

Lineage-specific regulation of allergic airway inflammation by the lipid phosphatase Src homology 2 domain-containing inositol 5-phosphatase (SHIP-1)

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Background: *Inpp5d* (Src homology 2 domain-containing inositol-5-phosphatase [*Ship1*])–deficient mice experience spontaneous airway inflammation and have enhanced sensitivity to allergen-induced airway inflammation.

Objective: We hypothesized that lineage-specific deletion of *Ship1* expression in cells known to be crucial for adaptive T_H2 responses would uncover distinct roles that could either positively or negatively regulate susceptibility to allergic airway inflammation (AAI).

Methods: *Ship1* expression was deleted in B cells, T cells, or dendritic cells (DCs), and the resulting *Ship1*^{ΔB} cell, *Ship1*^{ΔT} cell, *Ship1*^{ΔDC}, or *Ship1*^{F/F} (wild-type) control mice were evaluated in a model of house dust mite (HDM)–induced AAI.

Results: Unlike germline panhematopoietic *Ship1* deletion, deletion of *Ship1* selectively in either the B-cell, T-cell, or DC lineages did not result in spontaneous airway inflammation. Strikingly, although loss of *Ship1* in the B-cell lineage did not affect HDM-induced AAI, loss of *Ship1* in either of the T-cell or DC lineages protected mice from AAI by skewing the typical T_H2 immune response toward a T_H1 response.

Conclusions: Although panhematopoietic deletion of *Ship1* leads to spontaneous lung inflammation, selective deletion of *Ship1* in T cells or DCs impairs the formation of an adaptive T_H2 response and protects animals from HDM-induced AAI. (J Allergy Clin Immunol 2015;■■■■:■■■-■■■.)

Key words: Allergy, asthma, dendritic cell, house dust mite, IgE, T_H2

Abbreviations used

AAI:	Allergic airway inflammation
AMΦ:	Alveolar macrophage
BAL:	Bronchoalveolar lavage
CFSE:	Carboxyfluorescein succinimidyl ester
DC:	Dendritic cell
HDM:	House dust mite
mLN:	Mediastinal lymph node
OVA:	Ovalbumin
PI3K:	Phosphatidylinositol 3-kinase
PI(3,4,5)P ₃ :	Phosphatidylinositol-3,4,5-triphosphate
SHIP-1:	Src homology 2 domain-containing inositol-5-phosphatase
Treg:	Regulatory T
WT:	Wild-type

Allergic asthma is a chronic inflammatory disease of the airways characterized immunologically by a T_H2-biased inflammatory response; high IL-4, IL-5, and IL-13 levels; eosinophilic infiltration into the airways and lung tissue; antibody class-switching to IgE; and increased mucus production. Despite the ever-increasing incidence of this disease, treatment options have remained stagnant over the years, with inhaled corticosteroids remaining the frontline treatment option. A greater understanding of the mechanisms surrounding allergic sensitization would facilitate the discovery of novel therapeutic targets.

The phosphatidylinositol 3-kinase (PI3K) pathway^{1,2} is pivotal to the activation, survival, and migration of allergy-promoting cells, and several studies have shown the therapeutic benefit of targeting this pathway in animal models.^{3–7} Unfortunately, the ubiquitous expression of PI3Ks makes them challenging to target clinically and potentially obscures cell type–specific effects of targeting this pathway at different stages of allergic airway inflammation (AAI) pathogenesis. In hematopoietic cells PI3K signaling is negatively regulated by the Src homology 2 domain-containing inositol-5-phosphatase 1 (*Ship1*, *Inpp5d*), which hydrolyzes the 5' phosphate of phosphatidylinositol-3,4,5-triphosphate (PI[3,4,5]P₃) generated by PI3K to produce phosphatidylinositol-3,4-bisphosphate, thereby inhibiting signaling downstream of PI(3,4,5)P₃. Importantly, *Ship1* is expressed selectively in hematopoietic cells, making it an attractive therapeutic target to regulate the PI3K pathway while limiting off-target effects on PI3K signaling in nonhematopoietic cells.⁸ Therapies aimed at targeting the PI3K pathway would benefit from a deeper understanding of how perturbing this hematopoietic-restricted negative regulator influences disease.

Ship1–deficient mice experience spontaneous airway inflammation,^{8,9} as well as a host of other hematologic abnormalities, including enhanced myelopoiesis and reduced lymphopoiesis

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TABLE I. Primer list

Primer	Forward (5'-3')	Reverse (5'-3')
<i>Actb</i>	GGCTGTATTCCTCCATCG	CCAGTTGGTAAACATGCCATGT
<i>Il4</i>	TCGGCATTTTGAACGAGGTC	CAAGCATGGAGTTTTCCTATG
<i>Il5</i>	GATGAGGCTTCCTGTCCCTACTC	TCGCCACACTTCTCTTTTGG
<i>Il13</i>	CCTGGCTCTTGCTTGCTT	GGTCTTGTTGATGTTGCTCA
<i>Ifng</i>	GGATGCATTCATGAGTATTGCC	CCTTTTCCGCTTCCTGAGG
<i>Prg2</i>	ATGGGTGACTCTGGATGCAAG	GCGGACTGGATTCCGAAGT
<i>Muc5ac</i>	CAGGACTCTCTGAAATCGTACCA	GAAGGCTCGTACCACAGGG
<i>Muc5b</i>	GTGGCCTTGCTCATGGTGT	CGCTCATGCTAGGGAAGACAG

and erythropoiesis,⁸ expansion of myeloid-derived suppressor cells¹⁰ and regulatory T (Treg) cells,¹¹ and altered natural killer cell development.¹² Although the diverse immune disorders caused by germline deletion of *Ship1* reveal a key role in hematopoietic homeostasis, they might mask subtle yet important functions in specific leukocyte subsets. To explore this possibility further, we selectively deleted *Ship1* in distinct hematopoietic lineages and examined the effects on the development of AAI. Strikingly, we found that loss of SHIP-1 in either T cells or dendritic cells (DCs) attenuated the development of T_H2 responses to house dust mite (HDM)-induced allergic inflammation, whereas loss in B cells showed no influence on the development of disease. Our data reveal an unanticipated therapeutic effect of targeting SHIP-1 in T cells and DCs and suggest that targeting this enzyme in a lineage-specific manner might offer a novel approach to therapy.

METHODS

Mice

Inpp5d^{F/F} mice (*Ship1*^{F/F}) were provided by Dr W. Kerr.¹² *Cd19-Cre* (B6.129P2[C]-*Cd19*^{tm1[cre]Cgn/J}), *Cd4-Cre* (B6.Cg-Tg[Cd4-Cre]1Cwi/BfluJ), *Itgax-Cre* (Cd11c-Cre, B6.Cg-Tg[Itgax-cre]1-1Reiz/J), C57BL/6J, and OT-II (B6.Cg-Tg[TcrαTcrβ]425Cbn/J) mice were obtained from the Jackson Laboratories (Bar Harbor, Me). Mice were bred and maintained in a specific pathogen-free environment at the Biomedical Research Centre. The Animal Care Committee of the University of British Columbia approved all protocols.

Histology

Lungs were fixed in 10% buffered formalin and paraffin embedded. Five-micrometer-thick sections were stained with hematoxylin and eosin or periodic acid–Schiff.

HDM-induced allergic airway disease

HDM (*Dermatophagoides pteronyssinus*) was obtained from Greer Laboratories (Lenoir, NC). Extracts contained 34 ng of Der p 1 and 0.095 endotoxin units/μg of total protein and were resuspended in sterile PBS. Mice were anesthetized with isoflurane, sensitized with 25 μg of HDM protein intranasally on days 0 to 2, and challenged with 5 μg of HDM on days 13 to 17. Mice were euthanized 24 hours after the last HDM challenge on day 18. Bronchoalveolar lavage (BAL) fluid was collected using 3 repeated instillations, and aspirations of 1 mL of sterile PBS and blood was collected by cardiac puncture for serum antibody analyses. BAL fluid cells were enumerated and classified by flow cytometry.

Flow cytometry

Samples were first incubated in staining buffer containing 10% goat serum and 5 μg/mL anti-CD16/CD32 to block nonspecific binding. IL-13,

IFN-γ, FoxP3, CD11c, MHC class II, CD4, CD11b, and CD45R/B220 antibodies were obtained from eBioscience (San Diego, Calif). CD103, CD40, Siglec-F, CD86, and CD45 antibodies were purchased from BD Biosciences (San Jose, Calif). Anti-neutrophil antibody (7/4) was purchased from Abcam (Cambridge, Mass). SHIP-1 antibody (P1C1) was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif) and conjugated to Alexa Fluor 647 (AbLab, Vancouver, British Columbia, Canada). Intracellular staining was performed with the Intracellular Fixation & Permeabilization Buffer Set or the FoxP3/Transcription Factor Buffer Set from eBioscience. Dead cells were excluded using eFluor fixable viability dyes (eBioscience). Samples were acquired on a BD LSR II, and data analysis was performed with FlowJo software (TreeStar, San Carlos, Calif).

