Entwistle, LJ; Pelly, VS; Coomes, SM; Kannan, Y; Perez-Lloret, J; Czieso, S; Silva Dos Santos, M; MacRae, JI; Collinson, L; Sesay, A; +5 more... Nikolov, N; Metidji, A; Helmby, H; Hui, DY; Wilson, MS; (2017) Epithelial-Cell-Derived Phospholipase A2 Group 1B Is an Endogenous Anthelmintic. Cell host & microbe, 22 (4). 484-493.e5. ISSN 1931-3128 DOI: https://doi.org/10.1016/j.chom.2017.09.006

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Cell Host & Microbe
Epithelial-Cell-Derived Phospholipase A2 Group 1B Is an Endogenous Anthelmintic

Graphical Abstract

Highlights
- Pla2g1b expression correlated with resistance to intestinal helminth infection
- PLAG1B is essential for resistance to intestinal helminth infection in mice
- PLAG1B directly reduces phospholipid abundance in infective larvae
- Pla2g1b is expressed by epithelial cells and is negatively regulated by IL-4Rα

Authors
Lewis J. Entwistle, Victoria S. Pelly, Stephanie M. Coomes, ..., Helena Helmby, David Y. Hui, Mark S. Wilson

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In Brief
Intestinal helminths are highly prevalent in developing countries, with chronic infection causing significant host morbidity. Entwistle et al. show that epithelial-derived phospholipase A2 group 1B (PLAG1B) acts as an endogenous anthelmintic and is essential for resistance to intestinal helminth infection via direct action on infective larvae.

Entwistle et al., 2017, Cell Host & Microbe 22, 484–493
October 11, 2017 © 2017 The Authors. Published by Elsevier Inc. https://doi.org/10.1016/j.chom.2017.09.006
Epithelial-Cell-Derived Phospholipase A2 Group 1B Is an Endogenous Anthelmintic

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SUMMARY

Immunity to intestinal helminth infections has been well studied, but the mechanism of helminth killing prior to expulsion remains unclear. Here we identify epithelial-cell-derived phospholipase A2 group 1B (PLA2g1B) as a host-derived endogenous anthelmintic. PLA2g1B is elevated in resistant mice and is responsible for killing tissue-embedded larvae. Despite comparable activities of other essential type-2-dependent immune mechanisms, Pla2g1b−/− mice failed to expel the intestinal helminths Heligmosomoides polygyrus or Nippostrongylus brasiliensis. Expression of Pla2g1b by epithelial cells was dependent upon intestinal microbiota, adaptive immunity, and common-gamma chain-dependent signaling. Notably, Pla2g1b was downregulated in susceptible mice and inhibited by IL-4R-signaling in vitro, uncoupling parasite killing from expulsion mechanisms. Resistance was restored in Pla2g1b−/− mice by treating infective H. polygyrus L3 larvae with PLA2g1B, which reduced larval phospholipid abundance. These findings uncover epithelial-cell-derived Pla2g1b as an essential mediator of helminth killing, highlighting a previously overlooked mechanism of anti-helminth immunity.

INTRODUCTION

Intestinal helminth infections are highly prevalent in developing countries, with chronic infections causing significant host morbidity (Hotez et al., 2008). With the emergence of drug-resistant helminths, a limited number of effective anthelmintics, and stalled vaccine efforts, new therapeutic avenues require a better understanding of anti-helminth immunity and killing. Expulsion mechanisms of intestinal helminths have been widely studied and reported; however, the mechanism of helminth killing in the tissue prior to expulsion remain unclear. Upon infection, activated epithelial cells secrete a suite of alarmins, including interleukin (IL)-25, thymic stromal lymphopoietin (TSLP), and IL-33, which promote activation and differentiation of innate and adaptive immune cells, leading to type 2 inflammation (Anthony et al., 2006; Katona et al., 1991; McCoy et al., 2008; Urban et al., 2009) and alternate activation of macrophages (Anthony et al., 2006). The resulting reorganization of intestinal tissue—with goblet cell hyperplasia, mucus hyper-secretion, and smooth muscle contraction (Gerbe et al., 2016; Hashimoto et al., 2009; Hasnain et al., 2010, 2011; Howitt et al., 2016; Murakami et al., 2016)—alongside type 2 cytokine-driven immunological changes such as B cell class switching (McCoy et al., 2008) and alternate activation of macrophages (Anthony et al., 2006) contributes to parasite expulsion. However, the precise mechanism of parasite damage and killing, whether in tissue or lumen, has remained unclear.

RESULTS AND DISCUSSION

To identify local tissue responses and novel mechanisms of intestinal helminth killing during anti-helminth immunity, we used the natural mouse intestinal helminth H. polygyrus (H.p.). Following oral infection, stage 3 larvae (L3) migrate to the duodenum and proximal jejunum, where they penetrate the mucosa and embed into the muscularis externa, undergoing developmental moults before emerging into the lumen as adult worms (Camberis et al., 2003; Valanparambil et al., 2014). C57BL/6 mice are naturally susceptible to a primary (1st) H.p. infection, establishing a chronic infection (Reynolds et al.,...
Correlate with Resistance to Intestinal Helminth Infection

(A) C57BL/6 mice were orally infected with 200 L3 H. polygyrus (H.p.) larvae on day 0. A cohort of mice were sacrificed 7 days after 1\(^{st}\) H.p. infection (H.p. 1\(^{st}\)). Remaining mice were drug treated (Rx) on days 14 and 15. Mice were then reinfected with H.p. on day 35 or day 56 and harvested 7 days after infection (H.p. 2\(^{nd}\)).

(B) H&E staining of the small intestine from H.p. 1\(^{st}\) and H.p. 2\(^{nd}\) (D42).

(C) Ratio-of-ratios analysis of differentially expressed genes in H.p. 1\(^{st}\) and H.p. 2\(^{nd}\) (D42) identified distinct gene clusters (C1-3).

(D) Top 10 pathways predicted to be activated more highly in and H.p. 2\(^{nd}\) than H.p. 1\(^{st}\) (both relative to naive, 2-fold filter, p < 0.05).

