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Group V Secretory Phospholipase A2 Reveals Its Role in House Dust Mite-Induced Allergic Pulmonary Inflammation by Regulation of Dendritic Cell Function

Giorgio Giannattasio,*†‡ Daisuke Fujioka,*† Wei Xing,*† Howard R. Katz,*† Joshua A. Boyce,*†‡§ and Barbara Balestrieri*†‡

We have previously shown that group V secretory phospholipase A2 (sPLA2) regulates phagocytosis of zymosan and *Candida albicans* by a mechanism that depends on fusion of phagosomes with late endosomes in macrophages. In this study, we report that group V sPLA2 (*Pla2g5*)-null mice exposed to an extract of house dust mite *Dermatophagoides farinae* had markedly reduced pulmonary inflammation and goblet cell metaplasia compared with wild-type (WT) mice. *Pla2g5*-null mice had also impaired Th2-type adaptive immune responses to *D. farinae* compared with WT mice. *Pla2g5*-null bone marrow-derived dendritic cells (BMDCs) activated by *D. farinae* had delayed intracellular processing of allergen and impaired allergen-dependent maturation, a pattern recapitulated by the naive lung DCs of *D. farinae*-challenged mice. Adoptively transferred *D. farinae*-loaded *Pla2g5*-null BMDCs were less able than *D. farinae*-loaded WT BMDCs to induce pulmonary inflammation and Th2 polarization in WT mice. However, *Pla2g5*-null recipients transferred with WT or *Pla2g5*-null *D. farinae*-loaded BMDCs exhibited significantly reduced local inflammatory responses to *D. farinae*, even though the transfer of WT BMDCs still induced an intact Th2 cytokine response in regional lymph nodes. Thus, the expression of group V sPLA2 in APCs regulates Ag processing and maturation of DCs and contributes to pulmonary inflammation and immune response against *D. farinae*. Furthermore, an additional yet to be identified resident cell type is essential for the development of pulmonary inflammation, likely a cell in which group V sPLA2 is upregulated by *D. farinae*, and whose function is also regulated by group V sPLA2. *The Journal of Immunology*, 2010, 185: 4430–4438.

Phospholipases A2 (PLA2s) are a family of enzymes that cleave the ester-bound fatty acids from membrane phospholipids (1, 2). PLA2-liberated arachidonic acid is the substrate for prostaglandin (PG) and leukotriene generation, a function that absolutely requires cytosolic PLA2α (3). Although members of the PLA2 family share common functions, such as generation of eicosanoids and lysophospholipids, some PLA2s have additional cell type-specific functions dictated by their subcellular localization. We have previously reported that group V secretory PLA2 (sPLA2) (sPLA2α) is expressed in the trans-Golgi network and recycling endosomes of mouse peritoneal macrophages (4, 5). Furthermore, group V sPLA2, but not cytosolic PLA2α, regulates phagocytosis of zymosan by peritoneal macrophages (5, 6). We have shown that mouse peritoneal macrophages lacking group V sPLA2 have delayed fusion of phagosomes with late endosomes and lysosomes, leading to defective phagocytosis and killing of *C. albicans* and increased susceptibility to *Candida* infection (7).

Phagocytes (neutrophils, macrophages, and dendritic cells [DCs]) are a heterogeneous population of cells that can ingest particles. The fate of the ingested particles depends on the characteristics of the phagocytic cell and of the particles ingested (pathogens, infected cells, apoptotic cells). Phagocytes derived from the monocyte lineage, such as macrophages, effectively clear pathogens and have modest Ag-presenting function. In contrast, DCs, also derived from the monocyte lineage, are potent APCs. Immature DCs reside proximal to mucosal surfaces, such as those lining the airways (8). When immature DCs encounter Ags in the context of a pathogen-associated molecular pattern (PAMP), they undergo maturation, migrate to the lymph nodes, and present the Ag to T cells (9, 10). This process initiates the adaptive immune response. The PAMP-induced activation step is absolutely required for DCs to undergo maturation and drive adaptive immune responses (8, 10, 11). Allergens are ingested by DCs through endocytosis, processed, and presented on MHC class II (MHC-II) to CD4+ Th cells. OVA, the most commonly used Ag in studies of allergen-induced pulmonary inflammation, activates DCs only in the context of exogenous adjuvants. Instead, natural allergens often activate DCs by carrying endogenous adjuvants that mimic PAMPs, thereby driving DC maturation and the subsequent adaptive immune response. In particular, allergens derived from house dust mites can directly activate DCs through protein and carbohydrate structures that mimic PAMPs and stimulate pattern recognition receptors on DCs and other cells (12–15).

