

CD8 α^+ , but Not CD8 α^- , Dendritic Cells Tolerize Th2 Responses via Contact-Dependent and -Independent Mechanisms, and Reverse Airway Hyperresponsiveness, Th2, and Eosinophil Responses in a Mouse Model of Asthma

John R. Gordon,^{1*} Fang Li,[‡] Aarti Nayyar,^{*} Jim Xiang,[†] and Xiaobei Zhang^{*}

Splenic CD8 α^+ dendritic cells reportedly tolerize T cell responses by inducing Fas ligand-mediated apoptosis, suppressing IL-2 expression, or catabolizing T cell tryptophan reserves through expression of IDO. We report in this study that CD8 α^+ , but not CD8 α^- , dendritic cells purified from the spleens of normal mice can tolerize the Th2 responses of cells from asthma phenotype mice through more than one mechanism. This tolerance could largely be reversed in vitro by anti-IL-10 or anti-TGF β Ab treatment. However, loss of direct dendritic cell-T cell contact also reduced tolerance, although to a lesser extent, as did adding the IDO inhibitor 1-methyltryptophan or an excess of free tryptophan to the cultures. Within 3 wk of reconstituting asthma phenotype mice with 1×10^5 OVA-pulsed CD8 α^+ , but not CD8 α^- , dendritic cells, the mice experienced a reversal of airway hyperresponsiveness, eosinophilic airway responses, and pulmonary Th2 cytokine expression. This data indicates that CD8 α^+ dendritic cells can simultaneously use multiple mechanisms for tolerization of T cells and that, in vivo, they are capable of tolerizing a well-established disease complex such as allergic lung disease/asthma. *The Journal of Immunology*, 2005, 175: 1516–1522.

Dendritic cells routinely patrol the airways for environmental Ags, phagocytosing, and processing these before migrating to the draining lymphoid tissues for direct MHC-peptide complex presentation to naive T cells (1). This presentation is, of course, dependent on appropriate reciprocal accessory and other signaling between the APCs and the T cells. For the majority of the population at least, respiratory dendritic cells launch a default tolerogenic program when exposed to innocuous aeroantigens (2, 3), and this makes sense from a teleologic perspective. Nevertheless, respiratory dendritic cells become highly immunogenic when exposed to pathogens (4). For some individuals seemingly predisposed to atopy, rather undefined circumstances lead to immunogenic responses to otherwise harmless allergens (2, 5).

A number of factors are known to affect the tolerogenicity of dendritic cells, including the expression by some of the tryptophan-catabolizing enzyme IDO or of IL-10 and/or TGF β by others, whereas some populations induce tolerance by virtue of their relative immaturity, for example. Tryptophan is critical to the genesis of T cell responses, so that IDO-driven tryptophan consumption by APCs can lead to deletion of the partnered T cells through induction of apoptosis (6–8). Interestingly, IDO expression can be up-regulated in CD8 α^+ dendritic cells through T cell CTLA4 signaling (9), which could perhaps be related to the abilities of some T cell populations to render dendritic cells tolerogenic (10). Ag presentation by IL-10- or TGF β -expressing cells can lead to tol-

erance through the ability of the cells to induce expansion of tolerogenic CD25 $^+$ or CD25 $^-$ T regulatory cells (11), and this has been proposed as a mechanism by which IL-10-secreting melanoma tumors may overcome antitumor immunity (12). CD8 α^+ dendritic cells have been reported to secrete some IL-10 (13), but their expression of IDO is presumed critical for their tolerization of T cell responses (7, 8). Dendritic cells generated in vitro from bone marrow precursors in the absence of inflammatory stimuli are immature or relatively immature (i.e., express only low levels of Ag-presentation costimulatory molecules) and are therefore tolerogenic, whereas they can become immunostimulatory after in vitro exposure to maturational factors (e.g., LPS and TNF) (14).

The demonstrations that dendritic cells are important for the induction of tolerance to aeroallergens, self-Ags, or model Ags in naive animals (2, 3, 15) has raised hopes that we should be able to similarly apply this technology for reversal of established clinically important disease complexes, such as autoimmune or allergic diseases (16). Cultured immature dendritic cells given prophylactically can delay graft rejection times (17), but allotransplant survival is best realized when immunosuppressive strategies are jointly administered with the dendritic cells (18, 19). Also, dendritic cells purified from the draining lymph nodes of mice previously tolerized by aeroallergen exposure can be used to prophylactically prevent subsequent allergen sensitization in passive transfer recipients (11). However, there have been few, if any, reports of successful use of dendritic cells to reverse an ongoing disease complex. We show in this study that splenic CD8 α^+ dendritic cells use IL-10, TGF β , and IDO expression to tolerize allergic asthma-like Th2 cytokine and Ab responses in vitro but, more importantly, that these dendritic cells can also reverse airway hyperresponsiveness (AHR),² pulmonary eosinophilic inflammation, and Th2 responses in a mouse model of asthma.

*Immunology Research Group, Department of Veterinary Microbiology, and [†]The Saskatoon Cancer Agency, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; and [‡]Department of Immunology, Dalian Medical University, Dalian, Liaoning, People's Republic of China

Received for publication December 8, 2004. Accepted for publication May 13, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Address correspondence and reprint requests to Dr. John R. Gordon, Department of Veterinary Microbiology, 52 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4 Canada. E-mail address: john.gordon@usask.ca

² Abbreviations used in this paper: AHR, airway hyperresponsiveness; alum, aluminum hydroxide; DMEM-10, DMEM/10% FCS/1% antibiotics/antimycotics; 1-MT, 1-methyltryptophan; BAL, bronchoalveolar lavage.

Materials and Methods

Reagents

The supplies used and their commercial sources were as follows: DMEM, FCS, HBSS, and 1% antibiotics/antimycotics (Invitrogen Life Technologies); collagenase and hyaluronidase (Worthington Biochemical); aluminum hydroxide (alum), FITC-dextran (40 kDa molecular mass), grade V OVA, methacholine, and Tween 20 (Sigma-Aldrich); Lymphocyte Separation Medium (Valeant Pharmaceuticals); Immulon-4 ELISA plates (Dynatech Laboratories); matched capture and biotinylated detection Ab pairs and recombinant protein standards for IL-2, IL-4, IL-5, IL-9, IL-12, IL-13, and TGF β (R&D Systems); FITC- or PE-conjugated monoclonal rat anti-mouse I-A^d, CD3, CD4, CD8 α , CD11c, CD19, CD25, CD40, CD54, CD80, CD86, CD152, goat anti-mouse IgE Ab, and biotinylated anti-mouse-IgG1, -IgG2a (BD Pharmingen), and -IgA detection Abs (Caltag Laboratories); anti-CD8 α -paramagnetic beads (Miltenyi Biotec); streptavidin-conjugated HRP (Vector Laboratories); and ABTS peroxidase substrate (Kirkegaard and Perry Laboratories). OVA was biotinylated as noted previously (20). BALB/c mice were purchased from our institutional breeding colony, which routinely renews its breeding stock from commercial sources (The Jackson Laboratory). All animal procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Preparation and characterization of splenic CD8 α^+ and CD8 α^- dendritic cells