RNA isolation and quantitative RT-PCR

Lungs were homogenized in Trizol (Life Technologies, Carlsbad, Calif) with a TissueLyser II (Qiagen, Valencia, Calif). Total RNA was extracted and reverse transcribed with a high-capacity cDNA synthesis kit (Life Technologies), and quantitative RT-PCR was performed with SYBR green (KAPA Biosystems, Woburn, Mass). Reactions were carried out in an ABI 7900 real-time PCR machine (Life Technologies), and values are expressed relative to β-actin (*Actb*). Primer sequences are shown in Table I.

Detection of serum IgE, IgG₁, and IgG_{2a}

ELISA for total serum IgE was performed according to the manufacturer's instructions (BD Biosciences). Detection of HDM-specific IgG₁ and IgG_{2a} was carried out as described previously.¹³ Briefly, plates were coated with 25 μg/mL HDM antigen in carbonate buffer, and antigen-specific IgGs were detected with horseradish peroxidase-conjugated antibodies to mouse IgG₁ or IgG_{2a} and TMB substrate (BD PharMingen, San Jose, Calif). Reactions were stopped by the addition of 1N HCl, and absorbance was measured at 450 nm.

Immune analysis

Lungs were minced and digested in 200 U/mL collagenase IV (Sigma, St Louis, Mo) for 1 hour at 37°C and passed through a 70-μm cell strainer. RBCs were lysed, and leukocytes were enriched with Percoll (Sigma) separation. Mediastinal lymph nodes (mLNs) were passed through a 70-μm cell strainer to form a single-cell suspension. Isolated leukocytes were resuspended in culture medium (Iscove modified Dulbecco medium with 10% FBS, penicillin/streptomycin, and 150 μmol/L monothioglycerol) containing 750 ng/mL ionomycin and 50 ng/mL phorbol 12-myristate 13-acetate (Sigma) in the presence of brefeldin A (eBioscience) for 4 hours before intracellular staining for flow cytometry. BAL fluid cytokine levels were quantified using the cytometric bead array (BD Biosciences).

In vivo DC migration

Mice were challenged intranasally with 50 μg of DQ-ovalbumin (OVA; Molecular Probes, Eugene, Ore) and 100 μg of HDM antigen or 100 μg of

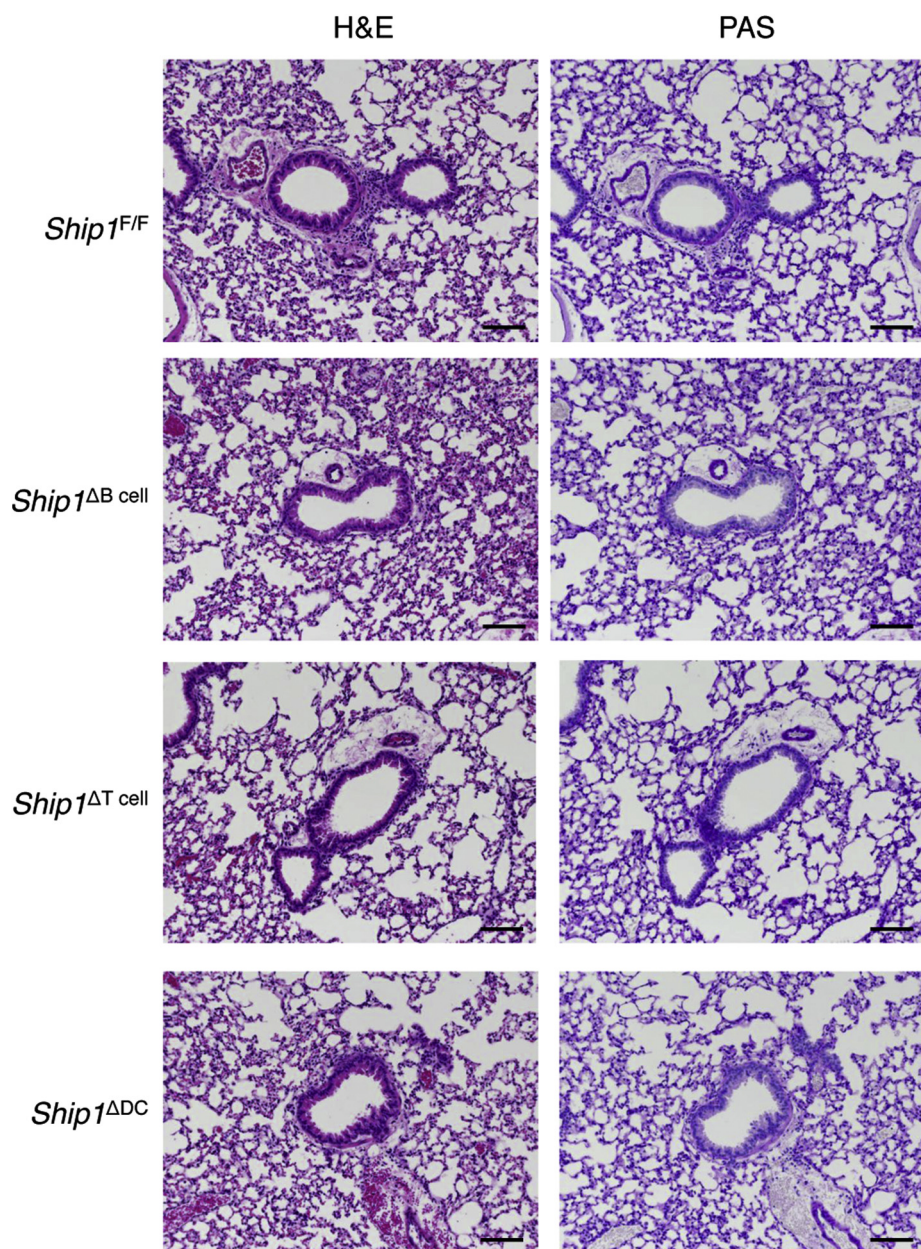


FIG 1. Lineage-specific deletion of *Ship1* does not result in spontaneous lung inflammation. Hematoxylin and eosin (H&E)- and periodic acid-Schiff (PAS)-stained lung sections from *Ship1^{F/F}* (WT control mice), *Ship1^{ΔB cell}*, *Ship1^{ΔT cell}*, and *Ship1^{ΔDC}* mice treated intranasally with PBS are shown (n = 3). Original magnification $\times 100$. Scale bars = 100 μm .