Previously, group V sPLA2 was shown to be necessary for the development of airway hyperresponsiveness in an OVA-induced mouse model of airway inflammation (16). The mechanisms and...
cell targets by which group V sPLA2 contributes to pulmonary inflammation were yet to be defined. Because we had previously demonstrated that group V sPLA2 is essential for phagocytic function of peritoneal macrophages, we postulated that it may also have a role in the processing of Ags by DCs. In the present study, we used an extract of the house dust mite *Dermatophagoides farinae*, which can drive maturation of endogenous lung DCs and induces pulmonary inflammation without need for systemic immunization, to investigate the role of group V sPLA2 in the maturation of DCs, Ag processing, and in the induction of the *D. farinae*-specific immune response and consequent pulmonary inflammation.

**Materials and Methods**

**D. farinae-induced pulmonary inflammation**

Groups of 7- to 9-wk-old C57BL/6 wild-type (WT) and *Pla2g5*-null (4) mice received *D. farinae* extract (3 μg) (Guer Laboratories, Lenoir, NC) in 20 μl NaCl 0.9% (containing <0.005 endotoxin unit/ml) (Sigma-Aldrich, St. Louis, MO) or saline alone intranasally on days 0, 4, 7, 11, 14, and 18 (14). Twenty-four hours after the last treatment, mice were euthanized and exsanguinated, and bronchoalveolar lavage (BAL) was performed. BAL fluid cells were cytocentrifuged onto slides, stained with Diff-Quick (Fisher 14, and 18 (14). LTE4) (GE Healthcare BioSciences, Buckinghamshire, U.K.), PGD2, and demonstrated that group V sPLA2 is essential for phagocytic func-

**Histologic assessment of pulmonary inflammation**

Left lungs were collected from the mice at the time of euthanasia, fixed for at least 8 h in 4% paraformaldehyde, washed twice with PBS containing 2% DMSO, suspended in 50 mM NH4Cl overnight at 4˚C, and finally embedded in glycolmethacrylate or paraffin. Two-micrometer-thick sections were stained by the chloroacetate esterase (CAE) reaction to assess inflammatory cell infiltrates. For histological study of the mucus-secreting cells of the airway epithelia (goblet cells), lung sections were stained with periodic acid-Schiff (PAS). Congo red (CR) dye was used to highlight eosinophil infiltrates. The extent of cellular infiltration of the tissue was evaluated on 15 bronchovascular bundles (BVBs) of comparable large-

**In vitro restimulation of parabronchial lymph node cells**

The parabronchial lymph node (PLN) was collected from the upper-right chest of each mouse and homogenized in complete medium (RPMI 1640, 10% FBS, 1% nonessential amino acids, 2 mM l-glutamine, 100 U/ ml penicillin, 100 μg/ml streptomycin, 50 μg/ml 2-ME, Sigma-Aldrich) plus 2.5 mM β-mercaptoethanol and 1 mM pyruvate. The cell suspension of 4 × 10^6 nucleated cells was incubated for 72 h with medium alone or containing 20 μg/ml *D. farinae* (14). Cytokine release in the supernatants was measured by ELISA (eBioscience, San Diego, CA).

**Measurement of serum IgG**

Total IgE levels were measured by ELISA (BD Biosciences, San Jose, CA). To measure *D. farinae*-specific IgE, mouse serum was incubated at 4˚C overnight in a 96-well plate coated with anti-mouse IgE (BD Biosciences). Bound *D. farinae*-IgE was detected by adding biotinylated *D. farinae*, streptavidin-HRP conjugate (BD Biosciences), and tetramethylbenzidine substrate solution (Invitrogen). Absorbance was read at 450–570 nm and the results were expressed as net optical density (O.D.) (i.e., sample O.D. – blank O.D.). *D. farinae*-specific IgG was measured by ELISA with plate-bound *D. farinae*, an alkaline phosphatase-conjugated anti-mouse IgG (SouthernBiotech, Birmingham, AL) and substrate solution (p-nitrophenyl phosphate; Sigma-Aldrich). Absorbance was read at 405 nm, and the results were expressed as net O.D.

