

No defect in T-cell priming, secondary response, or tolerance induction in response to inhaled antigens in Fms-like tyrosine kinase 3 ligand-deficient mice

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Background: Respiratory tract dendritic cells (DCs) are crucial for the regulation of immune responses to inhaled antigens. However, the precise function of the multiple DC subsets present in the lungs and the lung-draining lymph nodes is unknown. Fms-like tyrosine kinase 3 ligand (FLT3L) is a hematopoietic growth factor that drives the development of multiple subsets of DCs in the lymphoid organs.

Objective: We sought to study the contribution of DC subsets in the regulation of the balance between tolerance and immunity against respiratory antigens by using *FLT3L* knockout mice.

Methods: Phenotypic analysis of DC subsets in the airways and lungs of *FLT3L* knockout mice was performed. By using various experimental models, the role of FLT3L-dependent DCs in the priming of naive T cells, the presentation of inhaled antigen to previously primed T_H2 cells, and intranasal tolerance induction was addressed.

Results: *FLT3L* knockout mice display a 90% reduction in lung parenchyma DCs but a normal number of airway DCs and blood monocytes. *FLT3L* knockout mice had a normal induction of eosinophilic inflammation in response to intranasal administration of allergen. FLT3L-dependent DCs were not required for the presentation of inhaled antigen to previously primed T_H2 cells, and normal induction of T-cell tolerance in response to inhaled antigen was observed in *FLT3L* knockout mice.

Conclusion: Airway DC development is independent of FLT3L. FLT3L-dependent DCs are not required for the development and maintenance of airway inflammation or for the induction of intranasal tolerance. Our results point to airway DCs as the major regulators of the balance between tolerance and immunity to inhaled antigens. (*J Allergy Clin Immunol* 2005;115:192-9.)

Key words: Dendritic cells, lung, inflammation, tolerance, knock-out

Abbreviations used

BAL: Bronchoalveolar lavage

CRA: Cockroach allergen

DC: Dendritic cell

FLT3L: Fms-like tyrosine kinase 3 ligand

LN: Lymph node

OVA: Ovalbumin

WT: Wild type

Dendritic cells (DCs) are established inducers of T-cell immunity and are also increasingly viewed as mediators of T-cell tolerance. In the absence of pathogens, most DCs are in an immature state, featuring the ability to capture antigens by means of endocytosis, phagocytosis, or both but poorly activating naive T cells and inducing T-cell anergy or regulatory T cells, which could be important for maintaining self-tolerance. When stimulated by pathogen products or by inflammation molecules, DCs undergo a maturation process that leads to the upregulation of costimulatory molecules, secretion of proinflammatory cytokines, and the capacity to induce antigen-specific naive T cells to differentiate into effector T cells capable of mediating immunity.^{1,2} In the past years, multiple DC subsets with various properties have been identified in lymphoid or nonlymphoid organs.³ Some of these subsets, such as plasmacytoid DCs or the CD8 α ⁺ DCs, have been suggested to be specialized in maintaining T-cell self-tolerance,³⁻⁵ whereas other DC subsets would be responsible for T-cell priming. The lack of reagents or genetic models allowing the selective depletion of specific DC subsets has, however, hampered the clear assignment of a defined function for each subset.

Allergic asthma is characterized by eosinophilic inflammation, mucus hypersecretion, and bronchial hyperactivity. Airway inflammation is controlled by T_H2 cells that secrete IL-4, IL-5, and IL-13, leading to the recruitment of eosinophils, mucus secretion, and isotype switching, which further leads to IgE secretion.⁶ DC depletion, as well as adoptive transfer studies, pointed to the DC as an essential cell type for the initiation and control of airway inflammation.^{7,8} Moreover, some DCs obtained from the lung-draining lymph nodes (LNs) of tolerized mice have the capacity to induce the development of antigen-specific

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regulatory T cells *in vitro* and mediate tolerance induced by respiratory exposure to antigen.⁹ Thus it is believed that DCs regulate the balance between tolerance and immunity against respiratory antigens.¹⁰ However, in the lung numerous DC subsets exist: in the airways, above and beneath the basement membrane (airway DCs), between the alveoli (interstitial DCs), or within the alveoli (alveolar DCs).^{7,11} What is unclear at present is the exact subtype of DC that mediates T-cell tolerance or immunity and whether the same subset can accomplish these functions.