(E) Lipid metabolism pathway predicted activation score (relative to naive, 2-fold filter, p < 0.05).

(F) Pla2g1b expression in small intestine from RNA sequencing data, confirmed by qPCR.

Data are represented as mean ± SEM; n = 8, *=p<0.05, ** = p < 0.01 determined using a one-way ANOVA with Dunnett’s multiple comparison analysis. See also Figure S1 and Tables S1–S3.

Following drug cure of a 1\(^{st}\) infection (Rx), C57BL/6 mice are resistant to a secondary (2\(^{nd}\)) H.p. challenge infection (Finkelman et al., 1997; model, Figure 1A). Resistance to H.p. 2\(^{nd}\) infection correlated with substantial inflammation and tissue remodeling (Figure 1B), and significantly more transcriptional activity in duodenal tissue than in 1\(^{st}\) infection (H.p. 1\(^{st}\)), with 665 genes differentially expressed in 2\(^{nd}\) infection compared to 145 genes in 1\(^{st}\) infection and 116 common genes (relative to naive, 2-fold filter, p < 0.05) (Figure S1A). Using a ratio-of-ratios analysis to specifically identify genes expressed in resistant mice (H.p. 2\(^{nd}\)), we identified three transcriptional clusters based on their expression relative to uninfected mice and relative to susceptible mice (H.p. 1\(^{st}\)) (Figure 1C, C1-C3; Tables S1–S3). Cluster 1 (C1) identified common and quantitative differences between 1\(^{st}\) and 2\(^{nd}\) infection, including several genes previously described in immunity to H.p. (Anthony et al., 2006; Herbert et al., 2009). Cluster 2 (C2) identified qualitative differences between susceptible and resistant mice highlighting genes upregulated in H.p. 2\(^{nd}\) infection only. Many of these genes have not previously been described in immunity to H.p. Cluster 3 (C3) identified qualitative differences downregulated in H.p. 2\(^{nd}\) infection only. Pathway analysis reflected quantitative and qualitative transcriptional differences with a greater increase in immune-activated pathways in resistant mice, as previously described (Allen and Maizels, 2011; Anthony et al., 2007; Maizels et al., 2012), in addition to an increase in lipid metabolism pathways in H.p. 2\(^{nd}\) that was not previously described during anti-helminth immunity (Figures 1D and S1B). Increased activation of lipid metabolism pathways was also evident in resistant mice with or without a 2\(^{nd}\) challenge infection and was maintained for up to 48 days after drug treatment (Figure 1E), correlating with long-term resistance to reinfection following drug treatment (Urban et al., 1991b). Within C2 genes, which were upregulated in H.p. 2\(^{nd}\) infection only, we identified group 1B phospholipase A\(_2\), Pla2g1b, a member of a large family of secreted (sPLA\(_2\)) enzymes that regulate lipid metabolism through hydrolysis of phospholipids (Labonte et al., 2006, 2010). Pla2g1b was significantly increased in drug-treated mice with or without challenge infection, correlating with lipid metabolism pathways and resistance to H.p. (Figure 1F). The enzymatic activity of PLA\(_2\) in the small intestine was marginally increased in susceptible mice but dramatically increased in resistant mice (Figure S1C), reflecting a broad increase in several PL\(_2\) enzymes in resistant mice (Figure S1D).

To formally test whether elevated PLA\(_2\)-g1B contributed to resistance to intestinal helminth infections, we infected Pla2g1b\(^{-/-}\) mice with a variety of small- or large-intestinal helminths. Strikingly, Pla2g1b was essential for resistance to H.p., with...
Pla2g1b−/− mice failing to expel a 21 H.p. infection and retaining a patent infection (Figures 2A and 2B). The absence of PLA2g1B did not affect the expression of other detectable PLA2 family members (Figure S1E). PLA2g1b was also required for expulsion of N. brasiliensis, which also infects the small intestine (Figure S2A); however, Pla2g1b was not required for expulsion of the cecum-dwelling whipworm Trichuris muris (Figure S2B).

IL-4 and type 2 immune responses orchestrate many of the known anti-helminth, immune-driven expulsion pathways (Hashimoto et al., 2009). We therefore assessed innate and adaptive type 2 immune responses in Pla2g1b−/− mice and, to our surprise, found that all measured type 2 immune responses were intact. Specifically, by crossing Pla2g1b−/− mice onto an Il4gfp reporter background or by measuring IL-4 protein by intracellular staining, we found that Th2 cell commitment and differentiation were equivalent between genotypes (Figures 2C and 2D). Parasite-specific, Th2-derived cytokines IL-5 and IL-13 were also comparable (Figure 2E), with no measurable difference in IFNγ+ or IL-17A+ T cells (Figure 2D). Lymphocyte populations in Pla2g1b−/− mice, both at baseline and following 21 H.p. infection, were comparable to WT mice, including both CD4+ and CD8+ T cells, ILC2s, which support early Th2 differentiation (Pelly et al., 2016) and regulatory T cells, which inhibit type 2 immune responses (Wilson et al., 2005; Figures S2C–S2J and S3A).
Alternatively activated macrophage-associated genes, which are also essential for immunity to H. p. (Anthony et al., 2006), were similar between genotypes in vivo (Figure 2F) and following polarization in vitro (Figure S3B). Finally, B cell frequencies and serum antibodies, including H. p.-specific IgG1 and IgE, which are important for preventing adult worm development (McCoy et al., 2008), were comparable between WT and Pla2g1b\(-/-\) mice (Figures 2G, S3C, and S3D). Physiological responses— including goblet cell hyperplasia and mucus hypersecretion, which correlate with expulsion— were also comparable between WT and Pla2g1b\(-/-\) mice (Figures 2H and S3E). RNA sequencing and pathway analysis of the transcriptome of duodenal tissue from naive and infected Pla2g1b\(-/-\) mice 7 days after H. p. infection identified that the transcriptional responses and associated pathways were very similar to WT mice with the exception of 2 genes, Pla2g1b and Lars2 (Figures 2I, 2J, and S3F). To our surprise, predicted activation of lipid metabolism pathways and synthesis of bioactive lipids, including cysteine leukotrienes and prostaglandin E 2 (PGE2), serum fatty acid metabolites, and serum Lysophosphatidylcholines (LPC), were unaffected in Pla2g1b\(-/-\) mice (Figures 2K and S3G–S3I), most likely due to unaltered expression of Pla2g1b in vivo and cg-dependent signaling was required for the upregulation of Pla2g1b in resistant mice (Figure 3D), suggesting that Pla2g1b is not regulated in an IL-4 or type-2 immune dependent manner. To test whether Rag-dependent, adaptive immune cells and common gamma chain (cg)-dependent innate immune cells and signaling were required for Pla2g1b expression, we infected and drug-treated WT and Rag2\(-/-\)/cg\(-/-\) mice. Following drug treatment, WT, but not Rag2\(-/-\)/cg\(-/-\), mice upregulated Pla2g1b, indicating that at this time prior to immunemediated active expulsion, adaptive and cg-dependent signaling was required for the upregulation of Pla2g1b in vivo (Figure 3E).