**Bone marrow-derived DC generation and adoptive transfer**

Bone marrow-derived DCs (BMDCs) were obtained as described (17). Briefly, bone marrow from femurs and tibiae of mice was collected, dis- associated, and resuspended at a concentration of 4 × 10^6/ml in RPMI 1640, 10% FBS, 1% nonessential amino acids, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml 2-ME, Sigma-Aldrich) plus 2.5 mM β-mercaptoethanol. Cells were incubated for 48 h with 2.5 μg/ml laminin (BD Biosciences) and 2 μg/ml *D. farinae* in complete medium containing 20 ng/ml recombinant murine GM-CSF (PeproTech, Rocky Hill, NJ). On days 3, 6, and 8, half of the culture supernatant was replaced by an equal volume of fresh medium containing GM-CSF. On day 9, the floating cells were harvested; an aliquot was cultured in complete medium with 100 μg/ml *D. farinae* (BMDCs-BMDCs) (18). After 24 h, cells were analyzed by flow cytometry or instilled intra-

**Flow cytometry**

BMDCs or *D. farinae*-BMDCs were fixed in 2% paraformaldehyde, blocked, and incubated (1 h, 4˚C) with the following Abs: FITC–MHC-II (clone RM134L; BioLegend, San Diego, CA), or with corresponding isotypes as controls. The acquisition was performed on a FACScanto flow cytometer with FACS Diva software (BD Biosciences), and data were analyzed by FlowJo (Tree Star, Ashland, OR).

**Processing of *D. farinae* by BMDCs**

BMDCs were seeded on glass-bottomed 35-mm dishes (World Precision Instruments, Sarasota, FL) and labeled with Cell Tracker Orange (5-(and-6)-[(4-chloromethyl)benzoyl]amino)tetramethylrhodamine) (CMTMR; Invitrogen) according to the manufacturer’s protocol. After 4 h, 100 μg/ml *D. farinae* labeled with an Alexa Fluor 488 labeling kit (Invitrogen) was gently pelleted on top of the BMDCs. These dishes were incubated for 45 min on ice, washed to remove unbound Ag, and transferred to a heated Nikon CI confocal system (7) equipped with a ×40 Planapo lens (NA, 1.3). Each image was volume rendered from 10 to 12 Z-stacks of 0.5 μm by a pathologist without knowledge of the particular mouse genotype or procedure. The goblet cells positively stained for the presence of mucus at the PAS reaction were enumerated in at least four independent BVBS of each lung, and data were expressed as the average of goblet cell counts stained in the bronchi of each section per millimeter of bronchial basal lamina, as measured by IM (National Institutes of Health image analysis soft-

**Real-time quantitative PCR of mRNA transcripts in the lung**

Right lungs were collected at time of euthanasia and snap frozen. Total RNA was isolated from tissue homogenates with TRI Reagent (Sigma-Aldrich), reverse transcribed into cDNA (RT2 First Strand kit; SABiosciences, Frederick, MD), and assayed by real-time quantitative PCR (qPCR) for IL-5, IL-13, IL-17A, IFN-γ, Muc5ac, Clca3/Gob5, and GAPDH on an Mx™ (Stratagene, La Jolla, CA) with the use of SYBR Green/ROX master mix (SABiosciences). The mRNA relative to the GAPDH mRNA was calculated with the ΔΔCt method. Primers (Supplemental Table I) were designed with PrimerQuest software (IDT, Coralville, IA).
regulating the local innate and adaptive immune responses. The intranasal administration of 3 μg of *D. farinae* twice weekly for 3 wk induced in WT mice significant increases in total BAL fluid cell numbers and eosinophils (Fig. 1A). In contrast, the total number of cells in the BAL fluid of *D. farinae*-treated *Pla2g5*-null mice was no different from the levels of the saline controls (Fig. 1A). Compared to *D. farinae*-treated WT controls, *Pla2g5*-null mice had significantly reduced numbers of total BAL fluid cells and eosinophils (47.35 ± 0.27 versus 8.28 ± 0.67 per 15 BVBs; *p* < 0.01) and markedly fewer mucus-producing goblet cells (47.35 ± 0.27 versus 9.57 ± 1.64/ mm of bronchial basal lamina; *p* < 0.01).

Histologic evaluation of the lung tissue (Fig. 1B) revealed that BVBs were invested with inflammatory infiltrates consisting of mononuclear cells along with eosinophils, neutrophils, lymphocytes and plasma cells, and mucus-producing goblet cells in the WT mice treated with *D. farinae*. Compared to the *D. farinae*-treated WT mice, the lung tissue of *D. farinae*-treated *Pla2g5*-null mice showed markedly reduced BVB inflammation and goblet cell metaplasia. Morphometric analysis of lung preparations revealed that compared with WT controls, *Pla2g5*-null mice had significantly fewer BVBs invested with cellular infiltrates (14.06 ± 0.67 versus 8.28 ± 0.67 per 15 BVBs; *p* < 0.01) and markedly fewer mucus-producing goblet cells (47.35 ± 0.27 versus 9.57 ± 1.64/ mm of bronchial basal lamina; *p* < 0.01).