The ligand for the receptor kinase fms-like tyrosine kinase 3 (FLT3L) is a growth factor for hematopoietic progenitors. Mice treated with FLT3L showed dramatic increases in the numbers of classical DCs and plasmacytoid DCs in blood and lymphoid tissues.^{12,13} FLT3L has also been shown to promote the development of DCs from bone marrow progenitors.¹⁴ Mice lacking FLT3L showed profound deficiencies in DCs. In particular, *FLT3L*^{-/-} mice have a 75% to 90% reduction in DCs, affecting all subsets in the spleen, as well as in the LNs.^{15,16} Our present report shows that *FLT3L*^{-/-} mice also have a 90% reduction in lung interstitial DCs, alveolar DCs, or both but that they have a normal number of airway DCs and blood monocytes.

In this article we took advantage of the unique features of *FLT3L*^{-/-} mice to better define the nature of DC subsets involved in either T-cell priming, secondary response, or T-cell tolerance to inhaled antigens. Our results suggest that FLT3L-dependent DCs are not required for any of these processes and point to airway DCs as the major regulators of the balance between tolerance and immunity to inhaled antigens.

METHODS

Mice

All mice were used at 7 to 10 weeks of age. Female C57BL/6 wild-type (WT) and *FLT3L*^{-/-} mice were purchased from Taconic (Germantown, NY). Female OT2 T-cell receptor–transgenic mice specific for chicken ovalbumin (OVA) peptide 323–339 (OT2p) in the context of I-A^b or OT2 crossed with congenic B6.PL-*Thy1a*/Cy mice (Thy1.1 obtained from The Jackson Laboratory, Bar Harbor, Me) were bred at Amgen Inc (Seattle, Wash). All mice were maintained under specific pathogen-free conditions, and an institutional animal care and use committee approved all experiments according to federal guidelines.

Flow cytometry

The cells were stained in FACS buffer (PBS containing 2% FBS, 1% normal rat serum, 1% normal hamster serum, 1% normal mouse serum, and 10 µg/mL 2.4G2 [a rat anti-mouse Fc receptor] mAb. The following mAbs (clone name given in parentheses) were used: CD4 (GK1.5), CD11b (M1/70), CD11c (HL3), CD45R/B220 (RA3-6B2), CD90.1 (OX-7), I-A^b (AF6-120.1), Ly-6C (AL-21), Gr-1 (RB6-8C5), Vα2 (B20.1), and Vβ5 (MR9-4) were from BD Biosciences (San Diego, Calif). F4/80 (CI:A3-1) was purchased from Caltag (Burlingame, Calif). CCR3 (83103) was purchased from R&D Systems (Minneapolis, Minn). Biotinylated mAb binding was detected with streptavidin-PerCp (BD Biosciences). Flow cytometric analyses were performed on a FACSCalibur with CellQuest software (both BD Biosciences).

Detection of airway DCs in whole mounts of the trachea

WT and *FLT3L*^{-/-} naive animals were anesthetized, and tracheal whole mounts were prepared as previously described.¹⁸ Briefly, tissues were fixed in formalin, permeabilized, and stained for MHC class II I-A^b. Tissues were then dehydrated in serial alcohol steps and cleared in HistoClear (National Diagnostics, Atlanta, Ga). The entire trachea was mounted in Vectamount mounting medium (Vector Labs, Burlingame, Calif). At least 8 tracheas from WT and *FLT3L*^{-/-} mice were stained, and the cellular density of the airway DC network (cells per field) was blindly assessed during microscopy by 2 separate investigators. The data represent the mean number of cells ± SEM per field counted in at least 10 separate fields.

Detection of lung DCs

WT and *FLT3L*^{-/-} mice were killed, and the pulmonary circulation was perfused through the heart with saline to remove the intravascular pool of cells. Mediastinal LNs, the thymus, and the heart were removed, and lungs were harvested. Lungs were cut in small pieces and digested in Dulbecco modified Eagle medium 5% FBS containing 200 U/mL collagenase (Invitrogen, Carlsbad, Calif) for 60 minutes with occasional pipetting. Cells were further dissociated in Versene (Invitrogen) and filtered on a 70-µm screen, washed, counted, and stained in FACS buffer. DCs were identified as low autofluorescent CD11c⁺ I-Ab⁺ or CD11c⁺ CD11b⁺ cells.¹⁹

Detection of blood monocytes

WT and *FLT3L*^{-/-} mice were anesthetized, and blood was collected in heparinized tubes. RBCs were lysed in ammonium, chloride, potassium buffer, and cells were washed and stained with anti-CD11b, anti-Ly-6C, anti-Gr1, and anti-F4/80 mAbs in FACS buffer. Blood monocytes were identified as CD11b⁺ Ly-6C^{high} cells, as recently described.²⁰

Bronchoalveolar lavage

One or 2 days (see below) after the last antigen challenge, mice were killed by means of (Avertin; 2,2,2 tribromoethanol; Sigma, St Louis, Mo) overdose, followed by exsanguination. BAL was performed with 2 × 0.5 mL of Ca²⁺- and Mg²⁺-free HBSS supplemented with EDTA. Bronchoalveolar lavage (BAL) specimens were centrifuged, and cells were resuspended in FACS buffer. Differential cell counts were either performed on cytospin preparations stained with Diff-Quick (Dade Behring, Newark, NJ) by means of classification of 200 cells on standard morphologic criteria or by means of flow cytometric analysis. Eosinophils were identified as CCR3⁺ CD11b⁺ F4/80⁻ and Gr-1^{int} cells, as previously described,¹⁸ and OT2 T-cell receptor–transgenic cells were identified as CD4⁺ Vα2⁺ and Vβ5⁺ cells.