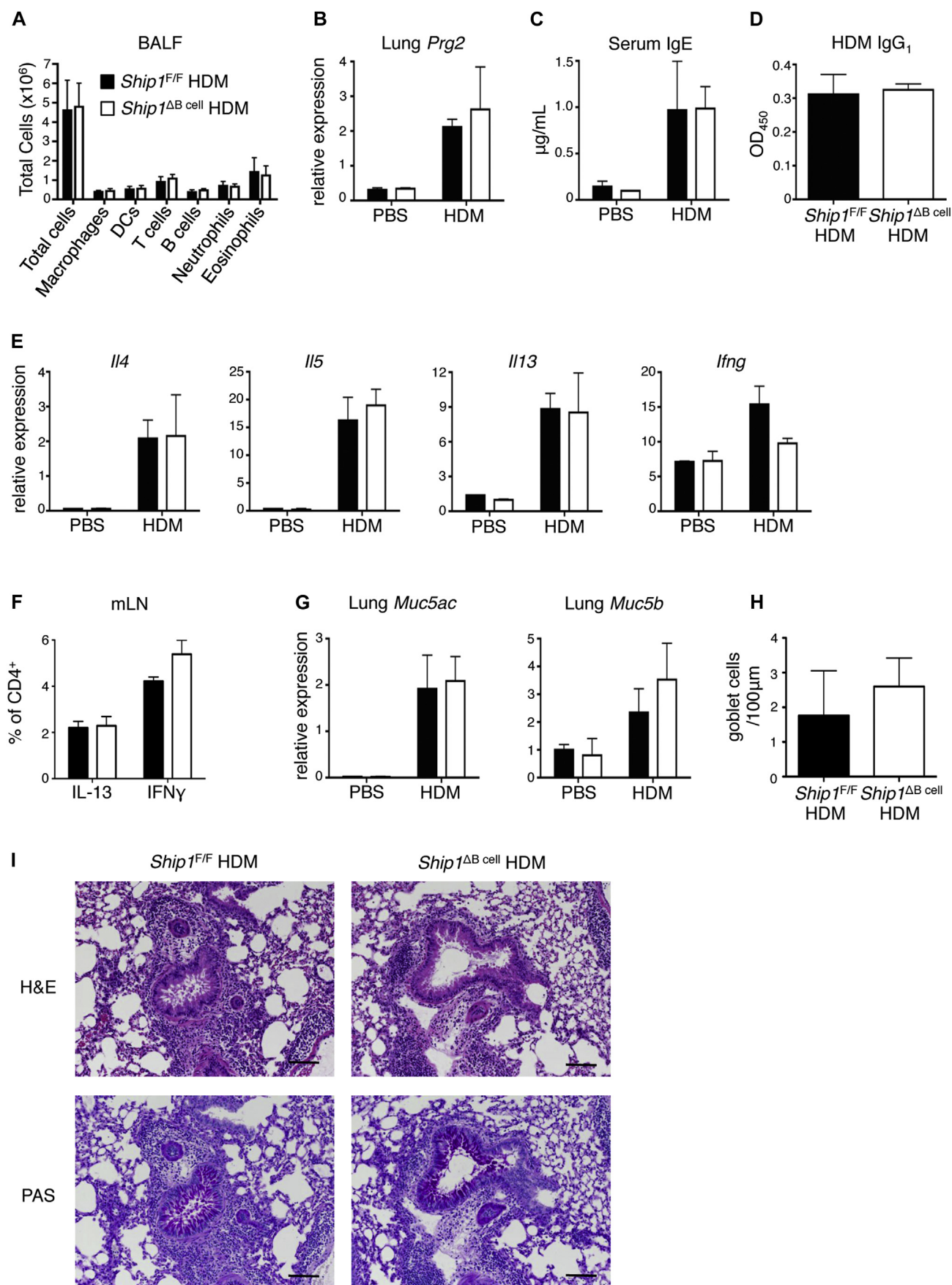
Alexa Fluor 647-labeled HDM. Draining mLNs were collected 24 hours later and analyzed by flow cytometry.

OT-II adoptive transfer

Mice were sensitized intranasally on days 0 to 2 with 25 μg of HDM and 100 μg of OVA (Grade V, Sigma) and again with 5 μg of HDM and 100 μg of OVA on days 13 to 15. Naive CD4^+ T cells were isolated from the spleens and lymph nodes of OT-II mice by magnetic separation (StemCell Technologies, Vancouver, British Columbia, Canada) and stained with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes). CFSE-labeled CD4^+ OT-II cells (2.5×10^6) were injected intravenously on day 13, and mice were euthanized on day 16.

DC culture and adoptive transfer

Splenic DCs were expanded *in vivo* by injecting Flt3L-expressing B16 melanoma cells subcutaneously (1×10^7 cells) into the lower backs of *Ship1^{F/F}* and *Ship1^{ΔDC}* mice. Splenic CD11c^+ DCs were purified by magnetic separation (StemCell Technologies). DCs were stimulated with 100 $\mu\text{g}/\text{mL}$ HDM antigen for 72 hours, and secreted IL-12p40 was quantified by ELISA (eBioscience). Adoptive transfer experiments were performed, as described previously,¹⁴ with some modifications. DCs were pulsed for 16 to 24 hours with 100 μg of HDM, and 2.5×10^5 of these DCs were delivered intranasally into naive C57Bl/6 mice. One week later, mice were challenged for 5 consecutive days with 10 μg of HDM and euthanized 24 hours after the final HDM challenge.



Statistics

Results are presented as means $\pm$ SEMs or means $\pm$ SDs. The Student *t* test and ANOVA were used to determine statistical significance.

RESULTS

Lineage-specific deletion of *Ship1* does not induce spontaneous lung inflammation

Ubiquitous deletion of *Ship1* leads to a myeloproliferative disorder, spontaneous lung inflammatory disease, and shortened lifespan, which prohibit evaluation of its role in adaptive immune responses to inhaled allergens. Mice carrying a *Ship1* gene flanked by loxP sites were crossed to mice expressing the Cre recombinase under the control of the *Cd19*, *Cd4*, and *Itgax* (*Cd11c*) promoters to examine *Ship1*'s role in specific leukocyte subpopulations. This led to selective deletion of *Ship1* expression in B cells (*Ship1*^{ΔB cell}), T cells (*Ship1*^{ΔT cell}), and DCs (*Ship1*^{ΔDC}), respectively (see Fig E1 in this article's Online Repository at www.jacionline.org). Interestingly, none of these mice had spontaneous lung inflammation (Fig 1 and see Fig E1), suggesting deletion of *Ship1* in another cell type (or a combination of cell types¹⁵) is required to induce the naive lung inflammation observed in ubiquitous knockouts.

B cell-specific deletion of *Ship1* has no effect on progression of AAI

SHIP-1 plays an important role in the negative regulation of B-cell receptor signaling through its association with the inhibitory motif of FcγRIIB. Previously, *Ship1*^{ΔB cell} mice were reported to have a mildly T_H1-biased antibody response and a lower threshold for negative selection, leading to reduced titers of antigen-specific antibodies after systemic immunization.¹⁶ Therefore we examined whether B cell-specific deletion of *Ship1* would alter the severity of HDM-induced AAI. Surprisingly, wild-type (WT; *Ship1*^{F/F}) and *Ship1*^{ΔB cell} mice exhibited similar numbers of total leukocyte and eosinophilic infiltrates into the alveolar space (Fig 2, A) and lung tissue, as measured by expression of the eosinophil-specific transcript for major basic protein (*Prg2*; Fig 2, B). *Ship1*^{ΔB cell} mice also had a normal humoral response to HDM challenge, producing WT titers of total IgE and antigen-specific IgG₁ (Fig 2, C and D). There was also no apparent defect in the formation of adaptive T_H2 responses because these mice exhibited WT levels of transcripts for the T_H2-associated cytokines *Il4*, *Il5*, and *Il13* and the T_H1-associated cytokine *Ifng* in total lung RNA (Fig 2, E). Correspondingly, they exhibited near-WT levels of cytokine production from CD4⁺ cells isolated from draining lymph nodes, as measured by intracellular flow cytometry (Fig 2, F, and see Fig E2 in this article's Online Repository at www.jacionline.org). In accordance with WT levels of T_H2 cytokines, there was no difference in mucus production in the

lungs, as measured by expression of *Muc5ac* and *Muc5b* transcripts (Fig 2, G) and quantification of goblet cells in the small airways (Fig 2, H). Additionally, there was no discernible difference in tissue inflammation in HDM-exposed *Ship1*^{F/F} and *Ship1*^{ΔB cell} mice (Fig 2, I). In summary, deletion of SHIP-1 in B cells does not alter the development of HDM-induced AAI.