To identify additional type 2 independent mechanisms of Pla2g1b upregulation, we tested whether the host microbiota contributed to Pla2g1b regulation. Antibiotic-treated mice completely failed to upregulate Pla2g1b (Figure 3F), indicating that intestinal microbiota are essential for elevated Pla2g1b expression. Intestinal microbiota changes following helminth infection (Giacomin et al., 2016; Rausch et al., 2013; Reynolds et al., 2014; Zaisz et al., 2015) have previously been reported. However, whether a microbiota-driven Pla2g1b axis has evolved to restore intestinal homeostasis and protect from small-intestine dwelling helminths is unclear.
In situ hybridization localized Pla2g1b expression to the epithelial layer following H. p. 2° infection (Figures 4A and S4A) rather than in the granuloma where larvae embed (Figure S4B). We confirmed that Pla2g1b was elevated in FACS-purified CD45 EpCam+ epithelial cells rather than CD45 EpCam− cells isolated from drug-cured mice compared to naive (Figure 4B). With the recent identification of tuft cells as important in anti-helminth immunity (Gerbe et al., 2016; Howitt et al., 2016; von Moltke et al., 2016), we analyzed the expression of tuft-cell-specific markers in our RNA sequencing data from the H. p. challenge infection model (Figure 1A). We identified significant upregulation of the tuft cell markers Dclk, Trpm5, Siglec5 and Pou2f3 in resistant H. p. 2° infected, but not in H. p. 1° infected mice (Figure S4C). Importantly, expression of tuft cell markers, which peaked transiently following infection, did not correlate with Pla2g1b expression, which was upregulated following drug cure of 1° infection and maintained with or without 2° challenge infection. These data suggest that Pla2g1b expression was not restricted to tuft cells, or at least tuft-cell-associated gene expression. In addition, although Pla2g1b is abundantly expressed in the pancreas (Eerola et al., 2006), it was not differentially expressed in the pancreas following infection, drug treatment, or reinfection (Figure S4D). Both our findings (Figures 2 and S3) and previous data (Hollie and Hui, 2011; Labonté et al., 2010) suggest that PLA2G1B does not contribute to altered dietary phospholipid digestion at steady state or during H. p. infection. To determine how Pla2g1b was regulated in epithelial cells, we generated ex vivo organoid cultures (Sato et al., 2009) and found that IL-4R signaling decreased Pla2g1b expression but increased the expulsion-related genes Relmβ (Relmβ) and Gob5 (Hashimoto et al., 2009; Herbert et al., 2009; Figure 4C). These data again uncouple Pla2g1b expression from type 2
immune pathways that drive expulsion mechanisms (Anthony et al., 2006; Herbert et al., 2009; Urban et al., 1991b) and suggest that type 2 immunity may negatively regulate Pla2g1b expression to protect host tissues from the potent effects of phospholipase enzymes (Murakami et al., 2011).

Other sPLA2 family members can degrade bacterial membranes and protect from fungal infections (Balestrieri et al., 2009; Degousee et al., 2002; Koduri et al., 2002; Weinrauch et al., 1998). We therefore asked whether PLA2g1B had a direct effect on \( H. p. \) by treating sheathed or exsheathed L3 \( H. p. \) larvae (to recapitulate the status of the larvae in the small intestine after passing through the stomach) (Sommerville and Bailey, 1973) and adult \( H. p. \) worms with recombinant PLA2g1B. No effect was identified on the fitness of treated L3 larvae, isolated L4 larvae, or adult worms, as determined by ATP concentration (Figures 5A, S5A, and S5B). To test the infectivity and viability of PLA2g1B-treated L3 larvae, we infected mice with PLA2g1B-treated L3 larvae and found that PLA2g1B-treated L3 larvae could embed into the intestinal wall (Figure 5B) However, significantly fewer treated larvae developed into adulthood (Figure 5C), impacting egg recovery in the feces, although failing to reach statistical significance (Figure S5C).

We therefore hypothesized that, for clearance of \( H. p. \), a combined PLA2g1B-mediated impact on larvae in concert with immune-mediated physiological responses would be required. To test this, we infected and drug cured WT and \( Pla2g1b^{+/+} \) mice to elicit robust type 2 immune responses (Figure 2) and challenged mice with vehicle- or PLA2g1B-treated L3 larvae (model, Figure 5D). WT mice cleared the majority of either vehicle or PLA2g1B-treated \( H. p. \) larvae. \( Pla2g1b^{-/-} \) mice failed to clear vehicle-treated L3 larvae, as expected (Figure 2). However, the protective effect of PLA2g1B-treated L3 larvae during a 1\(^{st}\) infection did not recapitulate the full killing, expulsion, and clearance of worms observed during 2\(^{nd}\) \( H. p. \) infection (Figure 2).