Spleens from healthy BALB/c mice were mechanically dispersed, then digested for 90 min at 37°C with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in DMEM/10% FCS/1% antibiotics/antimycotics (DMEM-10). The cells were washed and allowed to adhere to petri dishes for 2 h using DMEM-10, then the nonadherent cells were discarded, and the adherent cells were incubated overnight at 37°C in DMEM-10. The next day, the overnight nonadherent (dendritic) cells were harvested and incubated with anti-CD8 α -conjugated paramagnetic beads and sorted by MACS into CD8 α^+ and CD8 α^- dendritic cell subsets. The purified cells were cultured in DMEM-10, either with or without OVA (50 μ g/ml) for 2 h, then were washed extensively and resuspended in either DMEM-10 or in saline (in vivo reconstitution experiments only). For FACS analysis of surface marker expression, the cells were incubated for 30 min on ice with FITC- or PE-labeled marker-specific or isotype control Ab, then were fixed by the addition of an equal volume of 2% paraformaldehyde, and processed for FACS analysis. The cell markers assessed by FACS included mouse I-A^d, CD3 (contaminating T cells), CD4, CD8, CD11c, CD19 (contaminating B cells), CD25, CD40, CD54, CD80, CD86, and CD152 (CTLA4). Macrophage contamination was assessed by morphology and staining with mAb F4/80, as noted previously (21). For characterization of endocytosis, the dendritic cells were incubated in 40-kDa FITC-dextran (50 μ g/ml) for 30 min at either 4 or 37°C, then washed extensively at 4°C, and fixed for FACS analysis as described above.

In vitro analysis of CD8 α^+ dendritic cell tolerization of asthma-like Th2 responses

To dissect the mechanisms mediating CD8 α^+ dendritic cell-mediated tolerization of asthma phenotype Th2 cells, we cocultured FCS- or OVA-pulsed CD8 α^+ , or CD8 α^- , dendritic cells (1×10^5 cells/ml) with spleen cells (2×10^6 cells/ml) from asthma phenotype mice for 72 h, then harvested the culture supernatants for cytokine or OVA-specific IgE, IgG1, or IgG2a Ab assays. The dendritic cell-lymphocyte populations were either physically separated from one another by use of Transwell inserts (Costar), or cultured together, but in the presence of anti-IL-10R (20 μ g/ml), anti-TGF β (20 μ g/ml), recombinant murine IL-2 (10 μ g/ml), 1-methyltryptophan (1-MT; 200 μ M), or tryptophan (250 μ M).

Animal sensitization and challenge

Sensitization. Mice were sensitized for asthma-like disease using a standard protocol, as noted previously (20). Briefly, they were given two i.p. injections of OVA-alum (2 μ g of OVA/mg alum, in 200 μ l of saline) on days 0 and 14, and 1% OVA in saline aerosols on days 30, 32, and 34 (20 min/day). By day 35, such mice displayed 40–70% airway eosinophilia, strong AHR to methacholine, high-level circulating OVA-specific IgE and IgG1 Abs, and pulmonary expression of Th2 cytokines (20).

Dendritic cell treatments. Asthma phenotype or control mice ($n = 5$ per group) were surgically anesthetized with ketamine/xylazine, then the skin and musculature over their trachea were carefully reflected surgically, and 1×10^5 dendritic cells in 25 μ l of saline were injected transtracheally using a 30-gauge needle. Dendritic cells given in this fashion have been shown

by others to migrate across the airway epithelium, to the draining lymph nodes, where they efficiently present their Ags for sensitization of the treated animals (22–24). The cutaneous incisions in our animals were closed with surgical staples.

In numerous experiments, we have found that for ~ 1 wk after exposure to aeroallergens, many of the eosinophils found in the airways of asthma phenotype mice display overt signs of activation (i.e., degranulation), unlike the eosinophils present in more quiescent asthma phenotype airways. We reasoned that delivery of tolerogenic dendritic cells into such an overtly inflammatory environment could potentially alter their phenotype and subvert their tolerogenic potential. Thus, we waited for 2 wk after the last OVA aerosol exposure before introducing our cells into the airways; at this time, the AHR of the mice had not waned appreciably, and the airway eosinophil numbers were still elevated (data not shown). Weekly after the dendritic cell transfers, the animals were assessed for AHR to methacholine (see *Measurement of AHR*). Based on repeated observations that the dendritic cell treatments did not have full effect in vivo for ~ 3 wk after transplant, we waited for 4 wk before sacrificing our animals to assess the in vivo effects of the treatments. The day before sacrifice, the animals were again exposed once for 20 min to a nebulized 1% OVA aerosol.

Measurement of AHR

AHR to methacholine was assessed by head-out, whole-body plethysmography in conscious animals, as noted previously (20). The bronchoconstriction data was gathered as running 2-s means of the airflow at the 50% point in the expiratory cycle (flow at 50% TV_e1; TV_e1 is the tidal volume, expiratory cycle, 1 s). This parameter has been demonstrated previously to accurately reflect bronchiolar constriction, as opposed to alveolar constriction or airway occlusion (25). Each mouse was sequentially exposed to aerosols of saline alone, and then doubling doses of methacholine (0.75–25 mg/ml saline) were given over ~ 15 min. In some cases, the concentration of methacholine inducing a 20% drop in airflow (20% provocation concentration (PC₂₀)) was interpolated from graphs of the changes in airflow as a function of the methacholine challenge dose.

Asthma phenotype mouse biological samples

All biological samples were collected as noted previously (20). Briefly, heparin-anticoagulated plasma for circulating Ab analyses was obtained by cardiac puncture of the euthanized animals. Cytochrome slides for differentials of bronchoalveolar lavage (BAL) airway cells were stained with Wright's solution, whereas the BAL fluids themselves were aliquoted and stored at -80°C until analyzed. Cranial right lung lobe tissues of each mouse were fixed, processed to paraffin sections, and stained with Giemsa solution for histopathology. Splenocytes for Th2 cell recovery from each mouse were dispersed from the intact organ by mechanical disruption, and the RBC were subjected to hypotonic lysis. In some experiments, we dispersed the lung parenchymal cells of the treated and untreated asthma phenotype mice by collagenase/hyaluronidase digestion (20) and isolated the mononuclear cells for assessments of mediator/Ab release. For these assays, splenocytes or lung mononuclear cells were cultured under standard conditions (2×10^6 cells/ml DMEM-10) for 72 h, with or without OVA (25 μ g/ml), then the culture supernatants were harvested for mediator assay.

ELISA

Our Ab and cytokine ELISA protocols have been reported in detail previously (20). For the OVA-specific Ab assays, OVA (IgA, IgG1, and IgG2a) or anti-IgE was used as the capture reagent; for the IgE assays, the samples were added to the anti-IgE coated wells, and then biotinylated OVA was used as the detection reagent. The ELISA results (except IgE) are presented in picograms of cytokine or Ab per milliliter, based on a recombinant protein standard curve. The IgE results are expressed in OD₄₀₅ units ($\times 100$). All cytokine ELISA were sensitive to 5–10 pg/ml recombinant cytokine standard.