Because group V sPLA2 has been reported to contribute to eicosanoid generation in certain circumstances (4, 20–22), and eicosanoids have been reported to play a pivotal role in promoting the development of a proallergic Th2-type immune response (23–25), we measured the concentration of eicosanoids in the BAL fluids of WT and *Pla2g5*-null *D. farinae*-treated mice. There were not marked differences between the two genotypes (data not shown).

We then evaluated the effect of the loss of group V sPLA2 on the expression by the lung of cytokines and other inducible transcripts associated with the inflammatory response to *D. farinae* by real-time qPCR. The lungs of *D. farinae*-treated mice from both genotypes showed induced expression of transcripts encoding the Th2 cytokines IL-5 and IL-13 (Fig. 1C), as well as IL-17A, and IFN-γ mRNA relative to their respective naive control mice groups. However, the levels of IL-5 and IL-13 mRNAs were significantly lower in *D. farinae*-treated *Pla2g5*-null mice than in WT mice (Fig. 1C). There were similar levels of IL-17A and IFN-γ in the lungs of *Pla2g5*-null mice and WT mice (data not shown). Transcripts encoding the IL-13–inducible mucus-associated proteins Muc5ac and Clca3/Gob-5 (26, 27) were strongly expressed in the lungs of the WT mice treated with *D. farinae*, and they were significantly lower in *Pla2g5*-null mice than in WT mice. In contrast, IFN-γ was not marked differences between the two genotypes (data not shown).

**FIGURE 1.** *D. farinae*-induced pulmonary inflammation in WT and *Pla2g5*-null mice. *A*, Total and differential cell counts from BAL fluid of WT (filled bars; *n* = 6 and 16 in NaCl- and *D. farinae*-treated groups, respectively) and *Pla2g5*-null (open bars; *n* = 7 and 18, respectively) mice. *B*, Tissue sections of lung showing BVBs from WT (NaCl, a–e; *D. farinae*, f–i) and *Pla2g5*-null (NaCl, c; *D. farinae*, d, h, j) mice treated with NaCl or *D. farinae* intranasally were stained by the CAE reaction (a–d), for assessing inflammatory cell infiltrates, or by PAS reaction (e–h), for depicting mucus-secreting cells (arrows) and by CR dye (i, j) (arrowheads) to demonstrate accumulation of eosinophils. Original magnification ×20 (CAE and PAS) and ×40 (CR). *C*, Expression of mRNA transcripts for IL-5, IL-13, Muc5ac, and Clca3/Gob-5 in the lung of NaCl- and *D. farinae*-treated WT (filled bars) and *Pla2g5*-null (open bars) mice, measured by real-time qPCR. *D*, Lung sections of WT and *Pla2g5*-null mice treated with NaCl (a, c) or *D. farinae* (b, d) intranasally showing staining for group V sPLA2 (red) and nuclei (blue). Original magnification ×40; scale bar, 25 μm. *B* and *D*, Images are from one representative mouse per group from one of two experiments. Data are expressed as ratio of mRNA expression relative to GAPDH. Values are mean ± SEM from two independent experiments.
much lower in the lungs of *D. farinae*-treated *Pla2g5*-null mice (Fig. 1C).

To determine whether group V sPLA₂ protein was constitutively expressed in the lung and upregulated in response to *D. farinae*, we performed immunohistochemistry using a polyclonal Ab to group V sPLA₂ (4, 5) on paraffin-embedded lung sections. Compared with the lungs of the naive WT controls, the lungs of *D. farinae*-treated WT mice showed strong staining for group V sPLA₂ (Fig. 1D). The staining for group V sPLA₂ localized to cells with the anatomic locations and morphologies typical of granulocytes, macrophages, and goblet cells. In contrast, there was no staining in the lungs of either naive or *D. farinae*-treated *Pla2g5* mice.