We therefore hypothesized that, for clearance of \( H. p. \), a combined PLA2g1B-mediated impact on larvae in concert with immune-mediated physiological responses would be required. To test this, we infected and drug cured WT and \( Pla2g1b^{-/-} \) mice to elicit robust type 2 immune responses (Figure 2) and challenged mice with vehicle- or PLA2g1B-treated L3 larvae (model, Figure 5D). WT mice cleared the majority of either vehicle or PLA2g1B-treated \( H. p. \) larvae. \( Pla2g1b^{-/-} \) mice failed to clear vehicle-treated L3 larvae, as expected (Figure 2). However,
H. p. killing, expulsion, and clearance were completely rescued in Pla2g1b mice when L3 larvae were directly treated with PLA2G1B (Figure 5E), suggesting that killing and expulsion of H. p. requires both PLA2G1B-mediated effects on L3 larvae and immune-mediated expulsion mechanisms.

The requirement for a combined functional immune compartment alongside direct PLA2G1B-mediated action was confirmed by infecting Rag-/-cg-/- mice with PLA2G1B-treated larvae. Upon 1° infection, Rag-/-cg-/- mice fail to expel PLA2G1B-treated larvae, unlike WT mice (Figure 5F). These data highlight the clear requirement of a competent immune compartment for parasite expulsion. This observation was reinforced when Rag-/-cg-/- mice that were infected, drug cured, and challenged with PLA2G1B-treated L3 larvae failed to expel H. p., unlike WT mice (Figure 5G).

Nevertheless, these data demonstrate that PLA2G1B has direct anthelminthic properties distinct from type 2 immune responses and that PLA2G1B is essential for intestinal helminth clearance during H. p. infection. Furthermore, these data add to other discovered anti-microbial properties of sPLA2 enzymes (Balestrieri et al., 2009; Degousee et al., 2002; Koduri et al., 2002; Maizels and Hewitson, 2016; Weinrauch et al., 1998) and to the site-specific arsenal of anti-microbial responses in the small intestine (Gallo and Hooper, 2012).

Finally, to identify the direct effects of PLA2G1B on H. p. L3 larvae, we treated larvae for 24 hr with PLA2G1B or control buffer and subjected the treated larvae to scanning electron microscopy (SEM) and lipid composition analysis using liquid chromatography-mass spectrometry (LC-MS). Although SEM did not reveal any structural changes or alterations in membrane integrity (Figure 5E), LC-MS analysis identified a significantly lower phospholipid abundance in the PLA2G1B-treated larvae when compared to untreated controls (Figure S6A). Of the 1,165 apolar features detected, only 112 were significantly different following PLA2G1B treatment; 6 were identified as phosphatidylethanolamines (PEs) (by comparing their precursor ion and MS/MS fragments with the LipidBlast library) (Figures 6A and S6A–S6E), with each being of lower abundance in the treated larvae. We also identified a similar trend in a number of putatively identified PEs (as determined by comparison of peak retention time with other identified PEs, together with intercluster mass shifts of 28 Da (CH2CH2) and intracluster mass shifts of 2 Da, indicative of differences in double bond number [fatty acid saturation]) with other, identified PEs. MS/MS could not be performed due to low abundance.

Data are shown as normalized intensities expressed in arbitrary units. Data are represented as mean ± SEM, n = 3. * = p < 0.05. TIC: Total ion current. See also Figure S7.
Anthony et al., 2006; Esser-von Bieren et al., 2015), although this greater immune cell recognition and larval trapping in the tissue explanation is that reducing larval phospholipids may allow for overlooked role for epithelial-cell-derived PLA2g1B as an anti-helminth expulsion mechanisms from the production of has not been tested here.

(Figure 6B). The remaining, significantly different apolar features were seen to be both increased and decreased following PLA2g1B treatment; however, these features were unable to be identified (examples shown in Figure S6F). PEs are highly abundant phospholipids found in membranes of bacteria, yeast, and mammals and are required for an array of cellular functions including membrane fusion, cytokinesis, cell division, membrane curvature, and as a substrate for subsequent products (Wellner et al., 2013). The relative reduction in PEs in PLA2g1B-treated H.p. L3 larvae provides one explanation for many putative downstream impacts on larval integrity, health, and infectivity. For example, studies in yeast and Caenorhabditis elegans identified that low levels of PE can cause ER stress and disrupt vesicle trafficking (Wang et al., 2014). Whether similar effects are observed in PLA2g1B-treated H.p. are currently unclear. Another possible explanation is that reducing larval phospholipids may allow for greater immune cell recognition and larval trapping in the tissue (Anthony et al., 2006; Esser-von Bieren et al., 2015), although this has not been tested here.

Taken together, our data uncouple type 2 immune-mediated anti-helminth expulsion mechanisms from the production of host enzymes that mediate direct anti-helminth activity, which are regulated by the microbiota and require competent innate and adaptive immunity. Specifically, we highlight a previously overlooked role for epithelial-cell-derived PLA2g1B as an essential endogenous anthelmintic that has direct effects on invading larvae, possibly by reducing phospholipid levels. Identifying mechanisms that regulate site-specific expression of Pla2g1b and the functional role of phospholipids in helminths may provide avenues toward greater protection from helminth infection.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Animal Strains
  - Organoid Culture
  - Bone Marrow-derived Macrophage (BMDM) Culture and Stimulation
- **METHOD DETAILS**
  - Parasite Infections and PLA2g1B Treatment
  - ATP Assay
  - Antibiotic Treatment
  - Antibody Treatment
  - Histology, In Situ Hybridization
  - RNA Sequencing and Analysis
  - Quantitative Real-time Polymerase Chain Reaction
  - PLA2 Activity Assay
  - Cell Isolation
  - Flow Cytometry and Cell Sorting
  - Ex Vivo Stimulations
  - ELISAs
  - Scanning Electron Microscopy (SEM)
  - Serum Chemistry
- **Lipid Extraction and Analysis**
- **Experimental Design**
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
- **DATA AND SOFTWARE AVAILABILITY**

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and four tables and can be found with this article online at https://doi.org/10.1016/j.chom.2017.09.006.