Lung histopathology

Mice were killed, and fixative (10% formalin) was gently infused through the trachea. Then the lungs were resected and fixed for an additional 4 hours. After routine paraffin embedding, sections were stained with hematoxylin and eosin for quantification of inflammatory infiltrates and periodic acid–Schiff to highlight the mucus-secreting cells.

Induction of airway inflammation

WT and *FLT3L*^{-/-} mice were anesthetized with isoflurane (Abbott Laboratories, Chicago, Ill) before intranasal instillation of 100 µg of American cockroach allergen (CRA; Greer Laboratories, Lenoir, NC) in 50 µL of saline. Mice were dosed 3 times a week for 2 weeks. All harvests occurred 48 hours after the last challenge.

Generation and adoptive transfer of OVA-specific OT2 T_H2 cells

LN and spleen cells from OT2 mice were pooled and cultured in T_H2-polarizing conditions for 4 days (OVA peptide, 5 µg/mL; IL-2, 1 ng/mL; IL-4, 20 ng/mL; anti-IFN-γ, 10 µg/mL; and anti-IL-12 p70, 1 µg/mL). At the end of the culture, CD4⁺ cells were isolated by means of negative selection (StemSep CD4⁺ T cell enrichment kit; StemCell Technologies, Vancouver, British Columbia, Canada), and 2 × 10⁶ cells were injected intravenously in naive WT and *FLT3L*^{-/-} mice. Starting 1 day after transfer, mice were challenged by means of intranasal instillation of 100 µg of OVA (chicken egg albumin; EMD Biosciences, San Diego, Calif) for 7 consecutive days. Samples were harvested 24 hours after the last challenge.

Induction of intranasal tolerance

CD4⁺ T cells from LNs and spleen from OT2 CD90.1 mice were purified by means of negative selection (StemSep CD4⁺ T cell enrichment kit, StemCell Technologies), and 1 × 10⁶ cells were injected intravenously in naive WT and *FLT3L*^{-/-} mice. One day after transfer, mice were challenged by means of intranasal instillation of 100 µg of OVA or saline for 3 consecutive days or left untreated. Eight days after the last intranasal challenge, mice were immunized with 100 µg of OVA emulsified in Ribi adjuvant (Corixa, Seattle, Wash) subcutaneously in the footpads or left untreated. Four days after immunization, popliteal LN cells of individual mice were collected, and OVA-specific OT2-transgenic expansion was measured by means of flow cytometry with anti-CD4, Vα2, and anti-CD90.1. LN cells were also restimulated *in vitro* with graded doses of OVA in the presence of C57BL/6 spleen cells as feeders in Iscove's modified Dulbecco's medium supplemented with 0.5% heat-inactivated normal mouse serum and additives (penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 2-mercaptoethanol, HEPES, and L-glutamine). Triplicate culture supernatants were assayed by means of 2-site ELISA (Pharmingen) for IL-2 after 24 hours of incubation and for IFN-γ after 72 hours of incubation. The detection limits were 20 pg/mL for IL-2 and 80 pg/mL for IFN-γ.

RESULTS

FLT3L^{-/-} mice have normal numbers and distribution of airway DCs and blood monocytes but reduced numbers of interstitial DCs, alveolar DCs, or both

Previous reports have documented the reduction of DCs in the lymphoid organs of *FLT3L*^{-/-} mice.^{15,16} By contrast, much less is known about the role of *FLT3L* for the development of DCs in nonlymphoid organs, such as the lung. Therefore the cellular density of the DC network lining the airways was first visualized in tracheal whole mounts prepared from WT and *FLT3L*^{-/-} mice by staining for MHC class II expression (Fig 1, A). No difference in the density of this network was observed between WT and *FLT3L*^{-/-} mice (Fig 1, A). Next, enzyme-digested lung cells were analyzed by means of flow cytometry for the presence of DCs assessed by means of double staining for CD11c and MHC class II and CD11c and CD11b expression. As described before, 2 populations of CD11c⁺ cells could be discriminated on the basis of autofluorescence and MHC class II expression. MHC class II-negative high autofluorescent cells