**Adaptive immune response to *D. farinae* in WT and *Pla2g5*-null mice**

Next, we investigated the contribution group V sPLA₂ to the adaptive immune response induced by *D. farinae* Ags in lymph node. PLNs collected from saline- or *D. farinae*-treated WT and *Pla2g5*-null mice were isolated and cells were counted and stimulated with 20 μg/ml *D. farinae* to evaluate the profile of cytokines released in the supernatant. The numbers of cells in the draining PLN were increased in both WT and *Pla2g5*-null mice receiving *D. farinae*. However, the number of cells from the PLN of *D. farinae*-treated *Pla2g5*-null mice was significantly lower than that from *D. farinae*-treated WT mice (Fig. 2A). PLN cells (equalized for cell number) from WT and *Pla2g5*-null mice restimulated with the *D. farinae* released IL-4, IL-5, IL-13, IL-17A, and IFN-γ, but the levels of IL-4, IL-5, and IL-13 from *Pla2g5*-null PLN cells were significantly lower than those from WT cells (Fig. 2B). The levels of IL-17A and IFN-γ in the supernatants of *Pla2g5*-null PLN cells compared with those from WT cells were not significantly different (Fig. 2B). The serum levels of *D. farinae*-specific IgE and *D. farinae*-specific IgG₁ significantly increased after treatment of WT mice with *D. farinae*. In contrast, the levels of these Abs and the total IgE were dramatically lower in the serum of the *Pla2g5*-null mice than those in the WT controls (Fig. 2C).

**Processing of *D. farinae* by WT and *Pla2g5*-null BMDCs**

To determine whether DCs lacking group V sPLA₂ could process *D. farinae* Ags normally, we pulsed BMDCs from WT (Supplemental Video 1) and *Pla2g5*-null mice (Supplemental Video 2) with Alexa Fluor 488-labeled *D. farinae* and followed the movement of the labeled Ag for 2 h to allow for the degradation of labeled *D. farinae*. Fluorescence decreased progressively in WT BMDCs, but it persisted in the *Pla2g5*-null BMDCs. At 2 h, the fluorophore was still visible in most of the *Pla2g5*-null BMDCs but was largely absent in the WT BMDCs.

**D. farinae-induced maturation of WT and *Pla2g5*-null BMDCs**

In another set of experiments we sought to evaluate the effects of the lack of group V sPLA₂ on the maturation of BMDCs induced by *D. farinae*. Naive BMDCs from WT and *Pla2g5*-null mice expressed comparable levels of CD40, CD80, CD86, and CD252, whereas the expression of MHC-II was slightly diminished on *Pla2g5*-null BMDCs (data not shown). After ex vivo pulse with *D. farinae* for 24 h (18), the surface expression of MHC-II and CD86 increased in WT *D. farinae*-BMDCs more than in *Pla2g5*-null *D. farinae*-BMDCs (Fig. 3B; as shown for one experiment, Fig. 3A). The surface expressions of CD40, CD80, and CD252 were slightly reduced on *Pla2g5*-null *D. farinae*-BMDCs than on WT *D. farinae*-BMDCs.

To determine whether the absence of group V sPLA₂ altered DC maturation in vivo, we isolated CD11c⁺ cells from the lungs of WT and *Pla2g5*-null *D. farinae*-treated mice by mechanical dispersion followed by a density gradient. The WT CD11c⁺ cells had ∼1.5-fold increased expression of MHC-II compared with *Pla2g5*-null cells in two experiments (data not shown).

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**FIGURE 2.** PLN cellularity, cytokine release from restimulated PLN cells, and serum IgGs in NaCl- and *D. farinae*-treated WT and *Pla2g5*-null mice. PLNs and serum were obtained from WT (filled bars; n = 6 and 16 in NaCl- and *D. farinae*-treated groups, respectively) and *Pla2g5*-null (open bars; n = 7 and 18, respectively) mice. A, Number of cells obtained from PLNs. ELISA measurement of (B) cytokine release from restimulated PLN cells and (C) total serum IgE and *D. farinae*-specific IgE and IgG₁. Values are mean ± SEM from two independent experiments.
Role of group V sPLA2 expression by DCs and resident lung cells in pulmonary inflammation and adaptive immunologic responses induced by D. farinae

To understand whether the defective function of DCs lacking group V sPLA2 could contribute to the impaired local and acquired immune response to D. farinae in vivo, WT naive mice were treated intranasally with saline or 3 μg of D. farinae after intratracheal engraftment with WT or Pla2g5-null BMDCs in vitro with D. farinae (28). The transfer of WT and Pla2g5-null D. farinae-BMDCs did not induce any response in the airways of WT recipient mice in the absence of intranasal challenge with D. farinae (Fig. 4A). However, the intranasal administration of D. farinae to WT mice that were engrafted with WT D. farinae-BMDCs elicited an increase in BAL fluid cellularity and eosinophils. In contrast, WT mice receiving Pla2g5-null D. farinae-BMDCs and challenged with D. farinae had substantially fewer total BAL fluid cells and eosinophils (Fig. 4A). These findings were mirrored in the histologic analysis of lung tissue, in which the transfer of WT D. farinae-BMDCs into WT recipients tended to elicit more infiltration of BVBs and goblet cell metaplasia after D. farinae challenge than did the transfer of Pla2g5-null D. farinae-BMDCs (Fig. 4B). Likewise, the transfer of WT D. farinae-BMDCs resulted in trends toward greater numbers of lymph node cells (data not shown), and restimulation of these cells with D. farinae resulted in higher levels of IL-4, IL-5, and IL-13 than did samples from mice receiving Pla2g5-null D. farinae-BMDCs (Fig. 4C). IL-17A and IFN-γ levels were not significantly different between the WT mice receiving WT and Pla2g5-null D. farinae-BMDCs (Fig. 4C).

