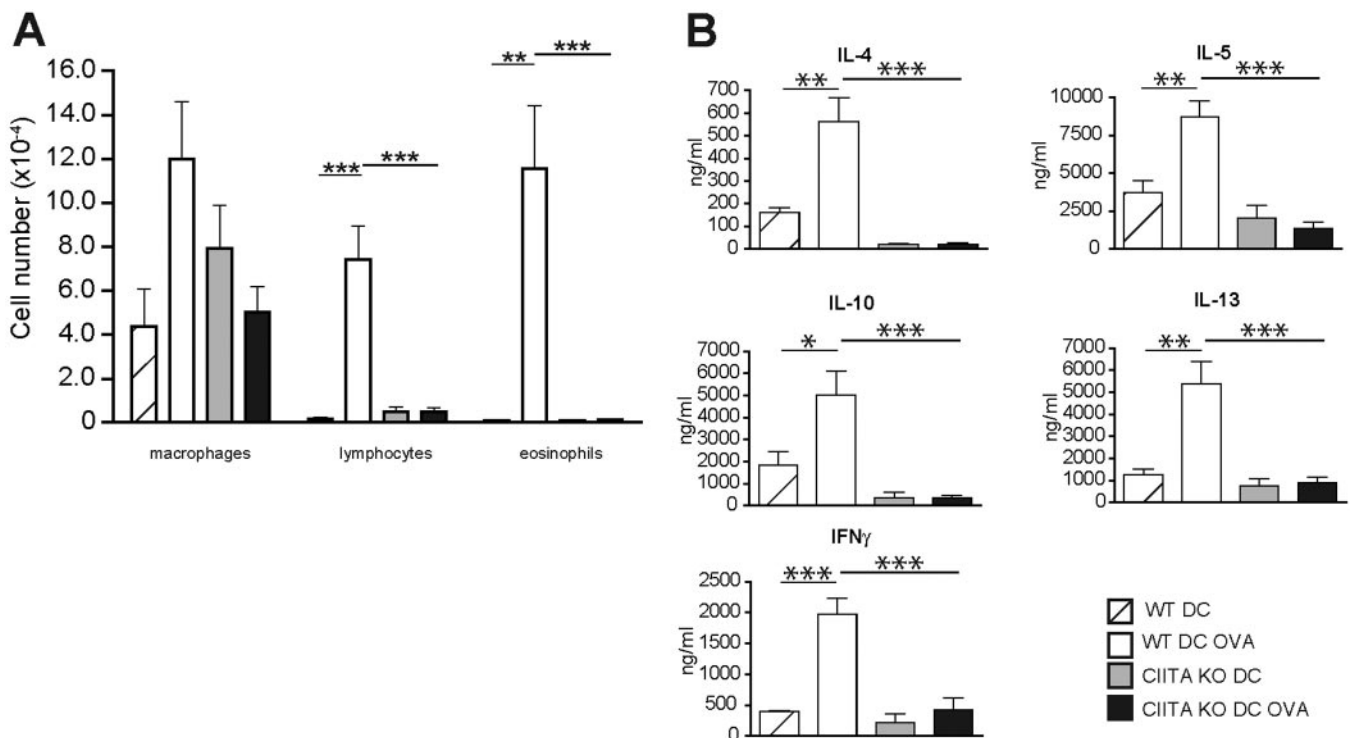


Fig. 5. CIITA KO DC fail to induce eosinophilic airway inflammation. On Day 0, groups of mice ($n=4-6$ per group) were immunized by i.t. administration of 1×10^6 WT DC or CIITA KO DC. On Days 10–13, mice were exposed to OVA aerosols for 30 min daily. At 24 h after the last exposure, mice were killed, and BAL and mediastinal lymph node cytokine production was analyzed as described in Materials and Methods. (A) Cellular composition of BALF. Alveolar macrophages were characterized by their light-scatter and autofluorescence properties. Eosinophils are defined by their CCR3⁺MHCII⁻ staining pattern. The lymphocyte cell fraction consists of CD3⁺ and CD19⁺ cells within the lymphocyte light-scatter region. (B) Cytokine production by mediastinal lymph nodes restimulated with OVA. Results are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

DC. We cannot fully explain this discrepancy with our findings, but their study differed with respect to route of administration (s.c.), DC dose, antigen loading, and model system, which might have led to increased antigen transfer. As a consequence, preventing antigen presentation by host DC in their experimental system could have a more dramatic impact on T cell priming. Furthermore, it has been demonstrated recently that *c2ta*-deficient mice have residual MHCII expression on a DC subset in the lymph nodes [31], which might have partially rescued CD4⁺ T cell priming by endogenous DC in our model system. Most importantly, irrespective of the physiological role of antigen transfer and CD4⁺ T cell priming in vivo, i.e. injection of antigen-loaded CIITA KO DC did not lead to eosinophilic airway inflammation. This demonstrates that CD4⁺ T cell priming in this model system is dependent on direct antigen presentation by exogenously injected DC, in agreement with previous findings [4].

The finding that CD4⁺ T cell stimulation occurs directly and exclusively as a result from antigen presentation by injected DC opens the possibility that DC can be modified ex vivo to alter the type of immune response induced and ultimately lead to new immunotherapies directed against asthma.

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