and MHC class II-positive low autofluorescent cells correspond to alveolar macrophages and intrapulmonary DCs, respectively (data not shown).¹⁹ We therefore restricted our analysis to low autofluorescent cells. At least 2 subsets of DCs can be identified on the basis of CD11b expression (Fig 1, B), but these DCs do not express CD4 or CD8α, as opposed to DCs found in lymphoid organs (data not shown). Ninety percent fewer DCs were observed in the lungs of *FLT3L*^{-/-} mice compared with WT mice (Fig 1, B). Finally, the percentage of blood monocytes was compared in WT and *FLT3L*^{-/-} mice because monocytes are known to migrate into nonlymphoid tissues and to differentiate into DCs in response to inflammatory signals. Monocytes were defined as Ly-6C^{high} CD11b⁺ cells, as recently described.²⁰ No significant difference in the percentage of monocytes was observed between WT and *FLT3L*^{-/-} mice (Fig 1, C). Thus *FLT3L*^{-/-} mice have normal numbers of airway DCs and blood monocytes but reduced numbers of interstitial DCs, alveolar DCs, or both and DCs residing in lymphoid organs.¹⁵

Normal induction of eosinophil accumulation and peribronchial inflammation in response to intranasal administration of CRA in *FLT3L*^{-/-} mice

The implication of *FLT3L*-dependent DCs in the priming of naive T cells in response to inhaled antigens was tested. Repetitive local administration of CRA was performed in WT and *FLT3L*^{-/-} mice (Fig 2, A). Forty-eight hours after the last CRA challenge, BAL specimens were collected from *FLT3L*^{-/-} and WT mice for further analysis. In contrast to saline-treated mice, CRA-challenged mice had a significant infiltration of inflammatory cells in BAL specimens composed primarily of eosinophils (70% of total cells). The total number of infiltrating cells and the percentage of eosinophils in BAL specimens were comparable in *FLT3L*^{-/-} and WT mice (Fig 2, B). There was no difference in the number of lymphocytes, neutrophils, or macrophages present in the BAL specimens between the groups of mice (Fig 2, B, and data not shown). Histopathologic analysis of affected lungs revealed significant perivascular and peribronchiolar infiltration of inflammatory cells, consisting primarily of mononuclear cells and eosinophils, and mucus-secreting cells, which was indistinguishable between *FLT3L*^{-/-} and WT animals (Fig 2, C). Thus these data show that *FLT3L*-dependent DCs are not required for the priming of CRA-specific naive T cells and the induction of airway inflammation in response to intranasal administration of CRA.

FLT3L-dependent DCs are not required for the presentation of inhaled antigen to previously primed T_H2 cells, leading to allergic airway disease

CD11c⁺ cells have been shown to be essential for presenting inhaled antigen to previously primed T_H2