To determine whether expression of group V sPLA2 by resident cells in the host tissue also influenced the response to D. farinae in the BMDC transfer protocol, Pla2g5-null mice received transfers of both WT D. farinae-BMDCs and Pla2g5-null D. farinae-BMDCs. In these experiments, D. farinae treatment of the recipient mice resulted in very little cellular response in the BAL fluid, regardless of the BMDC genotype (Fig. 5A). The recipients of WT D. farinae-BMDCs had similar number of monocytes, lymphocytes, neutrophils, and eosinophils compared with the recipients of Pla2g5-null D. farinae-BMDCs (Fig. 5A). The infiltration of BVBs and the metaplasia of goblet cells were very mild and not evidently different in mice receiving WT D. farinae-BMDCs and Pla2g5-null cells (Fig. 5B). The numbers of cells obtained from the PLNs of mice receiving WT D. farinae-BMDCs were not significantly different from those of mice receiving Pla2g5-null D. farinae-BMDCs (data not shown). However, the restimulated PLN cells from the Pla2g5-null mice receiving WT D. farinae-BMDCs released significantly more IL-4, IL-5, and IL-13 than did the PLN cells from the recipients of Pla2g5-null D. farinae-BMDCs. There were no differences in IL-17A or IFN-γ levels (Fig. 5C).

Discussion

We have previously shown that group V sPLA2 regulates phagocytosis through different receptors and that this function is related to the ability of group V sPLA2 to regulate phagosome maturation in macrophages (5, 7). This mechanism also contributes to phagocytosis and killing of C. albicans and to the in vivo innate immune response against the pathogen, which are greatly impaired in Pla2g5-null mice (7). D. farinae is an extract from a clinically relevant house dust mite that activates innate resident APCs as a prerequisite to the adaptive immune response and inflammation (8, 11). Group V sPLA2 contributes to the development of airway hyperresponsiveness in an OVA-induced model of pulmonary disease in which allergic sensitization is induced by i.p. injection of OVA plus alum as an adjuvant (16). We postulated that group V sPLA2 might play a prominent role in a D. farinae-induced model due to its demonstrated importance in macrophages and the ability of D. farinae to activate endogenous APCs in the lung. We first compared the cellular components of BAL fluids obtained from WT and Pla2g5-null mice treated with intranasal D. farinae at a dose sufficient to induce moderate pulmonary inflammation in WT C57BL/6 mice. The importance of group V sPLA2 in this model was supported by the markedly reduced numbers of total

FIGURE 3. D. farinae-induced maturation in WT and Pla2g5-null BMDCs. A, Expression of MHC-II, CD40, CD80, CD86, and CD252 (solid lines) versus the isotype controls (dashed lines) on gated CD11c+ D. farinae-BMDCs from one experiment representative of three. B, Net (negative control subtracted) mean fluorescence intensity (MFI) of MHC-II, CD40, CD80, CD86, and CD252 expression on WT (filled bars) and Pla2g5-null (open bars) D. farinae-BMDCs evaluated by flow cytometry and expressed as fold increase versus Pla2g5-null D. farinae-BMDCs. Values are mean ± SEM from three independent experiments.
cells and granulocytes (Fig. 1A) in the BAL fluid of the *D. farinae*-treated *Pla2g5*-null mice relative to the *D. farinae*-treated WT controls. This included a near absence (95% reduction) of eosinophils, a cellular surrogate of a Th2-type immune response. These findings were further supported by the reduced bronchovascular cellular infiltrates (Fig. 1B). The markedly decreased numbers of goblet cells (Fig. 1B) and associated goblet cell transcripts (Fig. 1C) in the lungs of the *D. farinae*-treated *Pla2g5*-null mice compared with WT controls are also consistent with the defective generation of Th2 cytokines observed in these animals. In particular, the decreased expression of IL-5 is consistent with the reduced numbers of eosinophils, whereas the reduction in IL-13 correlated with the diminution in goblet cell metaplasia (Fig. 1C). *D. farinae* contains ligands for C-type lectin receptors, protease-activated receptors, and likely other pattern recognition receptors that contribute to breaking tolerance to the allergen by inducing expression of cytokines and enzymes in resident cells in the lung. We speculate that group V sPLA2 is one of these inducible products, since its expression is strikingly upregulated in the lung of *D. farinae*-treated WT mice in several target cell types (Fig. 1D). These data indicate that group V sPLA2 plays a robust role in local inflammatory responses induced by a clinically relevant Ag, and its absence impairs local expression of Th2 cytokines and its downstream markers typical of *D. farinae*-driven pulmonary inflammation (29).