Statistical analyses

The data were analyzed by ANOVA with post hoc protected least significant difference testing (StatView 4.1; Abacus Concepts). All data are reported as the mean $\pm$ SEM.

Results

Allergen-pulsed splenic CD8 α^+ dendritic cells display markers of tolerogenic cells

Purified CD8 α^+ dendritic cells displayed a largely homogeneous appearance of cells with irregular nuclei and smaller dendritic processes. They expressed high levels of MHC class II, moderate levels of CD11c, CD40, CD80, and CD86, and lower levels of DEC-205, and were avidly endocytotic, readily taking up 40 kDa molecular mass FITC-dextran (Fig. 1). These characteristics compare well with those published previously for CD8 α^+ splenic dendritic cells, although some report higher levels of DEC-205 expression than we observed (reviewed in Ref. 26). Our purified CD8 α^+ cell populations did not stain significantly with CD4, CD25, CD152 (i.e., CTLA4), F4/80 (macrophage marker), CD19 (B cell marker), or CD3 (T cell marker) (data not shown). The CD8 α^- dendritic cells were much like the CD8 α^+ dendritic cells morphologically, but were strongly positive for MHC class II, CD40, CD80, and CD86 (data not shown). Neither before nor after allergen pulsing did either population of cells secrete significant amounts of IL-4, IL-5, IL-9, IL-13, IFN- γ , or TNF- α in culture ($p \geq 0.05$ vs medium alone; data not shown). Otherwise unstimulated CD8 α^- dendritic cells secreted IL-12 (383 ± 22 pg/ 10^5 cells), whereas CD8 α^+ cells secreted both IL-10 (207 ± 17 pg/ 10^5 cells) and TGF- β (109 ± 19 pg/ 10^5 cells) over 18 h in culture. Allergen exposure itself did not induce these responses, because cells of each population secreted identical levels of these cytokines with or without allergen exposure (data not shown). CD8 α^+ cells

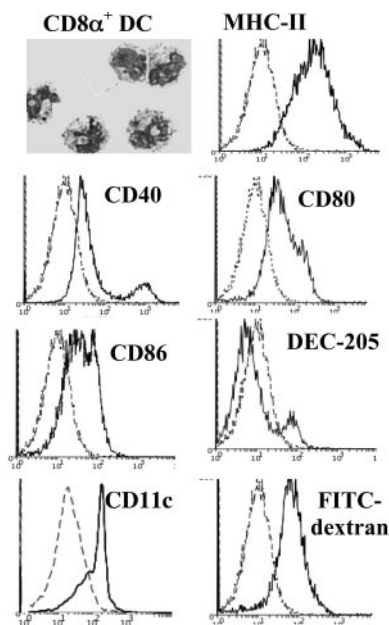


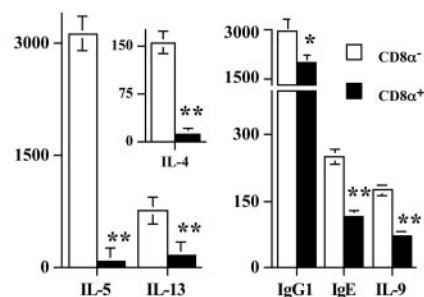
FIGURE 1. Splenic CD8 α^+ dendritic cells express costimulatory markers and are endocytically active. CD8 α^+ dendritic cells purified by differential adherence and MACS from the spleens of normal BALB/c mice were stained with Wright's solution (upper left panel) and also analyzed by FACS for numerous dendritic cell surface markers, including CD11c, CD40, CD80, CD86, CD205 (DEC205), and MHC class II (MHC-II) using FITC-labeled specific (solid lines) or isotype-matched irrelevant specificity (light lines) Abs. In addition, the endocytic activity of the cells was assessed by exposure to 40-kDa FITC-dextran (50 μ g/ml) for 30 min at 37°C (heavy line) or 4°C (negative control, light line), and measuring by FACS the relative amounts of fluorescent probe they had acquired. This data arises from a single experiment representative of two (phagocytosis panel) or five (surface markers) performed.

did not secrete IL-12, and CD8 α^- cells did not secrete IL-10 or TGF- β , either before or after allergen pulsing.

CD8 α^+ dendritic cells tolerize OVA-specific Th2 responses of lymphocytes from asthma phenotype mice through contact-independent and contact-dependent processes

The addition of 1×10^5 OVA-pulsed CD8 α^+ dendritic cells to 2×10^6 asthma phenotype mouse splenocytes depressed the expression by these cells of IL-4, IL-5, IL-9, IL-13, as well as IgE and IgG1 anti-OVA Ab release (Fig. 2), whereas OVA-specific IgG2a production was increased from 1852 ± 228 pg/ml to 3520 ± 402 pg/ml. The fact that efficient tolerization of the Th2 responses required presentation of OVA was confirmed by demonstrating that CD8 α^+ cells exposed to FCS Ags, but not OVA,

A CYTOKINE SECRETION (pg/ml $\pm$ SEM)



B

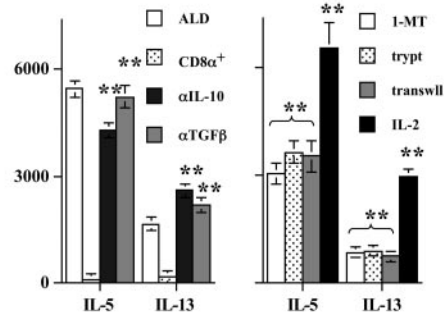


FIGURE 2. OVA-pulsed CD8 α^+ dendritic cells tolerize asthma phenotype mouse Th2 cytokine responses through contact-independent and -dependent mechanisms. Asthma-like disease was induced by parenteral exposure of mice to OVA-alum conjugates followed by exposure to OVA aerosols, such that the mice suffered $\sim 60\%$ airway eosinophilia and marked AHR. Two to 4 wk later, splenocytes (2×10^6 /ml) from these mice were cultured with 1×10^5 OVA-pulsed CD8 α^+ , or CD8 α^- , dendritic cells generated as in Fig. 1 (A). In B, the allergic lung disease splenocytes were cultured alone (ALD; 50 μ g/ml OVA was added to these wells) or with 1×10^5 CD8 α^+ dendritic cells (all other conditions). The dendritic cells and splenocytes were either cultured together or separated by Transwell inserts (transwl), then after 72 h the levels of the indicated cytokines or OVA-specific Abs in the culture supernatants were assessed by ELISA. Anti-IL-10 or anti-TGF β Ab (20 μ g/ml), the IDO inhibitor 1-MT (200 μ M), an excess of tryptophan (250 μ M), or rIL-2 (10 μ g/ml) was added to some of the cultures. OVA-presenting CD8 α^+ dendritic cells nearly abrogated the expression of IL-4, IL-5, and IL-13 by the Th2 cells from the asthma phenotype mice and significantly blunted their IL-9, IgE, and IgG1 responses. Physical separation of the dendritic cells and T cells, or the addition to the cocultures of anti-IL-10 or anti-TGF β Abs, 1-MT, tryptophan, and IL-2 each differentially antagonized the dendritic cell-driven tolerance in this system. * or **, $p \leq 0.05$ or $p \leq 0.01$, respectively, vs CD8 α^- -treated splenocytes cultures (A) or vs CD8 α^+ dendritic cell-treated cultures (B). The data depicted are expressed in picograms per milliliter culture supernatant ($\pm$ SEM) and arise from one experiment representative of two.