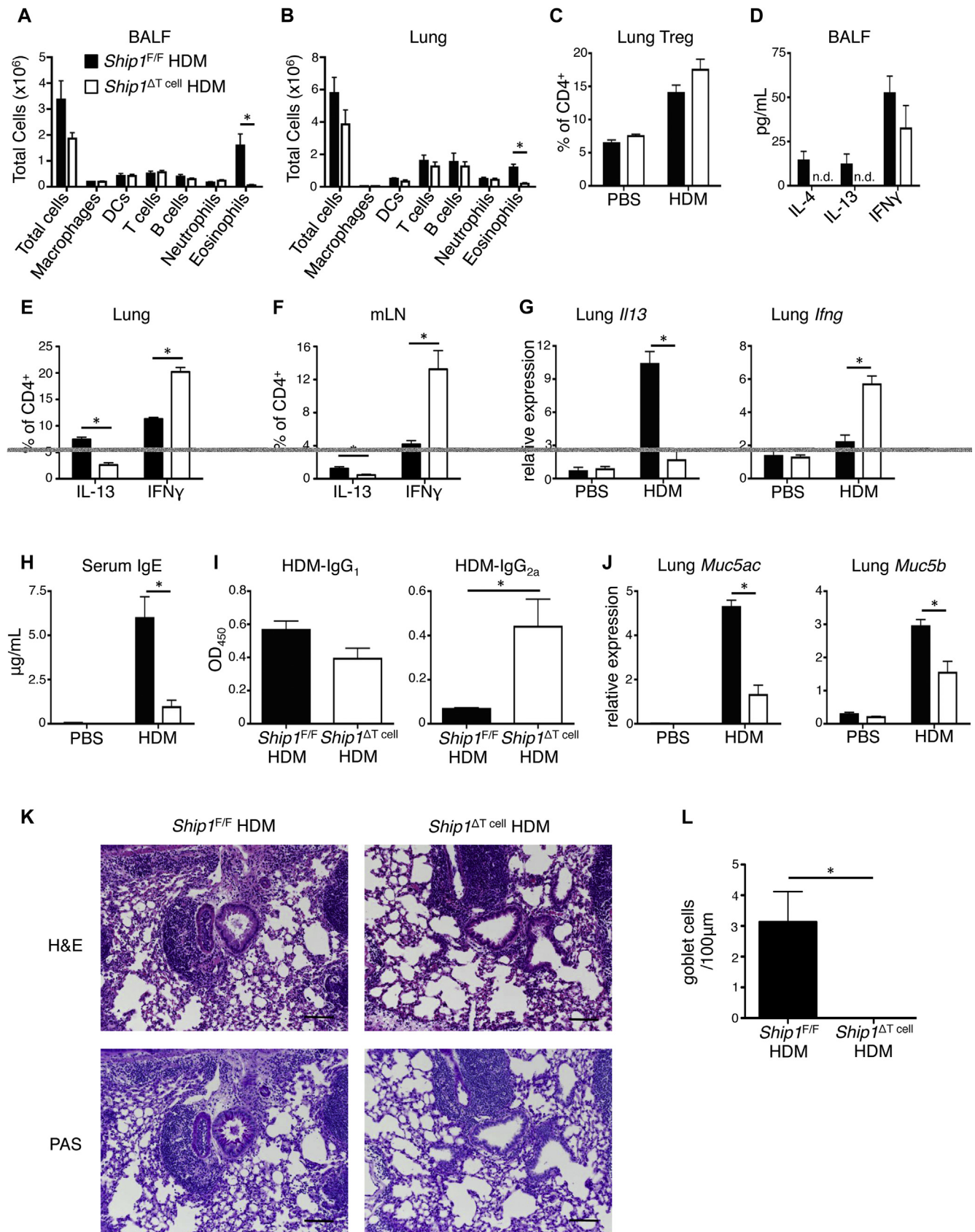
T cell-specific deletion of *Ship1* leads to a T_H1-biased response and protection from AAI

Although conventional SHIP-1-deficient mice (*Ship1*^{−/−}) exhibit an expansion of the Treg cell pool and a myeloproliferative disorder, mice with a T cell-specific deletion of SHIP-1 develop normally but have an increase in cytotoxic T-cell numbers and a biased polarization toward a T_H1 response, in part due to increased expression of T-bet.¹⁷ *Ship1*^{ΔT cell} mice were primed and challenged with HDM antigen to determine how this influences AAI. In contrast to WT mice, *Ship1*^{ΔT cell} mice exhibited reduced eosinophilic inflammation in both the airways and lung parenchyma (Fig 3, A and B). This reduction was not due to changes in lung Treg cell frequencies as we found no significant difference between WT and *Ship1*^{ΔT cell} mice (Fig 3, C). *Ship1*^{ΔT cell} mice also did not develop robust T_H2 responses, and we were unable to detect the T_H2 cytokines IL-4 and IL-13 in the BAL fluid (Fig 3, D) and observed significantly reduced levels of T_H2 cells in the lungs and draining mLNs of HDM-exposed animals. These were replaced by a significant increase in T_H1 cell numbers in HDM-exposed *Ship1*^{ΔT cell} mice, as measured based on CD4⁺ IFN-γ⁺ cells in the lungs and mLNs (Fig 3, E and F, and see Fig E2) and in lung tissue RNA (Fig 3, G). Consistent with the enhanced IFN-γ expression, *Ship1*^{ΔT cell} mice were found to have reduced titres of IgE and increased titres of HDM-specific serum IgG_{2a} (Fig 3, H and I). Finally, transcript levels of goblet cell-associated mucus genes and goblet cell hyperplasia was also attenuated in HDM-challenged *Ship1*^{ΔT cell} mice (Fig 3, J and L). Although *Ship1*^{ΔT cell} mice had reduced T_H2 responses to HDM exposure, they still exhibited significant tissue inflammation; however, mucus production was significantly reduced (Fig 3, K). In summary, the data suggest that *Ship1*^{ΔT cell} mice are protected from HDM-induced AAI through a generalized skewing of their adaptive immune response away from a T_H2 response toward a T_H1-biased response.

DC-specific deletion of *Ship1* protects against AAI

Although *in vitro* studies have suggested an important role for SHIP-1 in regulating DC activation and maturation,^{18–20} the role of SHIP-1 in DC function *in vivo* has yet to be explored. In response to HDM challenge, *Ship1*^{ΔDC} mice also displayed reduced eosinophilic infiltration in both the airways and lung parenchyma (Fig 4, A and B). This was accompanied by a slight

FIG 2. B-cell expression of *Ship1* does not influence HDM-induced AAI. **A**, BAL fluid (BALF) leukocyte differentials and cell counts from HDM-exposed mice. **B**, Lung eosinophil *Prg2* mRNA expression relative to *Actb*. **C** and **D**, Levels of serum IgE (Fig 2, C) and HDM-specific IgG₁ (Fig 2, D). **E**, Lung mRNA expression of *Il4*, *Il5*, *Il13*, and *Ifng* relative to *Actb*. **F**, Intracellular cytokine expression of mLN CD4⁺ cells measured by flow cytometry. **G**, Lung mRNA expression of mucus-associated mRNA *Muc5ac* and *Muc5b* relative to *Actb*. **H**, Periodic acid–Schiff positive goblet cells were enumerated and expressed per 100 μm of basement membrane. **I**, Hematoxylin and eosin (H&E)– and periodic acid–Schiff (PAS)–stained lung sections. Original magnification $\times 100$. Scale bars = 100 μm. Data are means $\pm$ SEMs and are representative of 2 independent experiments (*n* = 3–5 mice per experiment).



but significant increase in lung macrophage and DC numbers. Lung DCs serve as important sentinels for inhaled pathogens and undergo an intricate maturation and migration process to efficiently prime an adaptive immune response. Resident lung DCs first phagocytose and process inhaled antigens, then upregulate expression of costimulatory molecules before migrating to the draining lymph nodes, where they induce naive T-cell proliferation and activation. SHIP-1's ability to regulate PI(3,4,5)P₃ levels could influence each of these steps because it has been reported to have functions in phagocytosis,²¹ maturation,¹⁸ directional migration,²² and antigen presentation.²⁰

Mice were exposed intranasally to fluorescently labeled HDM antigen (or a mixture of HDM antigen and fluorescently labeled OVA [DQ-OVA]) to track the phagocytic and migratory activity of resident WT and *Ship1*^{ΔDC} DCs *in vivo*. Then, 24 hours later, we evaluated DC migration by enumerating fluorescently labeled cells in the draining mLN by flow cytometry. Interestingly, *Ship1*^{ΔDC} mice exhibited no defect in the ability to uptake and process antigen and migrate to the mLN (Fig 4, C); there was no difference in the relative frequency of antigen-positive CD11b⁺ or CD103⁺ DC subsets in the mLN (Fig 4, D and E) or in the total influx of DCs into the mLN (Fig 4, F). Additionally, there was no difference in expression of the surface markers CD40 and CD86 on mLN DCs after HDM exposure (Fig 4, G). Thus we conclude that the protective effect of *Ship1* loss was not due to a defect in DC antigen uptake or processing, migration, or activation. The ability of DCs to induce antigen-specific T-cell proliferation was also examined *in vivo* by using adoptively transferred CFSE-labeled CD4⁺ T cells from OT-II transgenic mice. Labeled OT-II cells were intravenously transferred into WT and *Ship1*^{ΔDC} mice sensitized with HDM and OVA antigens, followed by subsequent intranasal challenge with HDM and OVA. After 72 hours, mLN were removed, and OT-II proliferation was examined by CFSE dye dilution. There was no detectable difference in T-cell proliferation in WT and *Ship1*^{ΔDC} mice (Fig 4, H), which further confirmed that SHIP-1 is not required for DC migration or activation after HDM exposure.