To directly test whether group V sPLA2 was involved in the generation of a Th2 response to *D. farinae*, we measured cytokine generation by restimulated PLN cells (mostly reflecting Ag-specific CD4+ T cells), as well as the levels of the Th2 signature Abs (IgE and IgG1) in the serum (30, 31). Both the total numbers of cells in the PLNs (Fig. 2A) and the generation of IL-4, IL-5, and IL-13, but not IFN-γ (Fig. 2B), were significantly lower in the *Pla2g5*-null mice than in the samples from the WT controls, suggesting that group V sPLA2 is required for the local induction of a Th2-type immune response. Furthermore, the production of the Th2-associated Abs was virtually absent in *Pla2g5*-null mice (Fig. 2C). IL-5 is required for eosinophilia (32, 33), whereas IL-13 is needed to induce goblet cell metaplasia (34, 35) and IL-4 is required for induction of
IgE and IgG1 (33). Thus, the reduced inflammatory response induced by D. farinae in the lungs of Pla2g5-null mice, along with the markedly diminished Ab responses, are consistent with the lack of pathogenetic Th2 cytokines. This suggests that group V sPLA2 regulates a proximal mechanism in the immune response to D. farinae.

Next we tested the hypothesis that the defective D. farinae-induced Th2 response in the Pla2g5-null mice reflected a requirement for this enzyme in Ag presentation by DCs, an essential proximal step in the pulmonary immune response to Ags. We have previously reported that group V sPLA2 localizes in the trans-Golgi network and in the recycling endosomes of peritoneal macrophages (5), and it regulates phagocytosis by a mechanism dependent on delay in fusion of phagosomes with late endosomes and lysosomes (7). Ag presentation by DCs likewise requires intracellular trafficking and degradation of Ags to reach late endosomes and lysosomes where the peptides generated during this process are loaded onto MHC-II (36). We investigated the role of group V sPLA2 in processing of Alexa Fluor 488-labeled D. farinae by BMDCs using live cell imaging. In WT D. farinae-BMDCs, the fluorescence emitted by the labeled Ags progressively diminished and was almost lost after 2 h of observation (Supplemental Video 1). In D. farinae-BMDCs derived from Pla2g5-null mice, the labeled Ag was still visible after 2 h in most of the cells (Supplemental Video 2). These data are consistent with defective or delayed degradation of the labeled Ag in Pla2g5-null BMDCs during the movement of D. farinae Ags from the surface to the late endosomes and lysosomal compartment.

When DCs take up Ags in the context of PAMPs, they undergo maturation, as indicated by increased expression of MHC-II and costimulatory molecules (10, 36, 37). D. farinae mimics PAMPs and therefore can directly activate DCs and induce their maturation (12, 13). Whereas in vitro stimulation of WT and Pla2g5-null BMDCs with D. farinae induced maturation and upregulation of costimulatory molecules in both genotypes (Fig. 3A), the Pla2g5-null D. farinae-BMDCs expressed significantly less MHC-II and CD86 (Fig. 3B). Thus, group V sPLA2 plays a critical role not only in Ag processing, but also in the maturation of BMDCs.
following *D. farinae* loading. Furthermore, the analysis of CD11c+ cells isolated from the lung of *D. farinae*-treated mice confirmed that the expression of group V sPLA2 is important for maturation of APCs. To our knowledge, this is also the first time that a secretory PLA2 has been shown to regulate the function of an APC.

