Received: 11 July 2018 | Revised: 25 October 2018 | Accepted: 26 November 2018

DOI: 10.1111/all.13700

ORIGINAL ARTICLE

Experimental Allergy and Immunology

WILEY Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

House dust mite drives proinflammatory eicosanoid reprogramming and macrophage effector functions

Fiona D. R. Henkel[1]* | Antonie Friedl[1]* | Mark Haid[2] | Dominique Thomas[3] | Tiffany Bouchery[4,5] | Pascal Haimerl[1] | Marta de los Reyes Jiménez[1] | Francesca Alessandrini[1] | Carsten B. Schmidt-Weber[1] | Nicola L. Harris[4,5] | Jerzy Adamski[2,6] | Julia Esser-von Bieren[1]

[1]Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany

[2]Institute of Experimental Genetics, Genome Analysis Center, Neuherberg, Germany

[3]Pharmazentrum frankfurt/ZAFES, Institute of Clinical Pharmacology, Goethe University Frankfurt, Frankfurt, Germany

[4]Laboratory of Intestinal Immunology, Global Health Institute, School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[5]Department of Immunology and Pathology, Central Clinical School, Alfred Medical Research and Education Precinct, Monash University, Clayton, Victoria, Australia

[6]Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising-Weihenstephan, Germany

Correspondence
Julia Esser-von Bieren, Center of Allergy and Environment (ZAUM), Technische Universität München and Helmholtz Zentrum München, Munich, Germany.
Email: Julia.esser@tum.de

Funding information
This study was supported by the German Research Foundation (DFG) (grants ES 471/2-1 and ES 471/3-1) and by the Else Kröner-Fresenius-Stiftung (grant 2015_A195). CSW receives grant support by the German Center for Lung Research (DZL; 82DZL00302). MH was supported by Innovative Medicines Initiative Joint Undertaking under Grant agreement number 115317 (DIRECT): "The work leading to this publication has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no 115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution."

Abstract

Background: Eicosanoid lipid mediators play key roles in type 2 immune responses, for example in allergy and asthma. Macrophages represent major producers of eicosanoids and they are key effector cells of type 2 immunity. We aimed to comprehensively track eicosanoid profiles during type 2 immune responses to house dust mite (HDM) or helminth infection and to identify mechanisms and functions of eicosanoid reprogramming in human macrophages.

Methods: We established an LC-MS/MS workflow for the quantification of 52 oxylipins to analyze mediator profiles in human monocyte-derived macrophages (MDM) stimulated with HDM and during allergic airway inflammation (AAI) or nematode infection in mice. Expression of eicosanoid enzymes was studied by qPCR and western blot and cytokine production was assessed by multiplex assays.

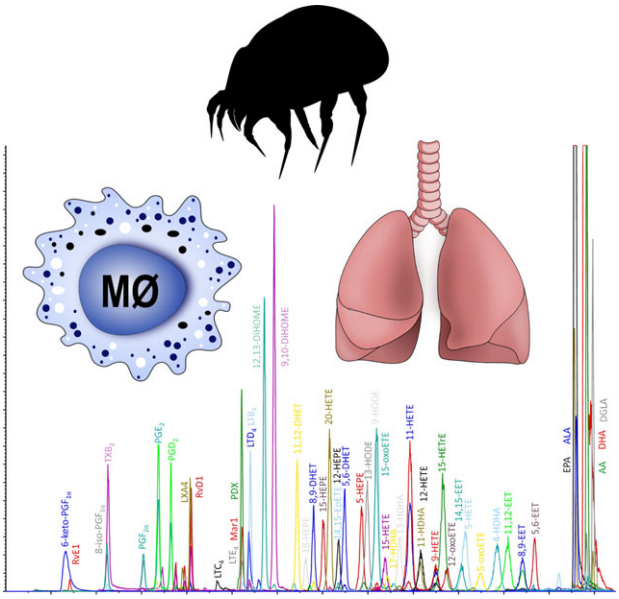
Results: Short (24 h) exposure of alveolar-like MDM (aMDM) to HDM suppressed 5-LOX expression and product formation, while triggering prostanoid (thromboxane and prostaglandin D_2 and E_2) production. This eicosanoid reprogramming was p38-dependent, but dectin-2-independent. HDM also induced proinflammatory cytokine production, but reduced granulocyte recruitment by aMDM. In contrast, high levels of cysteinyl leukotrienes (cysLTs) and 12-/15-LOX metabolites were produced in the airways during AAI or nematode infection in mice.