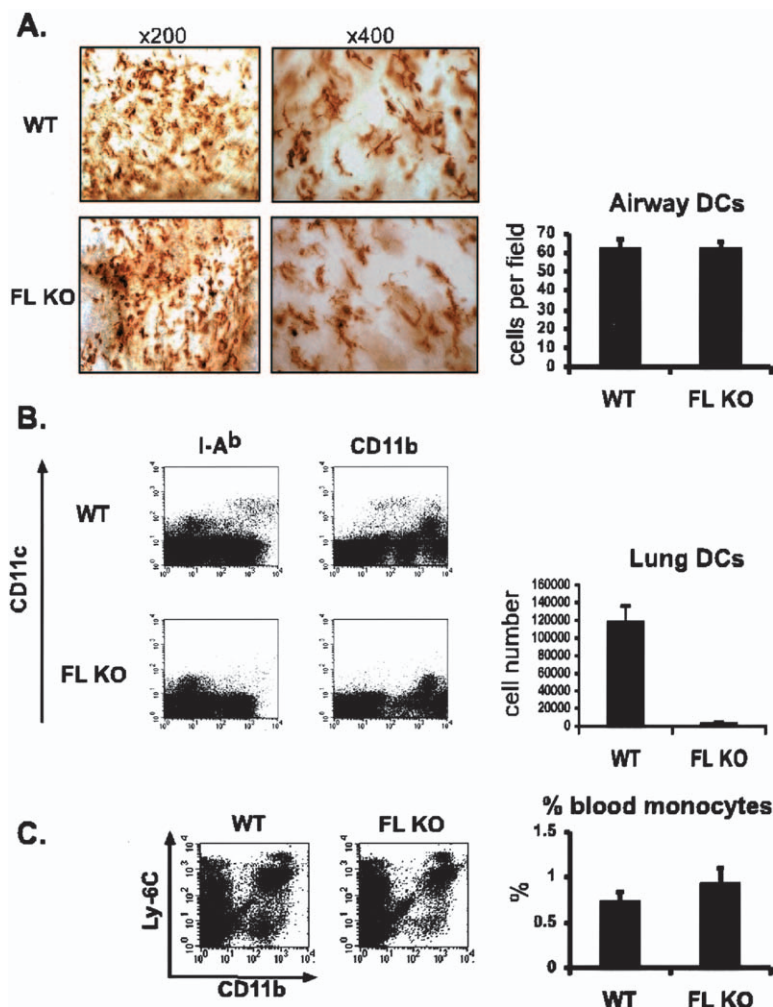


FIG 1. *FLT3L*^{-/-} mice have normal numbers of airway DCs and blood monocytes but reduced numbers of interstitial DCs, alveolar DCs, or both. **A**, Tracheal whole mounts from WT and *FLT3L*^{-/-} (FL KO) animals were stained with anti-MHC class II I-A^b, and the number of DCs was quantified. **B**, Total lung cells from WT and *FLT3L*^{-/-} animals were stained with anti-CD11c and anti-I-A^b or anti-CD11b mAbs. An electronic gate was performed on autofluorescent-negative cells to exclude alveolar macrophages, and CD11c versus I-A^b or CD11b expression was analyzed. The graph represents the total number of CD11c⁺ I-A^b⁺ DCs per lung ± SEM from 5 to 6 animals per group. **C**, Total blood cells were stained with anti-Ly-6C, anti-CD11b, anti-F4/80, and anti-Gr1 mAbs, and Ly-6C versus CD11b expression was analyzed. The graph represents the percentage of blood monocytes (Ly-6C^{high} CD11b⁺) ± SEM combined from 2 independent experiments with 2 to 3 animals per group.

cells in the lung, leading to chronic eosinophilic airway inflammation.⁷ WT and *FLT3L*^{-/-} mice were adoptively transferred with OVA-specific OT2 T_H2 effector CD4⁺ T cells (see experimental procedures) and challenged intranasally for 7 days, starting the day after adoptive transfer (Fig 3, A), to test the implication of FLT3L-dependent DCs in this process. Twenty-four hours after the last challenge, mice were killed, and the total number of infiltrating cells in the BAL specimens was enumerated. As shown in Fig 3, B, there was no difference in the total number of cells, eosinophils, or OVA-specific T_H2 cells present in the BAL specimens from OVA-challenged *FLT3L*^{-/-} mice compared with that seen in specimens from WT mice. Histologic examination of

lung sections did not reveal any differences in the degree of lung inflammation, tissue eosinophilia, and mucus-secreting cells between the 2 groups of mice (Fig 3, C). Some animals were killed and analyzed 24 hours after the last of 3 OVA challenges to test for an early difference in responsiveness to antigen challenge between WT and *FLT3L*^{-/-} mice. Lung eosinophilia was reduced compared with that seen in animals challenged for 7 days, but no significant difference was observed between WT and *FLT3L*^{-/-} mice (data not shown). Thus these data show that FLT3L-dependent DCs are not required for the presentation of inhaled antigen to previously primed T_H2 cells, leading to allergic airway disease.

