Group V Secretory Phospholipase A\textsubscript{2} Reveals Its Role in House Dust Mite-Induced Allergic Pulmonary Inflammation by Regulation of Dendritic Cell Function

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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

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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 native 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-activated 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 PLA2α (sPLA2) 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) (Greeer 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 cytospun onto slides, stained with Diff-Quick (Fisher exsanguinated, and bronchoalveolar lavage (BAL) was performed. BAL fluid cells were cytospun onto slides, stained with Diff-Quick (Fisher Diagnostics, Middletown, VA), and differentially counted. Cell-free BAL fluid was assayed for the content of cytokine leukotrienes (LTCA₄, LTDB₄, LTE₄) (GE Healthcare BioSciences, Buckinghamshire, U.K.), PGE₂, and PGE₃ (Cayman Chemical, Ann Arbor, MI) by enzyme immunoassay. All animal studies described here were approved by the Animal Care and Use Committee of Dana Farber Cancer Institute (Boston, MA).

### 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 glycol methacrylate or paraffin. Two in glycol methacrylate 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-caliber preterminal bronchi (diameter, 200–200 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 ImageJ (National Institutes of Health) image analysis software [http://rsweb.nih.gov/ij)] (14). For immunofluorescence, lung sections were deparaffinized and rehydrated. Ag retrieval was performed with Target Retrieval Solution (Dako, Glostrup, Denmark) at 97°C for 30 min. After blocking with 10% chicken serum (Santa Cruz Biotechnology, Santa Cruz, CA), the rabbit anti-mouse group V sPLA₂ Ab (4) was added and incubated at 37°C for 1 h. Samples were washed and incubated at 37°C for 1 h with the secondary Ab Alexa Fluor 594 chicken anti-rabbit IgG (1/1000; Invitrogen, Carlsbad, CA) and nuclear staining reagent Hoechst 33342 (Invitrogen). The sections were washed and covered with FluoroShield mounting media (Electron Microscopy Sciences, Hatfield, PA). Lung sections were imaged with an Eclipse 80i microscope (Nikon Instruments, Melville, NY) and images were acquired by a Hamamatsu C10800 digital camera and HCImage software 1.1.3.1 (Hamamatsu, Bridgewater, NJ).

### 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, Ccl23/Gob-5, and GAPDH on an Mx4000 thermal cycler (Stratagene, Santa Clara, 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).

### In vitro restimulation of parabronchial lymph node cells

The parabronchial lymph node (PLN) was collected from the upper-right chest of each mouse and frozen in complete medium (RPMI 1640, 10% FBS, 1% nonessential amino acids, 2 ml/glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml 2-ME, Sigma-Aldrich) plus 2% PE-10 and 1 mg/ml BSA (15). The PLNs were enzymatically dissociated by gentle trituration with a 10 ml pipet, and 4 × 10⁶ nucleated cells were cultured 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 IgS

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 (Innitrogen). Absorbance was read at 450–570 nm and the results were expressed as net optical density (OD.) (i.e., sample O.D. – blank O.D.). D. farinae-specific IgG₃ was measured by ELISA with plate-bound D. farinae, an alkaline phosphatase-conjugated anti-mouse IgG₃ (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, disaggregated, and resuspended at a concentration of 4 × 10⁶/ml in RPMI 1640, 10% FBS, 1% nonessential amino acids, 2 ml/glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml 2-ME, Sigma-Aldrich) plus 2% PE-10 and 1 mg/ml BSA (15). Cells were then plated into 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 (D. farinae-BMDCs) (18). After 24 h, cells were analyzed by flow cytometry or instilled intratracheally (15 × 10⁶/mouse) into anesthetized mice (19). Mice that received D. farinae-BMDCs received saline or 3 μg D. farinae intranasally on days 11 and 14 and were euthanized on day 15.

### 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 M5/114.15.2), PE-Cy7-CD11c (N418), allophycocyanin-CD40 (RM134L; BioLegend, San Diego, CA), or with corresponding isotypes as controls. The acquisition was performed on a FACSCanto flow cytometer with FACSDiva software (BD Biosciences), and data were analyzed with 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 the BMDCs. The 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 with a Nikon EZ-C Gold version 3.40 build 691 (7). Time series were acquired at a rate of 1 min per frame; frames were 3 min apart. Volumes were made using Photoshop CS3 to animate the volume-rendered TIFF images.

### Statistics

To compare three or more groups we used the Kruskal-Wallis nonparametric test with Dunn’s posttest to correct for multiple comparisons, using Prism software (GraphPad Software, La Jolla, CA). Variation between two groups with unequal variance was determined using the Mann-Whitney U test. All of the other comparisons used a two-tailed Student t test. The p values <0.05 were considered significant.

### Results

#### Effects of group V sPLA₂ deficiency on D. farinae-induced pulmonary inflammation

We used a mouse model of allergic pulmonary inflammation induced by D. farinae to elucidate the role of group V sPLA₂ in...
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 (Fig. 1A).

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 ± 2.67 versus 8.28 ± 0.67 per 15 BVBs; *p* < 0.01) and markedly fewer mucus-producing goblet cells (47.35 ± 2.48 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

**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; *D. farinae*, b, 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. Naïve 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).

FIGURE 2. PLN cellularity, cytokine release from restimulated PLN cells, and serum Igs 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 loaded 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 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 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
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.

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**FIGURE 5.** Pulmonary inflammation and cytokine release from restimulated PLNs in *Pla2g5*-null mice adoptively transferred with WT and *Pla2g5*-null *D. farinae*-BMDCs. A, Total cell and differential counts from BAL of *Pla2g5*-null mice transferred with WT (filled bars; n = 2 and 6 in NaCl- and *D. farinae*-treated groups, respectively) or *Pla2g5*-null (open bars; n = 2 and 5, respectively) *D. farinae*-BMDCs. B, Sections of lung showing BVBs from *Pla2g5*-null mice receiving with WT (NaCl, a, e, f) or *Pla2g5*-null (NaCl, c, g; *D. farinae*, d, h) *D. farinae*-BMDCs and treated with NaCl or *D. farinae* intranasally were stained by the CAE (a–d) or by the PAS (e–h) reactions. C, ELISA measurement of cytokine release from restimulated PLN cells of *D. farinae*-treated *Pla2g5*-null mice receiving with WT (filled bars; n = 4) or *Pla2g5*-null (open bars; n = 4) *D. farinae*-BMDCs. Images in B are from one representative mouse per group from one of two experiments. Original magnification ×20. Other data are mean ± SEM from two independent experiments.
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 engulfment. The transfer of *Pla2g5*-null *D. farinae*-BMDCs resulted in substantially less BAL fluid cellularity and granulocyte lacking group V sPLA2 is a key determinant of the immunologic with the hypothesis that the impaired APC function of BMDCs knowledge, that group V sPLA2 contributes to the pathogenesis of in different strains of mice.

D. farinae

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**