AUTHOR CONTRIBUTIONS

L.J.E. performed and analyzed the majority of experiments. V.S.P., S.M.C., J.P.-L., S.C., and Y.K. assisted with mouse studies and flow cytometry experiments; A.S. contributed to the design of the RNA sequencing experimental design; N.N. assisted with the analysis of the RNA sequencing data; and A.M. assisted with organoid experiments. L.C. assisted with SEM experiments. M.S.d.S. and J.I.M. performed and analyzed LC-MS experiments. H.H. kindly provided N. brasiliensis L3 larvae, and D.Y.H. donated Pla2g1b−/− mice. L.J.E. and M.S.W. designed experiments and wrote the manuscript.

ACKNOWLEDGMENTS

The authors thank The Francis Crick Institute Advanced Sequencing Facility, especially Abdul Sesay, Leena Bhaw, and Harsha Jari, for the RNA sequencing critical to this work. We would like to thank The Francis Crick Institute Flow Cytometry facility (Bhavik Patel, Graham Preece, Wayne Turnbull, and Phil Hobson) for cell sorting services and Gitta Stockinger for critically reading the manuscript. We are indebted to The Francis Crick Institute Procedural Service Section for the production of GA lines; to Biological Services, especially Trisha Norton, Keith Williams, and Adebambo Adekoya, for animal husbandry and technical support; to Radma Mahmood, Radika Anand and Bradley Spencer-Dene for help with histology and in situ hybridisation; and to Riccardo Guidi for constructive discussions and technical assistance. This work was supported by the Francis Crick Institute (grant number FCI01) which receives its core funding from Cancer Research UK, the UK Medical Research Council (MC_UP_A253_1028), and the Wellcome Trust. D.Y.H. was supported by NIH grant RO1 DK112657. M.S.W. is an employee of Gentech Inc.

Received: January 29, 2017

Accepted: September 14, 2017

Published: October 11, 2017

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## STAR METHODS

### KEY RESOURCES TABLE

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<td>Mouse: <em>Pla2g1b</em> <em>–/–</em> <em>Il4</em>&lt;sup&gt;gfp&lt;/sup&gt; (C57BL/6 background)</td>
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<td>Mouse: C57BL/6</td>
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<td>Heligmosomoides polygyrus bakeri</td>
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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Mark Wilson (wilson.mark@gene.com)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal Strains

All mice used in this study were maintained under specific pathogen-free conditions at the Mill Hill Laboratory, The Francis Crick Institute (London, UK). C57BL/6, Pla2g1b+/−/−/− (Richmond et al., 2001), 4get (Mohrs et al., 2001), Rag2−/−cg−/− and Rag2−/− mice were bred and maintained at The Francis Crick Institute. Pla2g1b+/−/−/−Il4gfp mice were generated by crossing Pla2g1b+/−/−/− and 4get mice at The Francis Crick Institute. All mice used were male and between 6-12 weeks old at the start of the experiment and were not involved in any previous procedures. Animal experiments were performed according to institutional guidelines and following UK Home Office regulations (project license 70/8809) and were approved by The Francis Crick Institute Ethical Review Panel.

Organoid Culture

Mouse organoids were established and maintained at 37°C as three-dimensional spheroid culture in Matrigel (R&D system) from isolated crypts collected from the duodenum of male C57BL/6 mice. The basic culture medium (ENR) contained advanced DMEM/F12 supplemented with penicillin/streptomycin, 10 mM HEPES, 2mM Glutamax, B27 (all from Life Technologies) and 1cmM N-acetylcysteine (Sigma) supplemented with murine recombinant EGF (life technologies), R-spondin1-CM (Trevigen) (10% final volume) and Noggin-CM (kindly provided by Dr. Hans Clevers, Hubrecht Institute, Utrecht, the Netherlands) (20% v/v). Wnt3a-CM was used at 50% (v/v) for 7 days at the beginning of the culture, then withdrawn. Organoids were stimulated with 20 ng/mL IL-4 (R&D) and 20ng/mL IL-13 (R&D) for 48 hr. RNA was extracted and qRT-PCR performed as described below.

Bone Marrow-derived Macrophage (BMDM) Culture and Stimulation

Bone marrow was isolated from the femur and tibia of mice and the red blood cells were lysed with ACK lysis buffer (GIBCO). The remaining cells were then cultured in DMEM (GIBCO) (with 20% L929 cell media (in-house preparation), 10% FCS (Invitrogen), 1% L-Glutamine (GIBCO), 100 U/mL Penicillin and 100 μg/mL Streptomycin (GIBCO), 10 mM HEPES (Lonza) and 0.05mM 2-mercaptoethanol (GIBCO)) in 10mls at a density of 5x10^5 cells/ml at 37°C. After 7 days of culture non-adhesive cells were removed before removing adherent BMDMs using 2.5mM edta (Invitrogen) in PBS (GIBCO) with 5% FCS (Invitrogen). Adherent BMDMs were washed and resuspended in DMEM (with 1% FCS (Invitrogen), 1% L-Glutamine (GIBCO), 100 U/mL Penicillin and 100 μg/mL Streptomycin (GIBCO), 10 μM HEPES (Lonza) and 0.05mM 2-mercaptoethanol (GIBCO)). Adherent BMDMs were then plated at a density of 2x10^6 cells/ml for 24 hr. The adherent BMDMs were then stimulated for 24 hr with either 20ng/ml IL-4 (R&D) and 20ng/ml IL-13 (R&D) before RNA extraction.

METHOD DETAILS

Parasite Infections and PLA2g1B Treatment

_Heligmosomoides polygyrus bakeri_

Mice were infected with 200 L3 infective _H. polygyrus_ larvae (p.o.) on day 0 (1° infection). Mice were drug cured (Rx) with the anthelminthic drug Pyrantel Embonate (2.5 mg/dose, Pfizer) (p.o.) on days 14 and 15. Mice were secondary (2°) challenge infected on day 35 or day 56 with 200 L3 infective _H. polygyrus_ larvae (p.o.). _H. polygyrus_ worms were counted in the wall of the intestine at day 5 post infection and luminal worms were counted 14 days-post 1° or 2° infection.