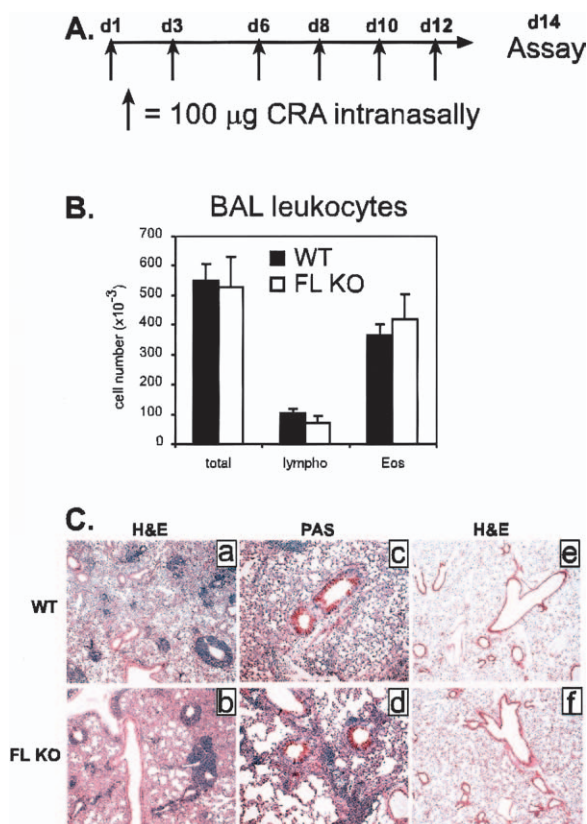


FIG 2. Normal induction of eosinophil accumulation and peribronchial inflammation in response to intranasal administration of CRA in *FLT3L*^{-/-} mice. **A**, Experimental protocol for treatment. Groups of 10 WT and *FLT3L*^{-/-} mice were treated with intranasal instillation of 100 µg of CRA in saline 3 times a week for 2 weeks. **B**, Total leukocyte numbers were enumerated in BAL fluid, and total numbers of lymphocytes and eosinophils were calculated from differential stained BAL cytospin preparations. Results are the mean number of cells ± SEM and are representative of 2 separate experiments performed with 10 mice per group. **C**, Lung tissue from WT and *FLT3L*^{-/-} mice challenged with CRA (*a* and *c* and *b* and *d*, respectively) and naive WT and *FLT3L*^{-/-} mice (*e* and *f*, respectively) were stained with hematoxylin and eosin (H&E; original magnification 40×; *a*, *b*, *e*, and *f*) for identification of inflammatory infiltrates and periodic acid-Schiff (PAS; original magnification 100×, purple-red staining; *c* and *d*) to highlight mucus-producing cells. *FL KO*, *FLT3L*^{-/-}.

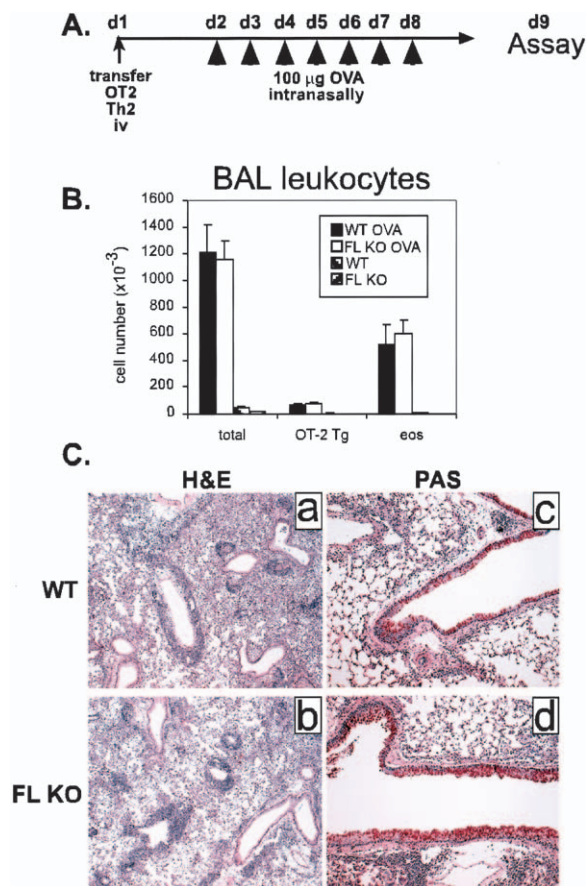


FIG 3. *FLT3L*-dependent DCs are not required for the presentation of inhaled antigen to previously primed Th2 cells, leading to allergic airway disease. **A**, Experimental protocol for treatment. OVA-specific Th2 effector cells were generated *in vitro* from OT2 T-cell receptor-transgenic mice, as described in the Methods section. Cells were injected intravenously into naive WT or *FLT3L*^{-/-} mice. Recipients were exposed the next day to intranasal instillation of 100 µg of OVA for 7 consecutive days. **B**, Total leukocyte numbers were enumerated in BAL fluid, and total numbers of OVA-specific OT2-transgenic and eosinophils were calculated from BAL specimens by means of flow cytometry. Results are the mean number of cells ± SEM and are representative of 4 separate experiments performed with 6 mice per group. **C**, Lung tissue from WT and *FLT3L*^{-/-} mice challenged with OVA (*a* and *c* and *b* and *d*, respectively) was stained with hematoxylin and eosin (H&E; original magnification 40×; *a* and *b*) for identification of inflammatory infiltrates and periodic acid-Schiff (PAS; original magnification 100×, purple-red staining; *c* and *d*) to highlight mucus-producing cells. *FL KO*, *FLT3L*^{-/-}.