Ship1*-deficient DCs preferentially prime T_H1 versus T_H2 responses *in vivo

Having ruled out a role for SHIP-1 in DC migration and activation, we examined its ability to influence an appropriately polarized adaptive T-cell response to HDM antigen. CD4⁺ T cells isolated from HDM-exposed *Ship1*^{ΔDC} mice exhibited significantly less IL-13 production and instead produced high IFN-γ levels (Fig 5, A and B, and see Fig E2). These data suggest that *Ship1*^{ΔDC} mice have an impaired ability to polarize naive CD4⁺ T cells to the T_H2 phenotype that is typically elicited in HDM-induced models of AAI and instead promote the formation of a T_H1 response. In further support of this notion, we found

significantly reduced expression of *Il4*, *Il5*, *Il13*, *Muc5b*, and *Muc5ac* transcripts in the lung tissues of HDM-exposed *Ship1*^{ΔDC} mice (Fig 5, C). This immune skewing was also observed in the humoral response, with reduced T_H2-associated IgE and IgG₁ levels and a compensatory increase in T_H1-associated IgG_{2a} levels (Fig 5, D and E) in HDM-exposed *Ship1*^{ΔDC} mice compared with their WT counterparts.

Although the T_H1-biased response in *Ship1*^{ΔDC} mice led to a reduction in HDM-induced BAL infiltrates (Fig 4, A), eosinophilic infiltration (Fig 4, A and B), and mucus production (Fig 5, C), these mice still exhibited similar numbers of total parenchymal infiltrates (Fig 4, B) and equivalent lung inflammation determined by histologic evaluation of sections from HDM-exposed *Ship1*^{F/F} and *Ship1*^{ΔDC} mice (Fig 5, F), although there was a significant reduction in goblet cell hyperplasia (Fig 5, G). This suggests that although *Ship1*^{ΔDC} mice are protected from HDM-induced eosinophilic infiltration into the airways (Fig 4, A), they still develop lung inflammation dominated by macrophages and lymphocytes.

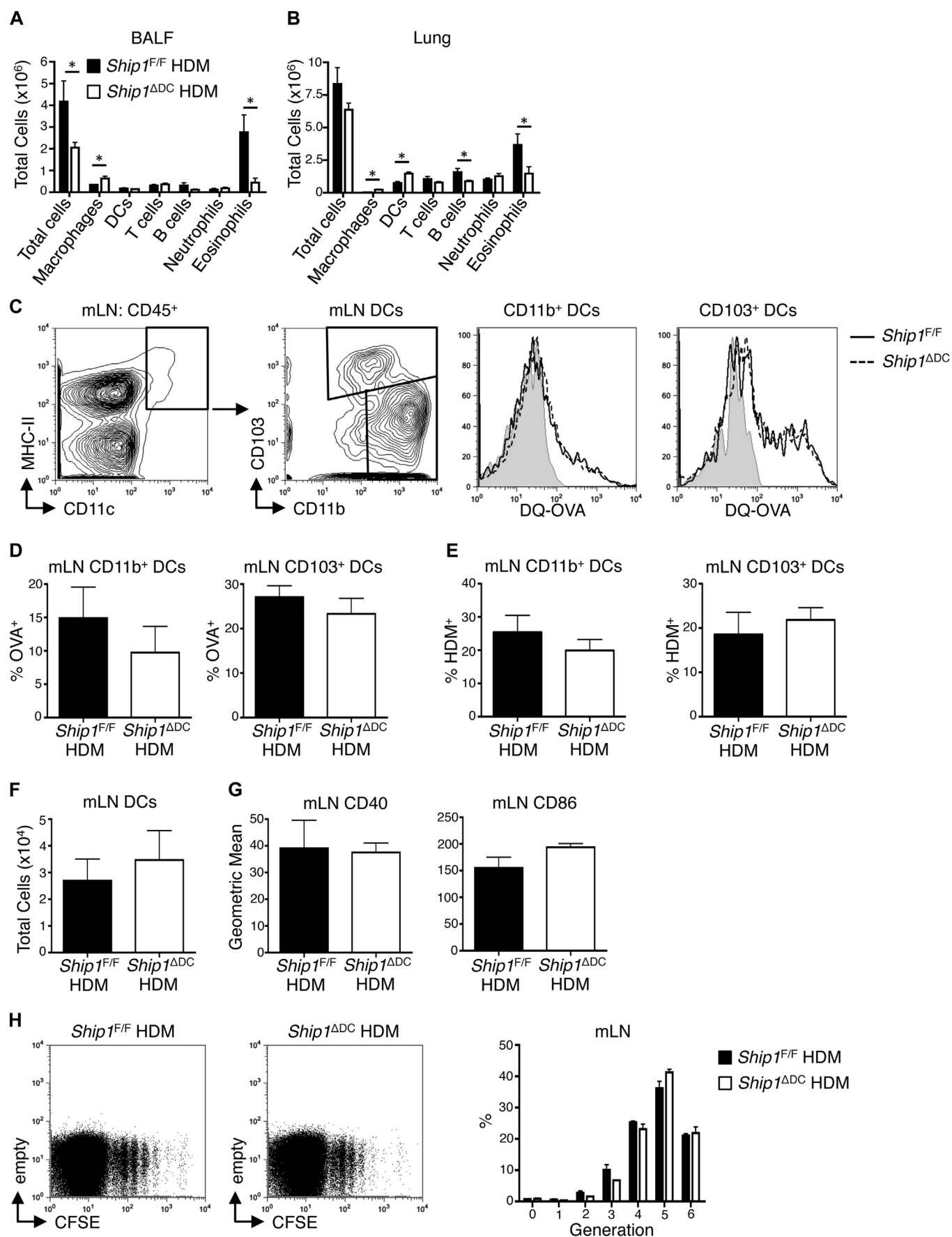
Ship1*-deficient DCs induce T_H1 polarization *in vitro* and *in vivo

Alveolar macrophages (AMΦs) constitutively reside in the lung and are integral to maintaining lung homeostasis. Because AMΦs express high levels of CD11c, it is likely that *Ship1*^{ΔDC} mice might also delete SHIP-1 in this macrophage population. Interestingly, expression of SHIP-1 increases in lung DCs, but not AMΦs, after HDM challenge, suggesting a potentially important role for SHIP-1 in regulating DC function in this model (Fig 6, A). To ensure that the T_H1 polarization phenotype observed in *Ship1*^{ΔDC} mice was due to loss of *Ship1* in DCs and not AMΦs, we isolated splenic DCs from *Ship1*^{F/F} and *Ship1*^{ΔDC} mice and evaluated their response to HDM stimulation *in vitro*. *Ship1*-deficient DCs produced significantly more IL-12p40 than their WT counterparts (Fig 6, B), suggesting an increased ability to promote T_H1 responses. Adoptive transfer of *in vitro* HDM-pulsed *Ship1*-deficient DCs into naive C57Bl/6 recipients resulted in reduced eosinophil infiltrates into the airways and lung parenchyma (Fig 6, C and D), an increased frequency of CD4⁺IFN-γ⁺ T cells in the mLN (Fig 6, E, and see Fig E2), and a significant reduction in serum IgE levels (Fig 6, F). Thus adoptive transfer of *Ship1*-deficient DCs into WT mice recapitulates the effects we observe in *Ship1*^{ΔDC} mice.

DISCUSSION

Allergen exposure activates the PI3K pathway and thereby facilitates inflammatory responses.²³ Correspondingly, inhibition of PI3K activity has been found to be effective in treating AAI in animal models.^{2-7,23} In leukocytes the lipid phosphatase SHIP-1

FIG 3. T-cell expression of *Ship1* restricts T_H1 adaptive responses to HDM. **A** and **B**, BAL fluid (BALF; Fig 3, A) and lung tissue (Fig 3, B) leukocyte differentials and cell counts from HDM-exposed mice. **C**, Flow cytometry of lung Treg cells (FoxP3⁺ CD25⁺) as a percentage of CD4⁺CD45⁺ leukocytes. **D**, IL-4, IL-13, and IFN-γ concentrations in BAL fluid were measured by cytometric bead array. **E** and **F**, Lung (Fig 3, E) and mLN (Fig 3, F) intracellular cytokine expression by CD4⁺ cells measured by flow cytometry. **G**, Lung expression of *Il13* and *Ifng* mRNA relative to *Actb*. **H** and **I**, Serum total IgE (Fig 3, H) and HDM-specific IgG₁ and IgG_{2a} (Fig 3, I) levels measured by ELISA. **J**, Lung mRNA expression of *Muc5ac* and *Muc5b* mRNA relative to *Actb*. **K**, Hematoxylin and eosin (H&E)- and periodic acid-Schiff (PAS)-stained lung sections. Original magnification ×100. Scale bars = 100 μm. **L**, Periodic acid-Schiff-positive goblet cells were counted and expressed per 100 μm of basement membrane. Data are means ± SEMs and representative of 2 independent experiments (n = 3-4 mice per experiment). *P < .05.



acts as a key negative regulator of PI3K signaling, and mice lacking this enzyme experience spontaneous airway inflammation and have a reduced lifespan. These severe hematologic defects could serve to obscure its function in specific leukocyte subsets, and here we have used lineage-specific deletion to examine its composite role in allergic disease. We show that although deletion of *Ship1* in the B-cell lineage has no effect, deletion of *Ship1* in either the T-cell or DC compartment protects mice from T_H2 disease by inducing a T_H1-biased adaptive response to the typically T_H2-inducing HDM allergen. Recent clinical studies with SHIP-1 agonists found a moderate therapeutic effect in patients with allergic asthma.²⁴ Although our results would suggest that activation of SHIP-1 in T cells and DCs would enhance disease severity, it is possible that SHIP-1 agonists have a net positive therapeutic effect through actions on specific leukocyte subsets, such as mast cells and basophils, where it has been shown to be an important regulator of their activation.²⁵⁻²⁸ Placing these observations in the context of the current work, it is likely that specific targeting of SHIP-1 activity, either positively or negatively, in a lineage-specific manner could lead to improved control of AAI.