_Nippostrongylus brasiliensis_

Mice were infected with 350 L3 infective _N. brasiliensis larva_ (s.c.) on day 0. Luminal _N. brasiliensis_ worms were counted on day 8.
Trichuris muris
Mice were infected with 200 embryonated T. muris eggs (p.o.) on day 0. Luminal T. muris worms were counted on day 35. For PLA2g1B treatment, infective L3 H. polygyrus larvae were exsheathed as previously described (Sommerville and Bailey, 1973). Briefly, 0.85% w/v NaCl (in distilled water) was bubbled with 40% CO2 in Nitrogen for 5 min, the tube was then sealed and incubated in a 37° C waterbath for a further 5 min. The pH was adjusted to pH2 using HCl and 5 mL was added to 30000-50000 L3 larvae before bubbling with 40% CO2 in Nitrogen for 30 s. The tube was then sealed and incubated in a 37° C waterbath for a further 30 min. Exsheathed L3 larvae were washed and counted before treatment with PLA2g1B. 8000 exsheathed L3 H. polygyrus larvae were treated with recombinant mouse PLA2g1B (Elabscience), recombinant mouse PLA2g1B plus manoalide (200 ng/mL final concentration, Santa Cruz Biotechnology) or heat inactivated recombinant mouse PLA2g1B in 1mL EnzChek® PLA2 reaction buffer (Invitrogen) at room temperature for 24 hr. Recombinant mouse PLA2g1B was heat inactivated by heating at 100°C for 4 hr. Following treatment, larvae were washed with MilliQ water prior to use in ATP assay (detailed below) or infection. Adult L5 H. polygyrus worms were isolated from C57BL/6 following primary infection between days 14 and 28 using a modified Baermann apparatus. Adult L5 H. polygyrus worms were treated with recombinant mouse PLA2g1B (Creative Biomart) or heat inactivated recombinant mouse PLA2g1B in EnzChek® PLA2 reaction buffer (Invitrogen) at room temperature for 24 hr.

ATP Assay
The ATP of infective L3 H. polygyrus larvae, L4 H. polygyrus larvae (removed from intestinal wall at day 7 post infection) and adult L5 H. polygyrus worms was measured using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Briefly, H. polygyrus adult worms, two L4 larvae or 100 L3 larvae were homogenized using a motorised pestle in 110 μL of PBS and 110 μL of CellTiter-Glo® Reagent. The homogenate was incubated for 10 min at room temperature before centrifugation at 1000 g for 3 min. 200 μL of the supernatant was transferred to a 96 well opaque-walled plate and incubated for 10 min at room temperature before recording luminescence. An ATP standard curve was generated by using recombinant ATP (Promega) as detailed in the CellTiter-Glo® Luminescent Cell Viability Assay instructions.

Antibiotic Treatment
The antibiotics Gentamicin sulfate salt (1 mg/mL, Sigma), Metronidazole (1 mg/mL, Sigma), Cefloxin sodium salt (1 mg/mL, Santa Cruz Biotechnology), Vancomycin hydrochloride (1 mg/mL, Sigma) were administered in the drinking water. Treatment was started 7 days prior to 1° H. polygyrus infection and maintained throughout the duration of the experiment.

Antibody Treatment
Anti-IL-4 antibody (0.5 mg/dose, BioXcell) was administered i.p. on days 13, 15, 17, 19 and 21 after 1° H. polygyrus infection. Mice were drug cured (Rx) with the anthelminthic drug Pyrantel Embonate (2.5 mg/dose, Pfizer) (p.o.) on days 14 and 15.

Histology, In Situ Hybridization
Small intestinal tissue was removed and fixed in 4% formaldehyde for 24 hr then washed in 70% ethanol. The tissues were embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin stain or Alcian blue/ periodic acid-Schiff stain. Small intestinal tissue was removed and fixed in 4% formaldehyde for 24 hr then washed in 70% ethanol. The tissues were embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin stain or Alcian blue/ periodic acid-Schiff stain.

RNA Sequencing and Analysis
RNA was extracted using the QIAGEN® miRNeasy Mini Kit, following the manufacturer’s instructions. RNA integrity was confirmed using Agilent’s 2100 Bioanalyzer. Total RNA libraries were created using the Encore® Complete RNA-Seq Library Systems kit (NuGEN), following manufacturer’s instructions. Total RNA libraries were sequenced using the Illumina® HiSeq 2500. The raw Illumina reads were analyzed as follows. First, the data quality was analyzed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Then the low quality bases were trimmed using Trimmomatic. The read pairs which passed the trimming quality filters were then aligned to mm10 (Ensembl version 75) using TopHat2. Counts were determined using htseq_count. Normalization and statistical analysis was performed using edgeR. Differential gene analysis was calculated from naive control group. Statistically significant genes with FDR < 0.05 are reported. Ingenuity Pathway Analysis® (IPA®): RNA sequencing datasets were uploaded to IPA® where fold change filters and pathway analysis algorithms were applied. Ratio of ratios plots were generated from the ratio of expression of genes from H.p. 2° to H.p. 1° (relative to naive, 2-fold filter, p < 0.05) (y axis) against the fold-change of each gene in both compared to naive (2-fold filter, p < 0.05) (x axis).

Quantitative Real-Time Polymerase Chain Reaction
RNA was extracted and purified from tissue or cells as described above. Reverse transcription was performed with 0.1-1 μg RNA using QIAGEN® Quantitect RT Kit following manufacturer’s instructions to create cDNA. Generated cDNA was used for quantitative real-time PCR analysis using Power SYBR® Green PCR Master Mix (Applied Biosystems) and quantified on the 7900HT (Applied
PLA$_2$ Activity Assay

PLA$_2$ activity was determined using EnzChek$^\text{TM}$ Phospholipase A$_2$ Assay Kit (Invitrogen), following manufactures instructions. Briefly, approximately 1cm of duodenal tissue was removed and homogenized in 300 $\mu$L of cOmplete protease inhibitor (Roche) before centrifugation. 25 $\mu$L of the supernatant was transferred to a 96 XXX well plate with 25 $\mu$L of the substrate-liposome mix then incubated at room temperature in the dark for 10 min. Fluorescence emission was measured at 515nm and reported after blank reduction.