Normal induction of T-cell tolerance in response to inhaled antigen in *FLT3L*^{-/-} mice

WT and *FLT3L*^{-/-} mice that had been adoptively transferred with OVA-specific OT2 naive CD4⁺ T cells were treated with intranasal OVA or PBS and then immunized subcutaneously in the footpad with OVA in Ribi to test for the importance of *FLT3L*-dependent DCs in tolerance induction, as depicted in Fig 4, A. The expansion of OT2 CD4⁺ T cells in response to subcutaneous OVA was measured in the draining LNs. As previously described, OT2 CD4⁺ T cells failed to expand in OVA-pretreated WT animals compared with in saline-pretreated WT mice. A similar phenomenon was observed

in OVA-pretreated *FLT3L*^{-/-} mice, except that the magnitude of OT2 expansion in saline-pretreated WT animals was slightly but not significantly lower in the saline-pretreated *FLT3L*^{-/-} mice, as shown in Fig 4, B. Draining LN cells from the different groups of animals were also restimulated *in vitro* with OVA, and their cytokine production was measured. T cells from the OVA-pretreated mice failed to produce cytokines in response to OVA *in vitro* in contrast to cells from mice that had received intranasal saline. No difference was observed

between WT and *FLT3L*^{-/-} mice. Thus these results show that FLT3L-dependent DCs are not required for the induction of tolerance in response to inhaled antigen.

DISCUSSION

DCs are now widely recognized as the most efficient professional antigen-presenting cells and are uniquely equipped to induce and regulate immune responses. In the airways DCs have been shown to reside in the airway epithelium, the lung interstitium, and the lung-draining LNs.^{7,11} DC depletion with the thymidine kinase–ganciclovir system has been shown to eliminate the asthmatic features induced by antigen aerosol.⁷ However, because this system induced the depletion of all DCs from the airways, LNs, and spleen, the relative contribution of each DC subset in the induction of airway inflammation in response to respiratory antigens is still unknown.

Our results show that DC number and localization in the airway epithelium (resembling DCs of the skin or Langerhans cells) is not affected by the absence of the DC growth factor FLT3L, whereas all other subsets of DCs in the lung interstitium (this report), LNs, and spleen^{15,16} are reduced by 70% to 90%. This suggests that the airway DCs are a particular subset of DCs and that their development or recruitment to the epithelium requires another growth factor. These results identify FLT3L knockout mice as the only mouse system in which the role of airway DCs in the regulation of immunity versus tolerance to inhaled antigens can be specifically addressed without manipulation, such as antibody treatment or drug injection. Our data show that the development and maintenance of airway inflammation, as well as the induction of intranasal tolerance, is similar in WT and FLT3L knockout mice. Because the only DCs present in the lungs of FLT3L knockout mice are airway DCs, our data strongly suggest that DCs mediating tolerance and immunity in our experimental conditions are airway DCs themselves. These results are consistent with and extend previous findings about airway-derived DCs but not resident LNs. DCs present fluorescent proteins after migrating into the draining LNs in the absence of inflammation.¹⁹ In addition, after aerosol challenge in a rat model, resident airway DCs, but not lung parenchymal DCs, acquire antigen and rapidly mature into potent antigen-presenting cells after cognate interactions with T memory cells in the late phase of asthma.²¹

Previous reports showed that the development of airway inflammation was accompanied by an increase in the number of DCs in the lungs, the trachea, and the draining LNs.^{18,22,23} We also observed an increase in the number of DCs in the lungs and draining LNs of WT mice and, to a slightly lesser extent, in *FLT3L*^{-/-} mice on challenge with OVA in our T_H2 adoptive transfer model (data not shown). These FLT3L-independent DCs could be monocyte-derived DCs locally expanded by an increase in GM-CSF, the only other known growth factor for DCs, because we show here that *FLT3L*^{-/-} mice have