To address whether the defect in Ag processing in *Pla2g5*-null BMDCs can contribute to the development of pulmonary inflammation in vivo, we used an adoptive transfer model of BMDC-dependent sensitization to *D. farinae* (28). WT and *Pla2g5*-null *D. farinae*-BMDCs were transferred to WT recipient mice, which then were challenged twice with *D. farinae* after *D. farinae*-BMDC engrafment. The transfer of *Pla2g5*-null *D. farinae*-BMDCs resulted in substantially less BAL fluid cellularity and granulocyte accumulation (Fig. 4A) after *D. farinae* challenge than did transfers of WT *D. farinae*-BMDCs. This pattern was recapitulated in the histologic assessments of inflammation and goblet cell metaplasia (Fig. 4B). The fact that the production of Th2 cytokines by restimulated PLN cells was selectively impaired in the WT recipients of *Pla2g5*-null *D. farinae*-BMDCs (Fig. 4C) is consistent with the hypothesis that the impaired APC function of BMDCs lacking group V sPLA2 is a key determinant of the immunologic phenotype in this model.

Since group V sPLA2 is expressed by resident cells in the lung other than DCs (epithelium, smooth muscle, and macrophages as well as infiltrating granulocytes; Fig. 1D) (16), we performed transfers of both WT *D. farinae*-BMDCs and *Pla2g5*-null *D. farinae*-BMDCs into *Pla2g5*-null mice transferred with WT recipients of *Pla2g5*-null *D. farinae*-BMDCs (Fig. 4D). This pattern was recapitulated in the adoptive transfer model of DC-dependent sensitization to *D. farinae*, we demonstrate, for the first time to our knowledge, that the BMDC-associated group V sPLA2 is the primary determinant of the *D. farinae*-induced Th2 immune response in this model, the expression of group V sPLA2 in at least one other resident lung cell is critical to subsequent development of *D. farinae*-driven pulmonary inflammation. It is important to note some limitations of our study. Further work is needed to define the other cell types in which group V S PLA2 plays an important role in this model, as well as the intracellular mechanisms by which group V PLA2 regulates DC maturation and Ag processing. Although *D. farinae* is an allergen clinically relevant to human asthma, the *D. farinae* model of pulmonary inflammation only reproduces some of the features of human asthma. Furthermore, the function of group V PLA2 could be different in other paradigms of allergic airway inflammation or in different strains of mice.

In summary, our study demonstrates, for the first time to our knowledge, that group V sPLA2 contributes to the pathogenesis of *D. farinae*-induced pulmonary inflammation through a mechanism that is unique among the enzymes of the PLA2 family: the presence of group V sPLA2 in DCs is critical for *D. farinae* processing and cell maturation, prerequisites to *D. farinae*-induced adaptive immune response and inflammation. Using an adoptive transfer model of DC-dependent sensitization to *D. farinae*, we demonstrate that the development of a pulmonary Th2 response to dust mite allergens depends on group V sPLA2 expression by DCs, but that expression of the same enzyme by a resident cell population is essential for subsequent pulmonary inflammation. Therefore, group V sPLA2 might represent a therapeutic target for pharmacologic intervention in the treatment of diverse types of human asthma.

Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Video 1:  *Df* processing in WT BMDCs. BMDCs from WT stained with CMTMR (red), were incubated with Alexa 488-labeled *Df* (green). Cells were imaged from 10 min to 2 h. Data are from one experiment representative of three.

Supplemental Video 2:  *Df* processing in *Pla2g5*-null BMDCs. BMDCs from *Pla2g5*-null mice stained with CMTMR (red), were incubated with Alexa 488-labeled *Df* (green). Cells were imaged from 10 min to 2 h. Data are from one experiment representative of three.
### Supplemental Table I. Real-time qPCR primers.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primers (5'-3')</th>
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| IL-5       | Forward: ATGAAGTGCTGGAGATGGAACCCA  
              Reverse: ACCCTGATGCAACGAAGGATGA  |
| IL-13      | Forward: TGAGGAGCTGAGCAACATCACACA  
              Reverse: TGCGGTTACAGAGGCCATGCAATA  |
| IL-17A     | Forward: TCCACCCHAATGAAGACCCTGATA  
              Reverse: ACCAGCATCTTCTCGACCCTGAAA  |
| IFN-γ      | Forward: GGCCATCAGCAAACAACATAAGCGT  
              Reverse: TGGGTTGTTCACCTCAAACCTTGGC  |
| Muc5Ac     | Forward: AAGCCACGAAATGTGGAGTGAGTG  
              Reverse: GGTGGCAGCTTGTCTTGTTCAAAA  |
| Clca3/Gob-5| Forward: AGAATGAACCCACCACGTCCCTGAA  
              Reverse: TCAGATTCACCAGGTCTGCCCCTT  |
| GAPDH      | Forward: TCAACAGCAAATCTCCACTCTTCCA  
              Reverse: ACCCTGGTCTGTAGCCGTATTCA  |