were not strong inducers of tolerance. FCS-pulsed CD8 α^+ dendritic cells reduced IL-5 and IL-13 expression by 42 and 53%, respectively, whereas the OVA-pulsed cells reduced these responses by 98 and 92%, respectively. The fact that these allergen-naïve cells had some tolerogenic effects in vitro might be expected, given that the addition of IL-10 by itself to cultures of PBMC from allergic subjects is known to dampen their Th2 phenotype responses (27). Although it is possible that the effects the CD8 α^+ dendritic cells had on anti-OVA Ig production were attributable to direct B cell tolerization, it seems quite feasible that reduced Th cell activity by itself could explain the reduced Ab responses.

To address the effector mechanisms behind this in vitro tolerance, we next tested the effects of adding anti-IL-10 or anti-TGF- β Abs, the IDO inhibitor 1-MT, an excess of tryptophan, or rIL-2 to the dendritic cell/asthma phenotype splenocyte cultures (Fig. 2B). The addition of anti-IL-10 or anti-TGF- β Abs dramatically reduced the tolerogenic effects of the dendritic cells, and inhibition of IDO with 1-MT, or addition of an excess of exogenous tryptophan, also partially reversed CD8 α^+ dendritic cell-dependent tolerance (Fig. 2B). We also examined the issue of whether direct CD8 α^+ dendritic cell-T cell contact was required and found that disallowing contact by use of Transwell inserts also partially displaced the CD8 α^+ dendritic cell-driven tolerance. The addition of IL-2 to the cocultures essentially negated tolerance (Fig. 2B). As with their effects on splenocyte cytokine expression, these treatments also affected IgE responses in the CD8 α^+ dendritic cell/asthma-like splenocyte cocultures. The IL-2, 1-MT, anti-IL-10, and anti-TGF- β treatments reduced IgE tolerization by 42–53%, whereas physical separation of the cells by use of Transwell inserts reduced tolerance by $27 \pm 4\%$.

CD8 α^+ dendritic cells tolerize eosinophilic inflammation and AHR in a mouse model of allergic asthma

We next asked whether CD8 α^+ dendritic cells could similarly tolerize an active disease complex (i.e., asthma-like disease) in vivo. The first parameter we assessed was AHR, in part because we could address this in nonterminal assays (i.e., whole-body plethysmography) but also because AHR is a primary symptom of asthma. We repeatedly found that the CD8 α^+ dendritic cell treatments had no detectable effect on AHR for 12–13 days posttreatment, but between 14 and 21 days after transplant, we observed highly significant and increasing reductions in AHR of the CD8 α^+ , but not CD8 α^- , dendritic cell recipients (Fig. 3). At 18 days, the concentration of methacholine provoking a 20% decline in airflow (PC₂₀) in normal mice was 6 mg/ml, whereas the PC₂₀ for the saline-treated asthma phenotype mice was 0.71 mg/ml. The PC₂₀ for the CD8 α^+ dendritic cell-treated asthma model mice was 2.3 mg/ml, whereas that for the CD8 α^- dendritic cell-treated mice was 0.75 mg/ml. We observed no further increases (or decreases) in AHR in the CD8 α^+ dendritic cell-treated mice between ~3 and 8 wk after transfer, but did not assess longer times in this study.

Based on this tolerization of AHR, we asked whether OVA-presenting CD8 α^+ or CD8 α^- dendritic cells also affected eosinophilic inflammation in our mouse model of asthma. Thus, 4 wk after the dendritic cell transfers, when tolerization of AHR was complete, we challenged all mice once with OVA aerosols, then assessed their pulmonary inflammatory responses the following day. Eosinophils dominated the airway responses of the saline-treated asthma phenotype animals (Fig. 4), such that they comprised ~50% of the BAL cells at this time, whereas the proportions of eosinophils in the airways of the CD8 α^+ dendritic cell-treated asthma model mice were reduced by 53% ($p \leq 0.01$ vs the saline-treated group). The CD8 α^- dendritic cell-treated animals experienced a 43% increase in airway eosinophilia ($p \leq 0.03$

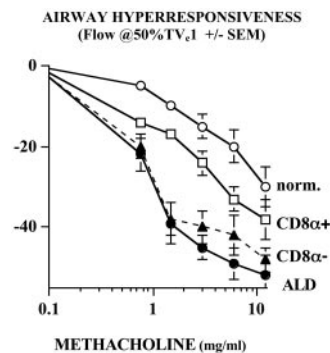


FIGURE 3. Treatment of asthma phenotype mice with CD8 α^+ , but not CD8 α^- , dendritic cells ameliorates AHR. Asthma-like disease was induced in mice as in Fig. 2, then the animal's lung inflammatory responses were allowed to wane for 2 wk before the animals were given 1×10^5 CD8 α^+ or CD8 α^- dendritic cells or carrier medium alone (allergic lung disease; ALD) via the airway. Over the following 3 wk, the AHR of these and saline-treated control mice (saline) to increasing doses of aerosolized methacholine was assessed by head-out, whole-body plethysmography, as indicated in *Materials and Methods*. The data, depicting AHR of the mice at 18 days posttreatment, are expressed as the airflow rate at the 50% point in the expiratory cycle. The CD8 α^- dendritic cell treatments had no effect on AHR in the asthma phenotype mice, whereas the CD8 α^+ cell treatments nearly normalized AHR (relative to the normal saline controls). The AHR of the CD8 α^+ dendritic cell-treated mice was significantly ameliorated relative to the untreated or CD8 α^- dendritic cell-treated asthma phenotype mice ($p \leq 0.01$). This data is from one experiment that is representative of five performed.

vs the saline-treated group). The CD8 α^+ dendritic cell treatments also reduced the peripheral blood eosinophilia of the animals. The proportions of eosinophils ($\pm$ SEM) in peripheral blood smears from our animals were as follows: normal mice, $4 \pm 0.5\%$; asthma phenotype mice, $16.5 \pm 4.5\%$; CD8 α^+ dendritic cell group, $10 \pm 4\%$; and CD8 α^- dendritic cell group, $24 \pm 2\%$. Thus, the CD8 α^+ dendritic cell treatments had a decidedly tolerogenic effect on the recipient animals' AHR and airway and circulating eosinophilia, whereas the CD8 α^- DC, which are considered to be immunostimulatory cells (28, 29), exacerbated these responses.