Abbreviations: 5-LOX, 5-lipoxygenase; COX, cyclooxygenase; cysLTs, cysteinyl leukotrienes; IS, internal standard; LC-MS/MS, liquid chromatography tandem mass spectrometry; LTA4H, leukotriene A4 hydrolase; LTB4, leukotriene B4; LTC4S, leukotriene C4 synthase; MDM, monocyte-derived macrophages; PGs, prostaglandins; PMN, polymorphonuclear leukocytes; SPM, specialized pro-resolving mediator.

*These authors contributed equally.

Conclusion: Our findings show that a short exposure to allergens as well as ongoing type 2 immune responses are characterized by a fundamental reprogramming of the lipid mediator metabolism with macrophages representing particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent a viable approach to prevent pathogenic lipid mediator profiles in allergy or asthma.

KEYWORDS
eicosanoids, house dust mite, LC-MS/MS, macrophages, type 2 inflammation



GRAPHICAL ABSTRACT

Macrophages respond to house dust mite (HDM) by increased cyclooxygenase (COX) metabolism, while the type 2 immune response to HDM or parasitic worms in the airways is characterized by increased lipoxygenase (LOX) metabolism

1 | INTRODUCTION

Lipid mediators govern immune responses in a multitude of infectious or chronic inflammatory settings.[1] In allergy and asthma, prostanoids and leukotrienes (LTs) derived from the polyunsaturated fatty acid (PUFA), arachidonic acid (AA), drive hallmark type 2 immune responses such as eosinophil accumulation.[2,3] AA metabolites (eicosanoids) have also been suggested to contribute to type 2 immunity during nematode infection.[4-6] Despite these important immunological functions, few studies have comprehensively assessed lipid mediator profiles during type 2 immune responses. This is possibly due to the limited availability of adequate LC-MS/MS workflows, which are required for the simultaneous quantification of a multitude of structurally similar but functionally distinct mediators. Indeed, most immunological studies in allergy or nematode infection have used immunoassays to quantify less than a handful of mediators.[5,7,8] However, LC-MS/MS analysis of 18 eicosanoids in macrophages from nematode-infected mice suggested abundant and plastic eicosanoid production during type 2 immune responses.[6] In addition, a number of studies have applied LC-MS/MS approaches to quantify up to 88 lipid mediators in *ex vivo* samples from allergy and asthma patients.[9-12] Moreover, using macrophages as a model system, targeted lipidomics approaches were applied to quantify more than 100 eicosanoid metabolites.[13] Due to their plasticity and abundant expression of eicosanoid biosynthetic pathways, macrophages present an attractive cellular model to study lipid mediator production in immunological settings.[14] In the context of inflammasome activation, targeted lipidomics workflows allowed for the characterization of an "eicosanoid storm" during macrophage activation.[15,16] However, despite these recent advances in lipidomics technologies, information about the lipid mediator profiles in type 2 immune responses remains scarce.

Here, we have established a targeted lipidomics workflow for the simultaneous quantification of 52 oxylipins from several PUFAs (AA, LA, and DHA). We applied this workflow to demonstrate that HDM exposure of human macrophages results in a pronounced eicosanoid reprogramming, characterized by high levels of prostanoids (particularly thromboxane), but low levels of 5-LOX products. This

eicosanoid reprogramming was dependent on p38 MAPK activation, but independent of dectin-2. We further show that HDM-driven eicosanoid reprogramming occurs on the mRNA and protein level and is associated with the production of proinflammatory cytokines and chemokines. However, HDM-exposed macrophages showed a reduced chemotactic potential toward granulocytes, correlating with suppressed LTB_4 production. Together, these findings suggest that HDM induces a proinflammatory macrophage phenotype with impaired effector function. Finally, we quantified mediator profiles in bronchoalveolar lavage fluid (BALF) from HDM-sensitized and nematode-infected mice, thus revealing profound changes in COX and LOX metabolites during type 2 immune responses *in vivo*. In summary, these data show that the AA metabolism is fundamentally reprogrammed during type 2 immune responses and suggest macrophage reprogramming as an attractive target in type 2 inflammation.

Highlights

- House dust mite triggers the production of cyclooxygenase metabolites (prostaglandin D_2, prostaglandin E_2, and thromboxane) as well as of proinflammatory cytokines in human macrophages.
- House dust mite-exposed macrophages produce low levels of 5-lipoxygenase metabolites (eg, leukotriene B_4) and suppress neutrophil recruitment.
- High levels of cysteinyl leukotrienes and 12-/15-lipoxygenase metabolites are produced during the type 2 immune response to house dust mite or nematode parasites in the airways.

2 | MATERIALS AND METHODS

Animal experiments were performed according to institutional guidelines and to Swiss federal and cantonal laws on animal protection.