SHIP-1 is known to be a key intermediary of signaling through the inhibitory motif of the FcγRIIB receptor²⁹ and plays an important role in B-cell development, survival, isotype class-switching, and high-affinity antibody production.^{16,30-32} Surprisingly, loss of *Ship1* in B cells did not affect HDM-induced AAI as *Ship1*^{ΔB} mice had similar levels of leukocyte infiltrates, mucus production, and T_H2-associated cytokines compared with their WT counterparts. There was also no observable defect in humoral response, with equal levels of both total serum IgE and HDM-specific IgG₁. Although this would appear to contradict previous reports of B cell-specific *Ship1*-deficient mice after infection or immunization,¹⁶ it is important to note that our work has focused primarily on airway T_H2 responses to inflammation. Our results do not rule out more subtle roles for SHIP-1 in B-cell development, antibody affinity maturation, or isotype switching. Indeed, it is noteworthy that although previous studies have noted enhanced levels of several immunoglobulin isotypes in B cell-specific *Ship1*-deficient mice, they exhibit WT levels of IgG₁ antibodies at steady state. Thus our data suggest that several aspects of B cell-specific *Ship1* deletion might be masked and less significant in the context of a T_H2 disease model.

Previously, lineage-specific deletion of *Ship1* in T cells has been shown to enhance CD8⁺ cytotoxic T-cell activity, increase T-box transcription factor expression, and impair the ability to mount an effective T_H2 response to helminth infections.¹⁷ In support of these reports, we found that *Ship1*^{ΔT} mice do not mount a robust T_H2 response to HDM antigen exposure and instead produce a strong T_H1 response. Protection from T_H2 disease is likely due to T_H1 skewing because we did not observe

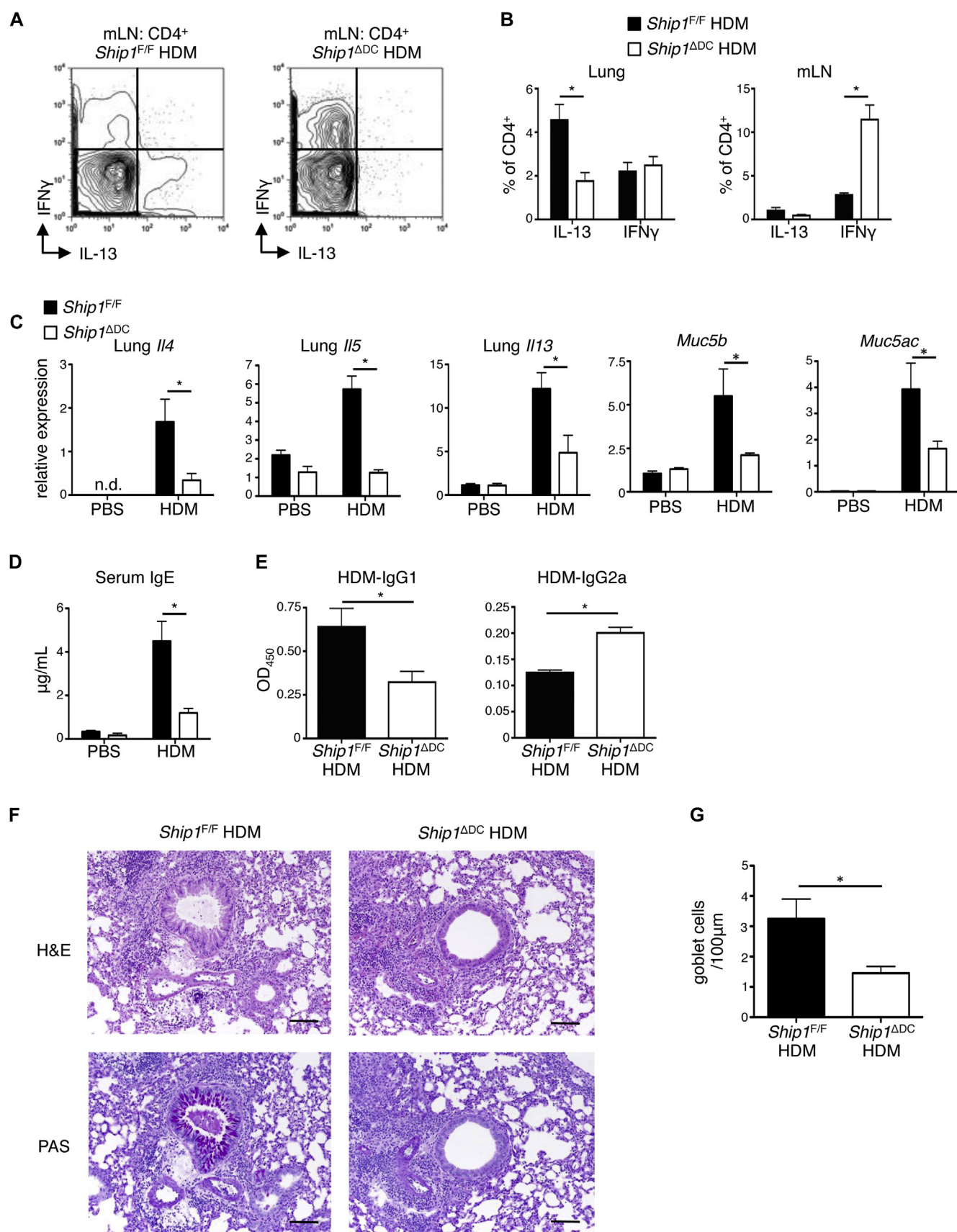
significant differences in Treg cell frequencies in *Ship1*^{ΔT} cell mice. Our data support previous findings and suggest that although deletion of *Ship1* in either the myeloid or early lymphoid lineages leads to increased Treg cell frequencies, deletion at later stages of lymphocyte development does not affect Treg cell numbers.^{17,33,34}

Although *in vitro* experiments have suggested a role for SHIP-1 in regulating DC development, maturation, and T-cell interactions, its role *in vivo* has not been explored.¹⁸⁻²⁰ In this study we used targeted deletion in DCs to evaluate SHIP-1's function in the development of adaptive immune responses. *Ship1*^{ΔDC} mice did not mount an effective T_H2 response to HDM antigen and instead produced an aberrant T_H1 response that led to reduced eosinophilia and mucus production, which are hallmarks of HDM-induced AAI. Although SHIP-1 deficient DCs were able to phagocytose and process antigen, migrate to the lymph node, and induce T-cell proliferation, they induced T_H1 rather than T_H2 polarization of naive CD4⁺ T cells. This could be due to alterations in the factors secreted by *Ship1*-deficient DCs because previous reports have found that loss of *Ship1* in myeloid cells can result in aberrant production of IL-12 and a skewed T_H1 response to intestinal helminth infection.³⁵ It is important to note that using Cre recombinase expressed under the control of the CD11c promoter likely leads to deletion of *Ship1* expression in AMΦs in addition to DCs. However, using *in vitro* and *in vivo* assays with DCs obtained from *Ship1*^{F/F} and *Ship1*^{ΔDC} mice, we showed that loss of *Ship1* in DCs alone was sufficient to induce T_H1 polarization because SHIP-1 deficient DCs produced high levels of IL-12p40 after HDM stimulation and preferentially induced T_H1 polarization of WT T cells *in vitro* and *in vivo*. However, this does not rule out a potential role for SHIP-1 in AMΦ development, survival, or function as we saw a small but significant increase in AMΦ numbers in the lungs of *Ship1*^{ΔDC} mice.