Cell Isolation

The spleen, mLNs and thymus were made into single-cell suspensions by gently mashing through a 40 micron filter (Thermo-Scientific, Loughborough, UK), and the red blood cells were lysed from the spleen single cell suspension with ACK lysis buffer (GIBCO). Single cell suspensions were used for ex vivo restimulations and flow cytometry analysis. For the isolation of small intestinal epithelial cells: adipose tissue was removed from the small intestine dissected longitudinally to remove faecal contents, and cut into 2cm segments. The epithelial layer was then dissociated by in incubating the intestine segments in PBS containing 10% FBS, 15mm HEPES, 5mm EDTA (Life Technologies, Paisley, UK), and 1cmmm dithiothreitol (Sigma, Gillingham, UK) for 30minutes at 37°C. The remaining intestinal tissue was removed using a wide mesh sieve and epithelial layer was retained. Cells were layered onto 20% isotonic Percoll (GE Healthcare, Little Chalfont, UK) to remove debris. Cells were then resuspended in cIMDM (complete Iscove’s Modified Dulbecco’s Medium (cIMDM) containing 1% fetal bovine serum (FBS), 1mM EDTA, 100U/ml Penicillin (GIBCO) and 100 $\mu$g/ml Streptomycin (GIBCO), 8mM L-glutamine (GIBCO) and 0.05mM 2-mercaptoethanol (GIBCO)) and prepared for cell sorting.

Flow Cytometry and Cell Sorting

Cell sorting was performed using a MoFlo XDP cell sorter (Beckman Coulter). Cell suspensions were stained for 25 min with antibodies in PBS with 1% FCS. To prepare for sorting, stained cells were diluted in phenol-red free IMDM (GIBCO) with 1% FCS, 2mM EDTA (Invitrogen), 100 U/mL Penicillin and 100 $\mu$g/mL Streptomycin (GIBCO), 8mM L-glutamine (GIBCO), and 0.05mM 2-mercaptoethanol (GIBCO). For flow cytometry analysis, cells were analyzed using a BD LSRFortessa X-20 (BD Biosciences) or BD LSRII (BD Biosciences) and data were analyzed using FlowJo software (Version 10, Treestar Inc). Cells were sometimes fixed in 2%–4% paraformaldehyde for FACS analysis. For cell sorting, viability of the cells was determined using Propidium Iodide (Sigma); for analysis, viability of the cells was determined using the LIVE/DEAD Fixable Blue kit (Life Technologies). Antibodies used include: CD3 (145-2C11; APC, (BioLegend)), CD4 (RM4-5; BV605, efluor450 (eBioscience), APC (BioLegend)), CD8 (53-6.7; PE-Cy7, APC (BioLegend)), CD11c (N418; APC (BioLegend)), CD11b (M1/70; APC (BioLegend)), CD19 (1D3; efluor450 (eBioscience)), CD16 (3D5; APC (BioLegend)), CD25 (PC61; APC-Cy7 (BioLegend)), APC (eBioscience)), CD44 (30-F11; FITC (Bioscience)), CD49b (DX5; APC (BioLegend)), CD62L (MEL-14; APC (eBioscience)), CD69 (H1.2.F3; PE (BioLegend), EpCam (G8.8; APC (eBioscience), Foxp3 (FJK-16S; PE (eBioscience)), Gr1 (RB6-8C5; APC (BioLegend)), IFN$\gamma$ (XMG1.2; PE (BD Biosciences)), IL-4 (PE, 11B11, efluor450 (eBioscience)), IL7a (17B7; PE-Cy7 (eBioscience)), KLRG1 (2F1; PerCpCel710 (eBioscience)), NK1.1 (PK136; APC (BioLegend)), Sca1 (E13-161.7; PB (eBioscience)), TCR$\gamma$ (GL3; APC (BioLegend)), TCR$\beta$ (H57-597; APC (eBioscience)), TCR$\alpha$ (H57-597; PerCpCel5.5 (BioLegend)), Ter119 (TER-119; APC (BioLegend), Thy1.2 (53-2.1; PE-Cy7 (BioLegend)). All staining was performed in the presence of FcR Blocking Reagent (Miltenyi Biotec). Intracellular cytokine staining (ICS) was performed following 6 hr of re-stimulation with 50ng/mL phorbol 12-myristate 13-acetate (PMA, Promega) and 1 $\mu$g/mL ionomycin (Sigma) and BD Golgi Stop and BD Golgi Plug (diluted 1:1000, BD Biosciences). Following surface staining, cells were incubated with eBioscience Fixation/Permeabilization buffer for 25 min followed by 25 min in Permeabilization buffer (for a further 30 min). ILCs were analyzed using the following strategy: Live, lymphocytes, CD45$^+$, Lineage$^-$ (CD3, CD4, CD8, CD19, CD11c, CD11b, NK1.1, TCR$\beta$, TCR$\gamma$, Gr-1, CD49b, Ter119), Thy1.2$^+$, KLRG1$^+$, and Sca1$^+$. Tregs were analyzed using the following gating strategy: Live, lymphocytes, CD4$^+$, TCR$\beta^+$, CD25$^+$ and Foxp3$^+$. Epithelial cells were sorted using the following gating strategy: Live, CD45$^+$ and EpCam$^+$. 

Ex Vivo Stimulations

mLNs were harvested and processed as above. Cells were plated at 2x10$^5$ cells per 200$\mu$L with 10$\mu$g/ml of H. polygyrus antigen extract (HEX). Supernatant was harvested after 4 days. Cytokines were detected in the supernatant using ELISAs.