2.1 | Material

Eicosanoids, PUFAs, and deuterated internal standards (ISs) were purchased from Cayman Chemical (Ann Arbour, MI, USA). An analyte and IS working solution were prepared as shown in Tables S1 and S2. LC-grade solvents [2-propanol, Carl Roth (Karlsruhe, Germany), acetonitrile, Thermo Fisher Scientific (Waltham, MA, USA), methanol, Applichem (Darmstadt, Germany)) and ultrapure H_2O (supplied through a MilliQ system (Merck Millipore, Darmstadt, Germany)] were used for mobile phase preparation.

2.2 | Isolation and culture of polymorphonuclear leukocytes (PMN) and peripheral blood mononuclear cells (PBMC)

Written informed consent in accordance with the Declaration of Helsinki was obtained from healthy volunteers before blood collection, which had been approved by the local ethics committee at the Technical University of Munich. PMN and PBMC were isolated and cultured in medium containing 10% heat-inactivated FBS and monocytes were differentiated to aMDM as described previously.[17,18] Supernatants were stored at −80°C in 50% MeOH for LC-MS/MS or undiluted for cytokine analysis.

2.3 | Chemotaxis assay

PMN were incubated for 30 min at 37°C with pooled conditioned medium of aMDM ± HDM ± indomethacin (100 μM, Cayman Chemical) ± DBM-1285. 2×10^5 PMN were transferred to transwells (3 μm pore size, Corning, NY, USA) and allowed to migrate for 3 h at 37°C toward conditioned medium containing chemoattractants: 2 ng/mL LTB_4, Cayman Chemical; 20 ng/mL IL-8; 2 ng/mL CCL5, both Miltenyi Biotec. Migrated PMN were counted microscopically.

2.4 | In vivo model of *Nippostrongylus brasiliensis* infection

Mice were infected subcutaneously with 200 larvae of *N. brasiliensis* (*Nb*), and BALF was collected on day 5 postinfection as previously described.[19,20]

2.5 | In vivo model of HDM-induced allergic airway inflammation

C57BL/6J mice were sensitized by bilateral intranasal (i.n.) instillations of extract from *Dermatophagoides farinae* ("HDM") (1 μg in 20 μL PBS; Stallergenes). Challenges were performed on days 8-11 with 10 μg HDM extract. Three days after the final challenge, BALF (600 μL) was collected, equal volumes of methanol were added, and samples were frozen immediately at −80°C until further processing.

2.6 | Real-time PCR

aMDM were lysed in RLT Buffer (Qiagen, Hilden, Germany) with 1% β-mercaptoethanol (Merck Millipore), followed by RNA extraction (Zymo Research, Irvine, CA, USA) and reverse transcription according to the manufacturer's instructions (Thermo Fisher Scientific). qPCR analysis was performed as described previously (primers shown in Table S3).[18]

2.7 | Western blotting

Western blotting was performed similarly to previously published procedures.[18] A detailed procedure can be found in the Appendix S1.

2.8 | Multiplex cytokine assay and ELISA

Multiplex cytokine assays were performed as detailed in the Appendix S1.

2.9 | Sample preparation for LC-MS/MS

Samples for method validation were prepared as triplicates in medium/MeOH (1:1) or PBS/MeOH (1:1) with an analyte concentration of 0.1, 1, or 10 ng/mL [10× higher concentrations for PUFAs (Tables S1 and S2)]. Automated solid phase extractions were performed with a Microlab STAR robot (Hamilton, Bonaduz, Switzerland). Prior to extraction, all samples were diluted with H_2O to a MeOH content of 15% and 10 μL of IS stock solution was added. Samples were extracted using Strata-X 96-well plates (30 mg, Phenomenex, Aschaffenburg, Germany) and eluted with MeOH. Samples were evaporated to dryness under N_2 stream and redissolved in 100 μL MeOH/H_2O (1:1).

2.10 | LC-MS/MS lipid mediator analysis

Chromatographic separation of eicosanoids was achieved with a 1260 Series HPLC (Agilent, Waldbronn, Germany) using a Kinetex C18 reversed phase column (2.6 μm, 100 × 2.1 mm, Phenomenex) with a SecurityGuard Ultra Cartridge C18 (Phenomenex) precolumn. The Sciex QTRAP 5500 mass spectrometer (Sciex, Darmstadt, Germany), equipped with a Turbo-V™ ion source, was operated in negative ionization mode. Identification of metabolites was achieved via retention time and scheduled multiple reaction monitoring (sMRM). Unique Q1/Q3 transitions were selected for each analyte by using single analyte injections and comparison with the literature.[14] Analytes with identical MRM transitions were differentiated by retention time (Figure S1). A more detailed method description can be found in the Appendix S1.