CD8 α^+ dendritic cell treatments ameliorate Th2 cytokine and OVA-specific IgE and IgG1 Ab responses in a mouse model of asthma

The airway inflammatory responses observed in asthma are considered to stem from the individual subjects' Th2 (e.g., IL-5 and IL-9) responses to aeroallergen challenge (30, 31). We examined then the pulmonary cytokine expression within each group of animals, testing BAL fluids for Th1 (IL-12 and IFN- γ) and Th2 (IL-5, IL-9, and IL-13) cytokines, as well as IL-10. In accordance with previous reports (20), the samples from the saline-treated asthma phenotype mice contained substantial levels of IL-5, IL-9, and IL-13, and low levels of IFN- γ , IL-12, and IL-10 (Fig. 5). The CD8 α^+ dendritic cell treatments reduced to near background levels the IL-5, IL-9, and IL-13 responses, and also reduced the IFN- γ component of the asthma-like response. In contrast, there was substantial IL-10 expression in the airways of these tolerized animals. The CD8 α^- dendritic cell treatments exacerbated the Th2 cytokine response, mirroring their effects on eosinophilic inflammation, with significant increases being observed in IL-5 and IL-9 expression. IL-13 expression remained more or less constant, whereas the IFN- γ and IL-12 responses increased, and the IL-10 levels were reduced in these animals.

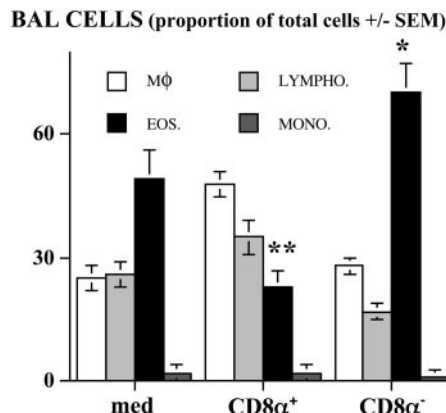


FIGURE 4. Treatment with CD8α⁺, but not CD8α⁻, dendritic cells dampens airway eosinophilia following airway allergen challenge in a mouse model of asthma. Asthma phenotype mice were given 1×10^5 CD8α⁺, or CD8α⁻, dendritic cells as in Fig. 3, then 4 wk later they were challenged once for 20 min with a 1% OVA aerosol. The following day, BAL were performed on the mice and the relative proportions of alveolar macrophages (MΦ), lymphocytes (LYMPHO.), eosinophils (EOS.), and monocytes (MONO.) were determined from counts of 300 cells/sample, using Wright's solution-stained cytocentrifuge preparations of the BAL cells. The data are expressed as proportion of the target population relative to the total nucleated cells. The CD8α⁺ dendritic cell treatments reduced the airway eosinophil responses by 53%, whereas the CD8α⁻ cell treatments increased this response by ~43%. This data is from one experiment that is representative of five with similar results. * or **, $p \leq 0.03$ or $p \leq 0.01$, respectively, vs the eosinophilia of medium-treated allergic lung disease mice.

We also examined the impact of the treatments on the levels of OVA-specific Abs in the plasma of our animals, and found that neither dendritic cell treatment significantly affected the 4-wk post-treatment levels of OVA-specific circulating IgE, IgG1, or IgG2a. To determine whether this apparent systemic lack of tolerance could potentially be related to our *in vivo* experimental approach (e.g., lack of dendritic cell access to the spleen or other compartments *in vivo*, or insufficient numbers of cells transferred to achieve full penetrance), as opposed to an inherent inability of CD8α⁺ dendritic cells to affect B cell Ab production *in vivo*, we assessed whether B cells isolated directly from the lungs of our treated animals (i.e., a compartment to which the cells had had access) exhibited signs of having been tolerized. Lung mononuclear cells from mice challenged 1 day earlier with aeroallergen were simply placed in culture for 48 h without an exogenous supply of OVA, then the culture supernatants were assayed for OVA-specific Abs. We found that the CD8α⁺ dendritic cell treatments had indeed dampened the expression by parenchymal B cells from the lungs of the asthma phenotype animals of OVA-specific IgG1, as a prototypical Th2-associated isotype, but augmented their expression of IgA. The cells from the saline, CD8α⁺, or CD8α⁻ dendritic cell-treated asthma-like mice produced 185 ± 25 , 103 ± 11 , or 196 ± 20 pg/ml, respectively, of OVA-specific IgG1 ($p \leq 0.02$ for the CD8α⁺ vs CD8α⁻ dendritic cell treatments), and 135 ± 9 , 185 ± 8 , and 251 ± 13 pg/ml, respectively, of OVA-specific IgA ($p \leq 0.01$ for the CD8α⁺ vs CD8α⁻ dendritic cell treatments). These data do suggest that our tolerogenic dendritic cell treatments did have local effects on the OVA-specific Ab response.

Discussion

OVA-pulsed CD8α⁺ dendritic cells were able to tolerize asthma phenotype Th2 responses *in vitro* by contact-dependent and con-

BAL CYTOKINE LEVELS (pg/ml +/- SEM)

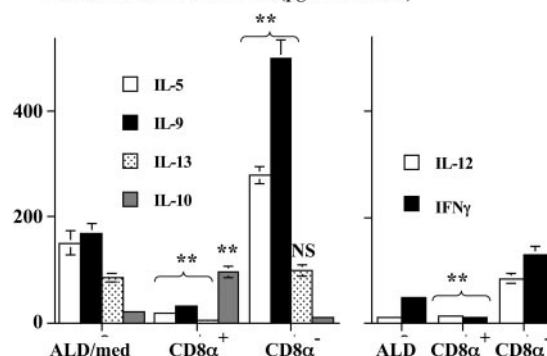


FIGURE 5. CD8α⁺ dendritic cell treatments also down-regulate BAL Th2 cytokine levels after airway allergen challenge in a mouse model of asthma. BAL fluids were obtained from asthma phenotype mice that were given 1×10^5 CD8α⁺ or CD8α⁻ dendritic cells as in Fig. 4, then assayed for their content of Th2 and Th1 cytokines by ELISA. As with the airway eosinophilia, the CD8α⁺ dendritic cell treatments uniformly reduced the airway IL-5, IL-9, and IL-13 responses, but increased local expression of IL-10 in the airways, whereas the CD8α⁻ cell treatments dramatically increased the expression of IL-5 and IL-9 in the airways, but had little effect on IL-10 or IL-13 responses. This data is from one experiment that is representative of five performed. **, $p \leq 0.01$, vs the value of medium-treated asthma phenotype mice.

tact-independent mechanisms. The purified dendritic cells secreted both IL-10 and TGFβ, and either anti-IL-10 or anti-TGFβ Abs substantially reversed dendritic cell-driven tolerance *in vitro*. Addition of the IDO inhibitor 1-MT or an excess of free tryptophan to the cocultures, or preventing direct contact between the dendritic cells and asthma phenotype T cells, somewhat less efficiently reduced the dendritic cell-driven tolerance. More importantly, the transfer of small numbers of CD8α⁺ dendritic cells was sufficient to reproducibly reverse asthma-like disease in the affected animals. It is known that exposure of naive animals to innocuous Ag aerosols induces a state of Ag-specific tolerance (reviewed in Refs. 2 and 4) mediated by rapidly induced tolerogenic γδ-TCR⁺CD8⁺ T cells (32) or that, as noted above, dendritic cells from the lymph nodes of mice tolerized by such aeroallergen exposure can prevent subsequent allergen sensitization in naive passive transfer recipients (11). However, we are not aware of any reports of tolerogenic dendritic cell-driven therapeutic reversal of an ongoing, immunopathologically complex disease process such as that which occurs in a mouse model of allergic asthma.