ELISAs

IFN$\gamma$, IL-5 and IL-13 were measured using DuoSet ELISA kits, according to the manufacturer’s instructions (R&D). Cysteinyl leukotrienes and prostaglandin E$_2$ were measured in small intestinal homogenate (see above) using ELISA kits, according to manufacturer’s instructions (Enzo), and normalized to total protein content. Total IgE ELISA was performed by coating with Purified Rat Anti-Mouse IgE (R35-72, BD PharMingen) at 2 $\mu$g/mL overnight, followed by overnight incubation with serum and standard (Purified Mouse IgE.k isotype Standard, BD PharMingen), and detection with Biotin Rat Anti-Mouse IgE at 1 $\mu$g/mL (R35-118, BD PharMingen), Streptavidin HRP at 1:000 (BD PharMingen) and ABTS One Component HRP Microwell Substrate (SurModics). H. polygyrus-specific IgG1 was detected by coating plates with 5 $\mu$g/mL H. polygyrus antigen overnight, followed by
overnight incubation with serially diluted serum and detection with Biotin Rat Anti-Mouse IgG1 (Invitrogen) and streptavidin and ABTS, as above.

Scanning Electron Microscopy (SEM)

H. polygyrus larvae were dehydrated stepwise in ethanol (2 × 5 min in 70, 90 and 100% ethanol) before transferring to 100% acetone (2 × 5 min). Larvae were critical point dried from 100% Ethanol in a CPD300 critical point drier (Leica Microsystems UK), mounted on a carbon sticky pad on a stub, sputter-coated with 5 nm of platinum, and imaged in a Phenom ProX benchtop scanning electron microscope (Phenom-World) with a secondary electron detector.

Serum Chemistry

Whole blood was collected from mice and the serum separated after clotting. The serum was sent to the MRC Harwell Institute (UK) for metabolite analysis. Lysophosphatidylcholine (LPC) was measured using the AZWELL LPC Assay Kit according to the manufacturer’s instructions (Cosmo Bio).

Lipid Extraction and Analysis

Lipids were extracted from H. polygyrus L3 larvae using a method adapted from a previous publication (Meyer et al., 1966). Following PLA2g1B treatment ~8000 H. polygyrus larvae were washed three times with MilliQ water, resuspended in 2 mL methanol and heated in a sealed tube under nitrogen at 55°C for 20 min. After cooling, 4 mL chloroform was added and the sample was agitated with a magnetic stirrer for 3 hr. The organic phase was removed and the residue ground (using a glass homogenizer) and extracted with 2 mL chloroform/methanol (2:1, v/v) for 2 hr.

For LC-MS, lipids were dried under nitrogen and redissolved in 100 μL of methanol/chloroform (1:1 v/v) and diluted 1:2 with solvent A (hexane:isopropanol, 70:30 [v:v], 0.02% [m/v] formic acid, 0.01% [m/v] ammonium hydroxide), centrifuged at 1,500 rpm for 5 min to remove trace non-lipidic materials prior to transfer to a glass autosampler vial (Agilent). 10 μL was injected onto a BETASIL diol column (5 μm x 150 mm x 2.1 mm, with BETASIL diol guard column (10 mm x 2.1 mm), held at 20°C) in an Ultimate 3000 HPLC system coupled to a Thermo Exactaive Plus Orbitrap MSfor full scan or Q Exactive Orbitrap MS for MS/MS scan. Lipids were eluted at 0.15 mL/min with a binary gradient from 0% to 100% solvent B (isopropanol:methanol, 70:30 [v/v], 0.02% [m/v] formic acid, 0.01% [m/v] ammonium hydroxide): 0–10 min, 0% B; 17–22 min, 50% B; 30–35 min, 100% B; 40–44 min, 0% B, followed by additional 6 min 0% B post-run. MS data were acquired in both polarities using a full scan method. The positive and negative HESI-II spray voltages were 4.5 and 3.5 kV, respectively; the heated capillary temperature was 250°C; the sheath gas pressure was 30 psi; the auxiliary gas setting was 20 psi; and the heated vaporizer temperature was 150°C. Both the sheath gas and the auxiliary gas were nitrogen. The parameters of the full mass scan were as follows: a resolution of 70,000, an auto gain control target under 3 × 10⁶, a maximum isolation time of 200 ms, and an m/z range 200–3000. To confirm the identification of significant features, samples were re-run in parallel reaction monitoring (PRM, mode, parameters as follows: a resolution of 17,500, an auto gain control target under 2 × 10⁵, a maximum isolation time of 100 ms, an isolation window of m/z 0.4 and normalized collision energy were optimized for each feature individually. Data were acquired using Xcalibur 3.0.63 (Thermo Fisher Scientific) and Progenesis (Nonlinear Dynamics) was used for data alignment and peak detection. Data were normalized against the total ion abundance.

Annotations were assigned to accurate masses with a maximum error of 5 ppm using Metlin, LipidMaps, Kegg and HMDB which were searched simultaneously using the CEU Mass Mediator engine (http://ceumass.eps.uspceu.es/mediator/).

Experimental Design

All experiments contained at least three biological replicates and are representative of at least two independent experiments (see figure legends for exact values). No strategy was employed for randomization, sample size estimation or data inclusion/exclusion criteria. The studies performed were also not blinded at any stage.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analysis for biological data was performed using GraphPad Prism (v6.02). Data was analyzed, where appropriate, with either an unpaired two-tailed t test, One-way ANOVA (Dunnett’s multiple comparison analysis), Two-way ANOVA (Sidak’s multiple comparison analysis) or Mann-Whitney test. n represents the number of biological replicates. Please see figure legends for statistical tests used and exact value of n. No methods were used to confirm whether the data met assumptions of the statistical approach used. Values are reported as the means ± SEM. * = p < 0.05, ** = p < 0.01, *** = p < 0.001 and **** = p < 0.0001.

DATA AND SOFTWARE AVAILABILITY

The raw and analyzed RNA sequencing data files have been deposited in the NCBI Gene Expression Omnibus database under ID code GEO: GSE102789. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102789

LC-MS metabolomics data have been deposited in the EMBL-EBI MetaboLights database under ID code MTBLS523 and PubMed PMID: 23109552. http://www.ebi.ac.uk/metabolights/MTBLS523