In summary, the severe pathologies found in panhematopoietic SHIP-1-deficient mice (*Ship1*^{-/-}) renders them difficult to evaluate in disease models and obscures cell-intrinsic roles for SHIP-1 in disease pathology. Here we have successfully used lineage-specific deletion of *Ship1* from either B cells, T cells, or DCs to further reveal its functional significance in allergic responses and have uncovered novel roles for SHIP-1 in DCs. In aggregate, our data suggest that the selective inactivation of *Ship1* in these lineages could prove therapeutic in instances of severe T_H2-driven lung inflammatory disease and justify further investigation of the efficacy of pharmacologic modulators of SHIP-1 activity.

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FIG 4. Loss of *Ship1* in DCs protects against development of HDM-induced AAI. **A** and **B**, BAL fluid (BALF; Fig 4, A) and lung tissue (Fig 4, B) leukocyte differentials and cell counts from HDM-exposed mice. **C**, Flow cytometry gating strategy for CD11b⁺ and CD103⁺ mLN DCs from HDM/DQ-OVA exposed *Ship1*^{F/F} and *Ship1*^{ΔDC} mice. **D**, Frequency of DQ-OVA⁺ CD11b⁺ and CD103⁺ DCs in mLNs 24 hours after HDM/DQ-OVA exposure. **E**, Frequency of HDM⁺ CD11b⁺ and CD103⁺ DCs in the mLNs 24 hours after HDM-Alexa Fluor 647 exposure. **F** and **G**, Total number and expression (geometric mean fluorescence intensity; Fig 4, F) of CD40 and CD86 on mLN DCs (CD11c⁺MHC-II⁺; Fig 4, G) 72 hours after HDM exposure. **H**, CFSE dilution of adoptively transferred OT-II CD4⁺ T cells in the mLNs of HDM/OVA-exposed mice. Data are means ± SEMs and representative of 2 independent experiments (n = 3-6 mice per experiment). *P < .05.



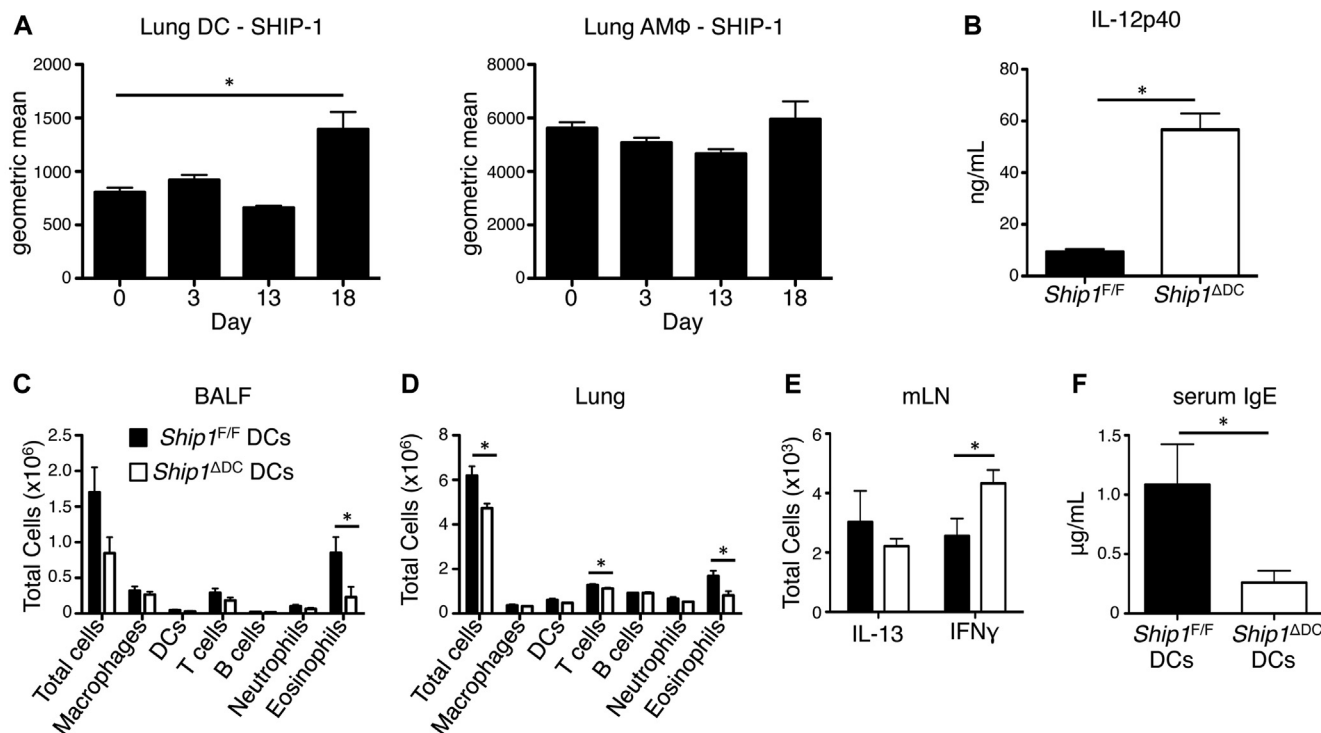


FIG 6. *Ship1*-deficient DCs induce T_H1 polarization *in vitro* and *in vivo* to HDM. **A**, WT mice were exposed to HDM over the 18-day disease time course, and lungs were collected at days 0, 3, 13, and 18. SHIP-1 expression in lung DCs ($CD45^+CD11c^+SiglecF^-$) and AMφs ($CD45^+CD11c^+SiglecF^+$) was measured by flow cytometry. **B**, IL-12p40 production from *Ship1^{F/F}* and *Ship1^{ΔDC}* DCs stimulated with HDM for 72 hours was measured by ELISA. **C** and **D**, BAL fluid (BALF; Fig 6, C) and lung tissue (Fig 6, D) leukocyte differentials and cell counts from mice adoptively transferred with HDM-pulsed WT or *Ship1^{ΔDC}* DCs. **E**, Frequency of intracellular cytokine-expressing $CD4^+$ T cells in the mLNs of HDM-exposed mice. **F**, Serum IgE levels in *Ship1^{F/F}* and *Ship1^{ΔDC}* DC recipient mice were quantified by ELISA. Data are means $\pm$ SEMs (Fig 6, A and C-F) or means $\pm$ SDs (Fig 6, B) and are representative of 1 independent experiment (Fig 6, A and C-F; $n = 5$ mice per group) or 3 independent experiments (Fig 6, B). * $P < .05$.

Key messages

- Chronic inflammation in *Ship1^{-/-}* mice masks important functions of SHIP-1 in specific leukocyte subsets.
- SHIP-1 expression in T cells and DCs facilitates appropriately polarized allergen-induced T_H2 responses.

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FIG 5. DC-expressed *Ship1* facilitates T_H2 sensitization to HDM. **A**, Gating strategy for intracellular IL-13 and IFN- γ expression in $CD4^+$ mLN lymphocytes. **B**, Frequency of intracellular cytokine-expressing $CD4^+$ T cells in the lungs and mLNs of HDM-exposed mice. **C**, Lung mRNA expression of *Il4*, *Il5*, *Il13*, *Muc5b*, and *Muc5ac* relative to *Actb*. **D** and **E**, Serum total IgE (Fig 5, D) and HDM-specific IgG₁ and IgG_{2a} (Fig 5, E) levels were measured by ELISA. **F**, Hematoxylin and eosin (H&E)- and periodic acid-Schiff (PAS)-stained lung sections. Original magnification $\times 100$. Scale bars = 100 μ m. **G**, Periodic acid-Schiff-positive goblet cells were counted and expressed per 100 μ m of basement membrane. Data are means $\pm$ SEMs and are representative of 2 independent experiments ($n = 4-6$ mice per experiment). *n.d.*, Not detected. * $P < .05$.