Purified CD8α⁺ dendritic cells have been reported previously to display either immunogenic (28, 29, 33, 34) or tolerogenic (35–37) properties, depending on the conditions under which the cells are manipulated *in vitro* or *in vivo*. Thus, whereas CD8α⁺ dendritic cells are relatively immature when freshly purified, on culture in GM-CSF or after LPS challenge both these and CD8α⁻ dendritic cells undergo maturational changes and become immunostimulatory for CD4⁺ and CD8⁺ T cells (28). GM-CSF-treated CD8α⁻ or CD8α⁺ dendritic cells have been reported to induce Th2- or Th1-type responses, respectively, *in vivo* (29), although others have reported that Flt-3 ligand induction of dendritic cell development *in vivo* skews the immunostimulatory functions of both CD8α⁻ and CD8α⁺ dendritic cells to the Th1 mode, such that either can efficiently induce transplant rejection responses (33). CD8α⁺ dendritic cells can also prime for protective cytotoxic T cell responses to influenza, herpes, or vaccinia virus infections (34). This suggests that a variety of stimuli can render CD8α⁺ dendritic cells immunostimulatory. Our cells were not given exogenous tolerogenic or maturational cytokines or stimuli (e.g., TNF and LPS) in

vitro before use as tolerogenic populations. Furthermore, we were careful to allow the intense eosinophilic inflammatory processes associated with airway allergen challenge of our asthma phenotype mice to wane for 2 wk before treating them, to circumvent any potential inflammation-associated maturational effects on the transferred cells.

CD8 α^+ dendritic cells can be tolerogenic, and this effect is not related to inadequate costimulatory molecule (e.g., CD40 and CD80) expression, per se, by these cells (35). This is in accordance with our data, wherein our CD8 α^+ cells expressed ample costimulatory molecules. CD8 α^+ dendritic cells can suppress CD8 $^+$ T cell responses through their abilities to limit IL-2 expression (35, 38), whereas in mixed lymphocyte reactions CD4 $^+$ T cell tolerization by these cells is reported to depend on Fas ligand-mediated apoptosis (39). CD8 α^+ dendritic cells are able to overcome the immunogenicity of CD8 α^- dendritic cells with which they are admixed, but secreted mediators reportedly do not mediate this suppression (35). This observation led to the conclusion that CD8 α^+ dendritic cell tolerance is contact-dependent, although this was not tested directly (35). CD8 α^+ dendritic cells have been shown previously to release IL-10, but it had been concluded that this secretion was not at levels sufficient to effect tolerance (13).

We directly tested the impact of allergen-presenting CD8 α^+ dendritic cells on asthma phenotype Th2 responses, and found that the T cell tolerance driven by our dendritic cells is in part contact-dependent, as well as being mediated in part by IL-10 and TGF β . The cells lost about half of their tolerance-inducing abilities when they could not directly contact the Th2 cells, or when we added to the cocultures the IDO inhibitor 1-MT or an excess of tryptophan. In contrast, we detected significant IL-10 and TGF β secretion by the CD8 α^+ , but not CD8 α^- , dendritic cells, and both of these mediators were implicated as contributing importantly to tolerization of the asthma phenotype mouse T cells. Thus, our evidence suggests that both of these cytokines can contribute importantly to CD8 α^+ dendritic cell tolerance, and both have been implicated as tolerogenic in multiple other settings (11, 40, 41). For example, as noted above, respiratory dendritic cells use IL-10 as a mechanism to tolerize naive T cell responses to innocuous environmental allergens (4). And constitutive TGF β expression by quiescent alveolar macrophages is speculated to provide tolerogenic signals to respiratory dendritic cells (16, 40).

Our in vitro tolerization protocols involved direct mixing of the dendritic cells and Th2 cells from the asthma model animals, and this approach was highly effective in reducing the expression of IL-5 and other Th2 cytokines in vitro (although physical separation of the dendritic cell and T cell populations significantly reduced this effect). In contrast, the in vivo CD8 α^+ dendritic cell treatments only reduced airway eosinophilia by half. We have no direct evidence that documents why we observed this discrepancy, but a number of factors could potentially have affected these processes. In vitro, the tolerogenic dendritic cells would potentially have had direct access to all of the T cells within the wells, whereas in vivo this would have been unlikely. Although we did not assess trafficking of our cells in vivo, others have shown that dendritic cells introduced into the airways traverse the airway-epithelial barrier, migrate to the draining lymph nodes, and there successfully present their Ags (e.g., Refs. 22–24). This rapid phase of T cell activation is Ag-specific and does not occur in nondraining lymph nodes or the spleen, but the activated cells do recirculate to other secondary lymphoid organs after a moderate delay (23), and this recirculation leads to subsequent systemic sensitization (22). We demonstrated in this study and have shown previously (20) that the lungs of our mice contain sizable numbers of allergen-specific T and B cells such that, whereas we did observe moderate systemic

effects of our treatments (the circulating eosinophilia of the CD8 α^+ dendritic cell-treated mice was ~50% reduced relative to asthma phenotype mice), we may simply not have allowed sufficient time for full penetrance of tolerance. Thus, although we did not observe significant reductions in the plasma levels of OVA-specific IgE, IgG1, and IgG2a Abs, the resident lung B cells from our CD8 α^+ dendritic cell-treated animals produced significantly less IgG1 than either the untreated mice or those given CD8 α^- dendritic cells. We cannot say whether the CD8 α^+ dendritic cell treatments directly affected plasma B cell Ab secretion, or whether they affected B cell responses by virtue of indirect effects on B cell-supportive T cell responses but, given the known importance of Th cells in B cell responses, we adopt the conservative hypothesis that the effects we observed were indirect. It is perhaps noteworthy that we have found in a parallel system that delivery into the airways of OVA-presenting bone marrow-derived dendritic cells rendered tolerogenic (by culture in IL-10) abrogates AHR and dramatically dampens both local and systemic Th2 responses, including OVA-specific IgE responses.³