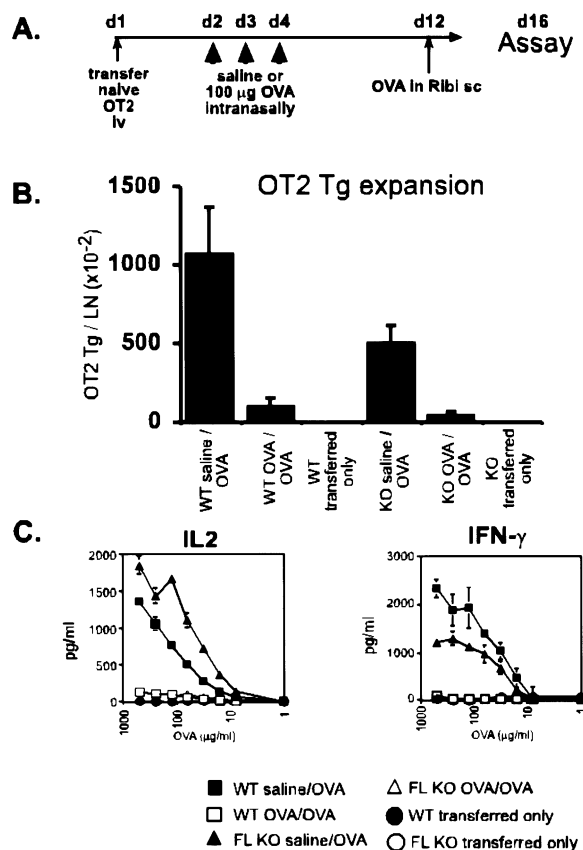


FIG 4. Normal induction of T-cell tolerance in response to inhaled antigen in *FLT3L*^{-/-} mice. **A**, Experimental protocol for treatment. CD4⁺ T cells from OVA-specific OT2 T-cell receptors were transferred intravenously into naive WT or *FLT3L*^{-/-} mice. Recipients were exposed the next day to intranasal instillation of saline or 100 µg of OVA for 3 consecutive days or left untreated. Eight days after the last instillation, mice were immunized with 100 µg of OVA emulsified in Ribi subcutaneously in the footpads or left untreated. **B**, Four days after immunization, T-cell expansion was measured by means of flow cytometric analysis of popliteal LN cells of individual mice. The figure represents the mean expansion ± SEM combined for 2 independent experiments with 3 to 4 mice per group. **C**, IL-2 and IFN-γ production by pooled LN cells restimulated *in vitro* with graded concentrations of OVA protein. FL KO, *FLT3L*^{-/-}.

the same proportion of blood monocytes as WT mice. Therefore it is possible that in the course of airway inflammation, these recruited DCs could participate in the amplification of the response, although it is not known whether they can capture and present the antigen. As supporting evidence, blocking GM-CSF has been shown to reduce the features of asthma.²⁴ Moreover, in human PBMC-reconstituted mice with severe combined immunodeficiency syndrome, it has been shown that monocyte-derived DCs might play a key role in the pathogenesis of the pulmonary allergic response by inducing T_H2 effector function after migration to the mesenteric LNs.²⁵ Thus naive T cell priming in airway-draining LNs could be initiated by airways DCs and then sustained by recruited FLT3L-independent monocyte-derived DCs as inflammation develops in the airways. Such cooperation between

different types of DCs has already been shown to occur in skin-draining LNs in the course of a T-cell response against an intradermally injected antigen.²⁶

Regarding tolerance, previous studies have suggested a role for certain DC subtypes, such as plasmacytoid DCs (pDCs) or CD8 α^+ DCs, in the induction or maintenance of T-cell tolerance. Other reports have shown in a mouse model that the superficial cervical and internal jugular LNs that drain the nasal mucosa are instrumental in the induction of tolerance against an inhaled antigen.²⁷ Umetsu and colleagues^{9,28} identified a population of ICOSL $^+$, IL-10-producing tolerizing DCs present in the bronchial LNs of mice exposed to respiratory allergen. The nature and origin of these DCs were not known. Surprisingly, our present data show that in the virtual absence of any potential tolerogenic DC subtype (pDCs and CD8 α^+ DCs) in lymphoid organs,¹⁶ the development of intranasal tolerance was similar in WT and FLT3L knockout animals, suggesting that airway DCs could directly regulate the balance between tolerance and immunity to inhaled antigens. As recently proposed, intranasal harmless OVA exposure could be sufficient to induce their partial maturation, leading to their migration to the LNs, where T-cell tolerance occurs.²⁹

FLT3L has been proposed to be used as an adjuvant because it could prevent peripheral tolerance induction to a systemically administered soluble antigen.³⁰ On the contrary, subsequent reports showed that FLT3L treatment could augment oral T-cell tolerance induction³¹ or reverse the late allergic response and airway hyperresponsiveness in a mouse model of allergic inflammation,³² making unclear the role of FLT3L dependent-expanded DCs in the induction of immunity versus tolerance. Our data demonstrate that FLT3L-dependent DCs do not control the balance between tolerance and immunity to an inhaled antigen. It is possible that dramatically increasing the number of immature DCs subsequent to FLT3L injection might alter the balance between tolerance induction and immunity, depending on the local environment. These data are in agreement with previous reports showing that FLT3L-expanded DCs have a tolerogenic or immunogenic potential, depending on their maturation stage.^{16,33} Thus they support a model in which the induction of tolerance or immunity to exogenous antigens does not rely on specialized subsets of DCs but rather on the inflammatory context in which DCs capture the antigen in tissues. Moreover, our present data clearly indicate that targeting FLT3L in asthmatic patients would not be a useful therapy because DCs involved in the initiation and development of airway inflammation are FLT3L independent. Because GM-CSF is the only other growth factor known to expand DCs *in vitro* and *in vivo*, it will be interesting to test the effect of blocking antibodies against GM-CSF on DC accumulation and the development of inflammation in the lungs of asthmatic individuals.

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