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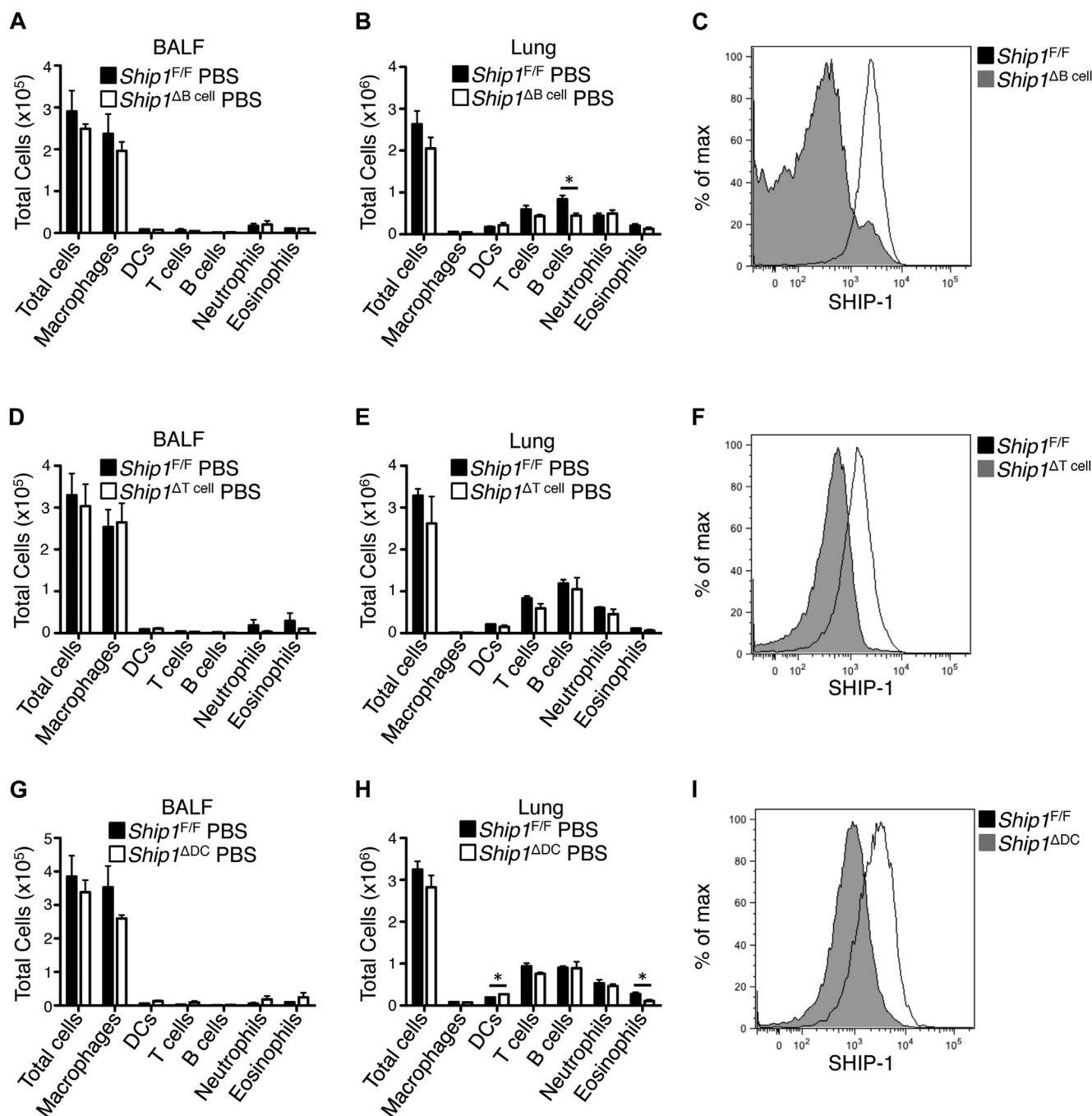


FIG E1. Naïve *Ship1^{F/F}*, *Ship1^{ΔB} cell*, *Ship1^{ΔT} cell*, and *Ship1^{ΔDC}* analysis. **A** and **B**, BAL fluid (BALF; Fig E1, **A**) and lung tissue (Fig E1, **B**) leukocyte differentials and cell counts from PBS-exposed *Ship1^{F/F}* and *Ship1^{ΔB} cell* mice. **C**, Intracellular SHIP-1 expression on lung tissue B cells ($CD45^+B220^+CD11c^-$) was measured by flow cytometry. **D** and **E**, BAL fluid (Fig E1, **D**) and lung tissue (Fig E1, **E**) leukocyte differentials and cell counts from PBS-exposed *Ship1^{F/F}* and *Ship1^{ΔT} cell* mice. **F**, Intracellular SHIP-1 expression on lung tissue T cells ($CD45^+CD3^+CD11c^-$) was measured by flow cytometry. **G** and **H**, BAL fluid (Fig E1, **G**) and lung tissue (Fig E1, **H**) leukocyte differentials and cell counts from PBS-exposed *Ship1^{F/F}* and *Ship1^{ΔDC}* mice. **I**, Intracellular SHIP-1 expression on lung tissue DCs ($CD45^+CD11c^+SiglecF^+$) was measured by flow cytometry. * $P < .05$.

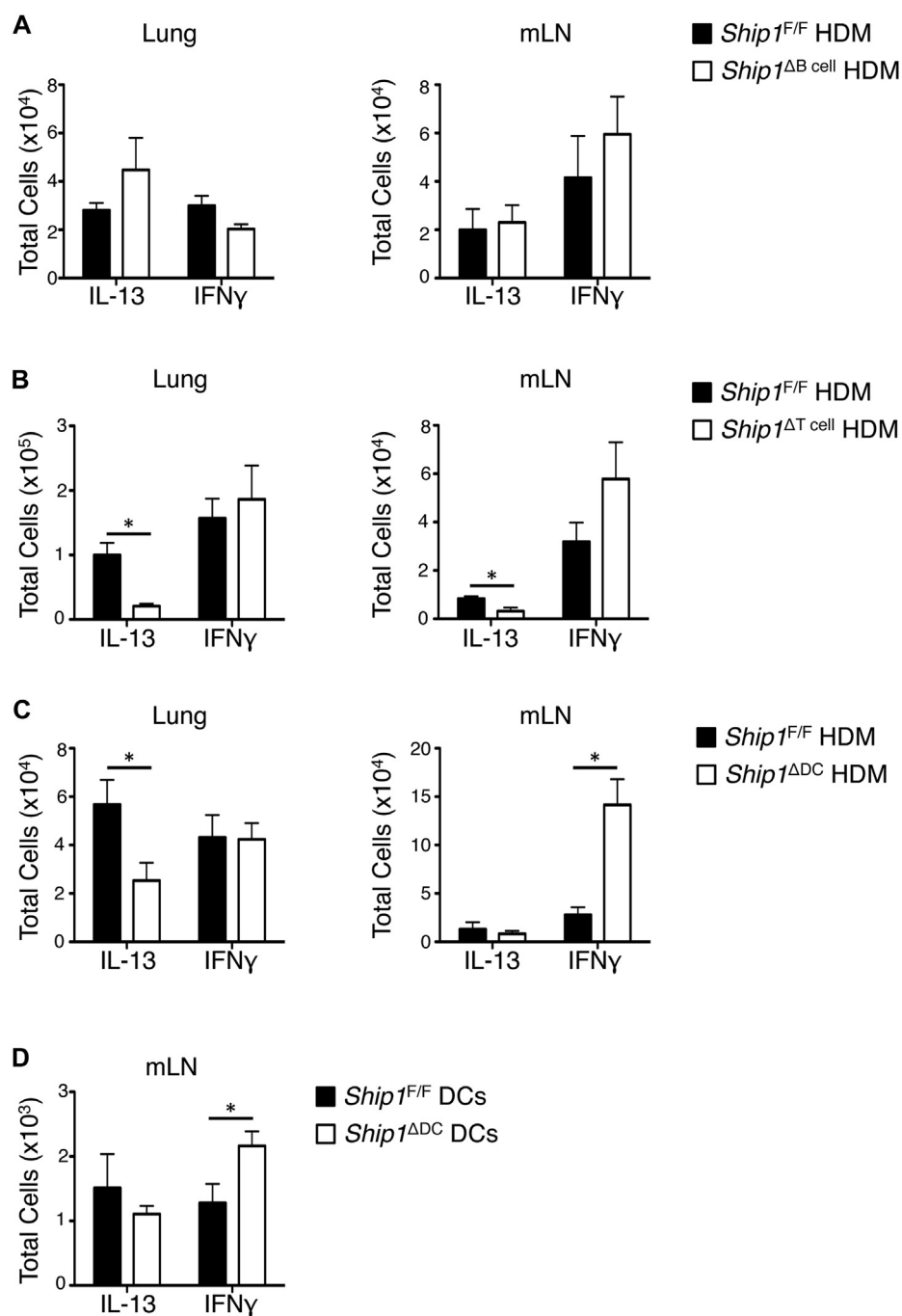


FIG E2. Lung tissue and mLN immune analysis. Lung and mLN intracellular cytokine production by CD4⁺ T cells was measured by flow cytometry in HDM-exposed *Ship1^{F/F}* and *Ship1^{ΔB cell}* mice (A), HDM-exposed *Ship1^{F/F}* and *Ship1^{ΔT cell}* mice (B), HDM-exposed *Ship1^{F/F}* and *Ship1^{ΔDC}* mice (C), and WT and SHIP-1 deficient DC adoptively transferred mice (D). **P* < .05.