Allergic asthma is the result of inappropriate immune responses to otherwise innocuous environmental allergens that are introduced into the lungs (2, 5). Nonasthmatics are exposed to identical levels of the same allergens, but have become allergen-tolerant, clearly exhibiting some responses to these Ags but suffering little, if any, pathology (42, 43). Experimental blockade of pathogenic responses to allergens has been achieved through either immune deviation (44) or prophylactic induction of immune tolerance (16, 20, 45). Delivery of CpG DNA (i.e., unmethylated bacterial DNA) (44, 46) or IL-12 (47) with allergen, for example, can induce a Th1 skewing of allergic sensitization, leading to reduced eosinophilic inflammation and AHR in experimental allergic asthma. However, Th1 displacement of allergic responses does not necessarily translate into a reduction in pulmonary inflammation, because aeroallergen-specific Th1 responses are also inflammatory in nature (48–50). In contrast, effective tolerance to innocuous respiratory Ags, which is proposed to occur for most individuals as a default response, is associated with Ag-specific nonpathogenic responses (2, 4). This suggests that tolerance induction might be a more desirable approach to therapy in allergic disease than immune deviation. Allergen-specific immunotherapy as a tolerization approach to management of atopic diseases can have clinical efficacy, but it is a cumbersome approach that can call for years of treatment to achieve high levels of efficacy (51, 52). Should dendritic cell-based therapy for asthma eventually prove clinically useful, as proposed by others (16, 53), its delivery may prove technically cumbersome, but therapeutically more satisfying in terms of both time to successful outcome and the depth of effect.

Acknowledgments

We thank Brian Chelack (Prairie Diagnostic Services, Saskatoon, Saskatchewan, Canada) for the FACS services provided.

Disclosures

The authors have no financial conflict of interest.

References

- Vermaelen, K. Y., I. Carro-Muino, B. N. Lambrecht, and R. A. Pauwels. 2001. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J. Exp. Med.* 193: 51–60.
- Stumbles, P. A., J. W. Upham, and P. G. Holt. 2003. Airway dendritic cells: co-ordinators of immunological homeostasis and immunity in the respiratory tract. *APMIS* 111: 741–755.

³ A. Nayyar, X. Zhang, and J.R. Gordon. Treatment of allergic asthma with IL-10-treated bone marrow-derived dendritic cells abrogates AHR and reverses Th2 responses. *Submitted for publication.*

3. McMenamin, C., and P. G. Holt. 1993. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J. Exp. Med.* 178: 889–899.
4. Holt, P. G., and P. A. Stumbles. 2000. Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. *J. Allergy Clin. Immunol.* 105: 421–429.
5. Holt, P. G., and J. W. Upham. 2004. The role of dendritic cells in asthma. *Curr. Opin. Allergy Clin. Immunol.* 4: 39–44.
6. Grohmann, U., R. Bianchi, M. L. Belladonna, S. Silla, F. Fallarino, M. C. Fioretti, and P. Puccetti. 2000. IFN- γ inhibits presentation of a tumor/self peptide by CD8 α^+ dendritic cells via potentiation of the CD8 α^+ subset. *J. Immunol.* 165: 1357–1363.
7. Grohmann, U., F. Fallarino, R. Bianchi, M. L. Belladonna, C. Vacca, C. Orabona, C. Uyttenhove, M. C. Fioretti, and P. Puccetti. 2001. IL-6 inhibits the tolerogenic function of CD8 α^+ dendritic cells expressing indoleamine 2,3-dioxygenase. *J. Immunol.* 167: 708–714.
8. Munn, D. H., M. D. Sharma, D. Hou, B. Baban, J. R. Lee, S. J. Antonia, J. L. Messina, P. Chandler, P. A. Koni, and A. L. Mellor. 2004. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J. Clin. Invest.* 114: 280–290.
9. Mellor, A. L., P. Chandler, B. Baban, A. M. Hansen, B. Marshall, J. Pihkala, H. Waldmann, S. Cobbold, E. Adams, and D. H. Munn. 2004. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int. Immunol.* 16: 1391–1401.
10. Vendetti, S., J. G. Chai, J. Dyson, E. Simpson, G. Lombardi, and R. Lechler. 2000. Anergic T cells inhibit the antigen-presenting function of dendritic cells. *J. Immunol.* 165: 1175–1181.
11. Akbari, O., R. H. DeKruyff, and D. T. Umetsu. 2001. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat. Immunol.* 2: 725–731.
12. Steinbrink, K., H. Jonuleit, G. Muller, G. Schuler, J. Knop, and A. H. Enk. 1999. Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8⁺ T cells resulting in a failure to lyse tumor cells. *Blood* 93: 1634–1642.
13. Grohmann, U., R. Bianchi, M. L. Belladonna, C. Vacca, S. Silla, E. Ayroldi, M. C. Fioretti, and P. Puccetti. 1999. IL-12 acts selectively on CD8 α^+ dendritic cells to enhance presentation of a tumor peptide in vivo. *J. Immunol.* 163: 3100–3105.
14. Chen, Z., S. Dehm, K. Bonham, H. Kamencic, B. Juurlink, X. Zhang, J. R. Gordon, and J. Xiang. 2001. DNA array and biological characterization of the impact of the maturation status of mouse dendritic cells on their phenotype and antitumor vaccination efficacy. *Cell Immunol.* 214: 60–71.
15. Belz, G. T., G. M. Behrens, C. M. Smith, J. F. Miller, C. Jones, K. Lejon, C. G. Fathman, S. N. Mueller, K. Shortman, F. R. Carbone, and W. R. Heath. 2002. The CD8 α^+ dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. *J. Exp. Med.* 196: 1099–1104.
16. Macaubas, C., R. H. DeKruyff, and D. T. Umetsu. 2003. Respiratory tolerance in the protection against asthma. *Curr. Drug Targets Inflamm. Allergy* 2: 175–186.
17. Lutz, M. B., R. M. Suri, M. Niimi, A. L. Ogilvie, N. A. Kukutsch, S. Rosner, G. Schuler, and J. M. Austyn. 2000. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo. *Eur. J. Immunol.* 30: 1813–1822.
18. Giannoukakis, N., C. A. Bonham, S. Qian, Z. Chen, L. Peng, J. Harnaha, W. Li, A. W. Thomson, J. J. Fung, P. D. Robbins, and L. Lu. 2000. Prolongation of cardiac allograft survival using dendritic cells treated with NF- κ B decoy oligodeoxynucleotides. *Mol. Ther.* 1: 430–437.
19. Min, W. P., D. Zhou, T. E. Ichim, G. H. Strejan, X. Xia, J. Yang, X. Huang, B. Garcia, D. White, P. Dutarte, et al. 2003. Inhibitory feedback loop between tolerogenic dendritic cells and regulatory T cells in transplant tolerance. *J. Immunol.* 170: 1304–1312.
20. Schneider, A. M., F. Li, X. Zhang, and J. R. Gordon. 2001. Induction of pulmonary allergen-specific IgA responses or airway hyperresponsiveness in the absence of allergic lung disease following sensitization with limiting doses of ovalbumin-alum. *Cell. Immunol.* 212: 101–109.
21. Gordon, J. R. 2000. Monocyte chemoattractant protein-1 (MCP-1) expression during cutaneous allergic responses in mice is mast cell-dependent and largely mediates monocyte recruitment. *J. Allergy Clin. Immunol.* 106: 110–116.
22. Sung, S., C. E. Rose, and S. M. Fu. 2001. Intratracheal priming with ovalbumin and ovalbumin 323–339 peptide-pulsed dendritic cells induces airway hyperresponsiveness, lung eosinophilia, goblet cell hyperplasia, and inflammation. *J. Immunol.* 166: 1261–1271.
23. Lambrecht, B. N., R. A. Pauwels, and B. Fazekas de St. Groth. 2000. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J. Immunol.* 164: 2937–2946.
24. Lambrecht, B. N., R. A. Peleman, G. R. Bullock, and R. A. Pauwels. 2000. Sensitization to inhaled antigen by intratracheal instillation of dendritic cells. *Clin. Exp. Allergy* 30: 214–224.
25. Vijayaraghavan, R., M. Schaper, R. Thompson, M. F. Stock, L. A. Boylstein, J. E. Luo, and Y. Alarie. 1994. Computer assisted recognition and quantitation of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. *Arch. Toxicol.* 68: 490–499.
26. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2: 151–161.
27. Akdis, C. A., T. Blesken, M. Akdis, B. Wuthrich, and K. Blaser. 1998. Role of interleukin 10 in specific immunotherapy. *J. Clin. Invest.* 102: 98–106.
28. De Smedt, T., E. Butz, J. Smith, R. Maldonado-Lopez, B. Pajak, M. Moser, and C. Maliszewski. 2001. CD8 α^+ and CD8 α^+ subclasses of dendritic cells undergo phenotypic and functional maturation in vitro and in vivo. *J. Leukocyte Biol.* 69: 951–958.
29. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. CD8 α^+ and CD8 α^+ subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med.* 189: 587–592.
30. Kay, A. B. 1991. T lymphocytes and their products in atopic allergy and asthma. *Int. Arch. Allergy Appl. Immunol.* 94: 189–193.
31. O'Byrne, P. M., M. D. Inman, and E. Adolph. 2004. Reassessing the Th2 cytokine basis of asthma. *Trends Pharmacol. Sci.* 25: 244–248.
32. McMenamin, C., C. Pimm, M. McKersy, and P. G. Holt. 1994. Regulation of IgE responses to inhaled antigen in mice by antigen-specific $\gamma\delta$ T cells. *Science* 265: 1869–1871.
33. Morelli, A. E., P. J. O'Connell, A. Khanna, A. J. Logar, L. Lu, and A. W. Thomson. 2000. Preferential induction of Th1 responses by functionally mature hepatic (CD8 α^+ and CD8 α^+) dendritic cells: association with conversion from liver transplant tolerance to acute rejection. *Transplantation* 69: 2647–2657.
34. Belz, G. T., C. M. Smith, D. Eichner, K. Shortman, G. Karupiah, F. R. Carbone, and W. R. Heath. 2004. Cutting edge: conventional CD8 α^+ dendritic cells are generally involved in priming CTL immunity to viruses. *J. Immunol.* 172: 1996–2000.
35. Winkel, K. D., V. Kronin, M. F. Krummel, and K. Shortman. 1997. The nature of the signals regulating CD8 T cell proliferative responses to CD8 α^+ or CD8 α^+ dendritic cells. *Eur. J. Immunol.* 27: 3350–3359.
36. Kronin, V., H. Hochrein, K. Shortman, and A. Kelso. 2000. Regulation of T cell cytokine production by dendritic cells. *Immunol. Cell Biol.* 78: 214–223.
37. Kronin, V., C. J. Fitzmaurice, I. Caminschi, K. Shortman, D. C. Jackson, and L. E. Brown. 2001. Differential effect of CD8⁺ and CD8⁺ dendritic cells in the stimulation of secondary CD4⁺ T cells. *Int. Immunol.* 13: 465–473.
38. Kronin, V., K. Winkel, G. Suss, A. Kelso, W. Heath, J. Kirberg, H. von Boehmer, and K. Shortman. 1996. A subclass of dendritic cells regulates the response of naive CD8 T cells by limiting their IL-2 production. *J. Immunol.* 157: 3819–3827.
39. Suss, G., and K. Shortman. 1996. A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. *J. Exp. Med.* 183: 1789–1796.
40. Lipscomb, M. F., A. M. Pollard, and J. L. Yates. 1993. A role for TGF- β in the suppression by murine bronchoalveolar cells of lung dendritic cell initiated immune responses. *Reg. Immunol.* 5: 151–157.
41. Enk, A. H., H. Jonuleit, J. Saloga, and J. Knop. 1997. Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. *Int. J. Cancer* 73: 309–316.
42. Li, Y., F. E. Simons, F. T. Jay, and K. T. HayGlass. 1996. Allergen-driven limiting dilution analysis of human IL-4 and IFN- γ production in allergic rhinitis and clinically tolerant individuals. *Int. Immunol.* 8: 897–904.
43. Aghayan-Ugurluoglu, R., T. Ball, S. Vrtala, C. Schweiger, D. Kraft, and R. Valenta. 2000. Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: a study performed with purified recombinant pollen allergens. *J. Allergy Clin. Immunol.* 105: 803–813.
44. Sur, S., J. S. Wild, B. K. Choudhury, N. Sur, R. Alam, and D. M. Klinman. 1999. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J. Immunol.* 162: 6284–6293.
45. Holt, P. G., M. Reid, D. Britten, J. Sedgwick, and H. Bazin. 1987. Suppression of IgE responses by passive antigen inhalation: dissociation of local (mucosal) and systemic immunity. *Cell. Immunol.* 104: 434–439.
46. Kline, J. N., T. J. Waldschmidt, T. R. Businga, J. E. Lemish, J. V. Weinstock, P. S. Thorne, and A. M. Krieg. 1998. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J. Immunol.* 160: 2555–2559.
47. Leonard, P., and S. Sur. 2003. Interleukin-12: potential role in asthma therapy. *BioDrugs* 17: 1–7.
48. Tsitoura, D. C., R. L. Blumenthal, G. Berry, R. H. Dekruyff, and D. T. Umetsu. 2000. Mechanisms preventing allergen-induced airways hyperreactivity: role of tolerance and immune deviation. *J. Allergy Clin. Immunol.* 106: 239–246.
49. Santeliz, J. V., G. Van Nest, P. Traquina, E. Larsen, and M. Wills-Karp. 2002. Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J. Allergy Clin. Immunol.* 109: 455–462.
50. Randolph, D. A., C. J. Carruthers, S. J. Szabo, K. M. Murphy, and D. D. Chaplin. 1999. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J. Immunol.* 162: 2375–2383.
51. Moverare, R., E. Vesterinen, T. Metso, R. Sorva, L. Elfman, and T. Haahtela. 2001. Pollen-specific rush immunotherapy: clinical efficacy and effects on antibody concentrations. *Ann. Allergy Asthma Immunol.* 86: 337–342.
52. Hinchin, M. S., L. K. Poulsen, F. Carat, C. Andre, A. B. Hansen, and H. J. Malling. 2004. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 59: 45–53.
53. Steinman, R. M. 2003. The control of immunity and tolerance by dendritic cell. *Pathol. Biol.* 51: 59–60.