2.11 | Data analysis

All data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) or R 3.4.3.[21] For LC-MS/MS analysis, all samples were normalized to their RNA content. Data were analyzed using Wilcoxon, Friedman, or Kruskal-Wallis test with respective post hoc test as specified in the Figure legends and considered statistically significant if $P < 0.05$.

3 | RESULTS

3.1 | Lipid mediators involved in type 2 immune responses can be detected with high accuracy, precision, and recovery by LC-MS/MS

Quantification of lipid mediators in type 2 immune settings has resulted in discrepancies, depending on the analytical method (EIA or LC-MS/MS).[5,22] Thus, we compared these methods for leukotrienes, PGE_2, and TXB_2 in supernatants of human PMN and aMDM. Quantification by EIA showed higher variability, particularly for LTs: SD = 8.48 (EIA) vs SD = 0.72 (LC-MS/MS) for LTB_4; SD = 6.44 (EIA) vs SD = 0.23 (LC-MS/MS) for cysLTs. Levels obtained by EIA were also significantly higher as compared to LC-MS/MS and did not correspond well to AA-metabolizing enzymes (Figure 1A).[17]

Thus, we established an LC-MS/MS workflow for the comprehensive and simultaneous quantification of PUFA metabolites involved in type 2 inflammation (Figure 1B, Figure S1, Tables S4-S9). At 1 ng/mL, we could detect 36 metabolites according to FDA guidelines (accuracy: ±15%, RSD <20%) (Table S4). This included eicosanoids (LTs, TXB_2, and PGD_2) as well as specialized pro-resolving mediators (SPMs) [resolvin E1/D1 (RvE1/RvD1) and protectin D1 (PDX)] (Figure 1C,D, Table S4). The recovery ranged from 69% to 127% for key lipid mediators of type 2 inflammation with a matrix effect in a similar range (Figure 1E, Table S5). Thus, at concentrations ≥ 1 ng/mL eicosanoid mediators of type 2 immunity (LTs, TXB_2, and PGD_2) and several SPMs could be quantified with good accuracy, precision, and recovery.

3.2 | Zymosan exposure reprograms the eicosanoid metabolism of myeloid cells

In order to validate our LC-MS/MS workflow in a well-characterized cellular model, we processed and analyzed culture supernatants from human PMN that were either left untreated or exposed for 24 h to zymosan prepared from fungal cell walls. First, a pool of PMN supernatants was measured in three technical replicates (Figure 2A-E), and second, levels of eicosanoids produced by PMN from different individuals (n = 5) were analyzed separately (Figure 2F). Untreated PMN produced mainly 5-LOX metabolites (5-HETE and LTB_4) at a concentration of around 1.4 ng/mL and low levels of cysLTs (Figure 2A). PMN preparations contained neutrophils and eosinophils and thus had the capacity to generate LTs and 15-LOX metabolites (Figure 2A,B). Treatment with zymosan resulted in reprogramming of the eicosanoid metabolism, characterized by reduced production of LTB_4, cysLTs, and 5-HETE ($P = 0.06$) (Figure 2A,D-F). In contrast, zymosan exposure triggered the formation of COX metabolites with a fivefold increase in TXB_2 levels. Additionally, zymosan-exposed PMN released PGE_2 and $PGF_2\alpha$ that were undetectable in unstimulated PMN (Figure 2C). Taken together, lipid mediator class switching could be tracked by the developed LC-MS/MS workflow, allowing us to reveal previously reported as well as unprecedented zymosan-induced changes in the eicosanoid profile.[23,24]

3.3 | TGFβ1 induces a macrophage phenotype that resembles alveolar macrophages and resists IL-4-mediated regulation of eicosanoid pathways

Based on recent studies showing key roles for GM-CSF and TGFβ1 in alveolar macrophage (AM) differentiation,[25,26] we differentiated human monocytes into alveolar-like macrophages (aMDM) and characterized their eicosanoid profile. At baseline, aMDM expressed high levels of 5-LOX and its respective oxylipin products (Figure 3B,E). In addition, aMDM expressed higher levels of 5-LOX and IL-1β as compared to MDM, suggesting that they adapted features of AM (Figure S2A,B).[27,28] IL-4 is known to reprogram the AA metabolism of macrophages by inducing 15-LOX, but suppressing 5-LOX and COX. We confirmed the IL-4-triggered induction of *ALOX15* in MDM from most donors during differentiation in the absence of TGFβ1 (Figure S2C). However, IL-4 had no significant impact on the eicosanoid profile of aMDM (Figure 3A-C), suggesting that aMDM

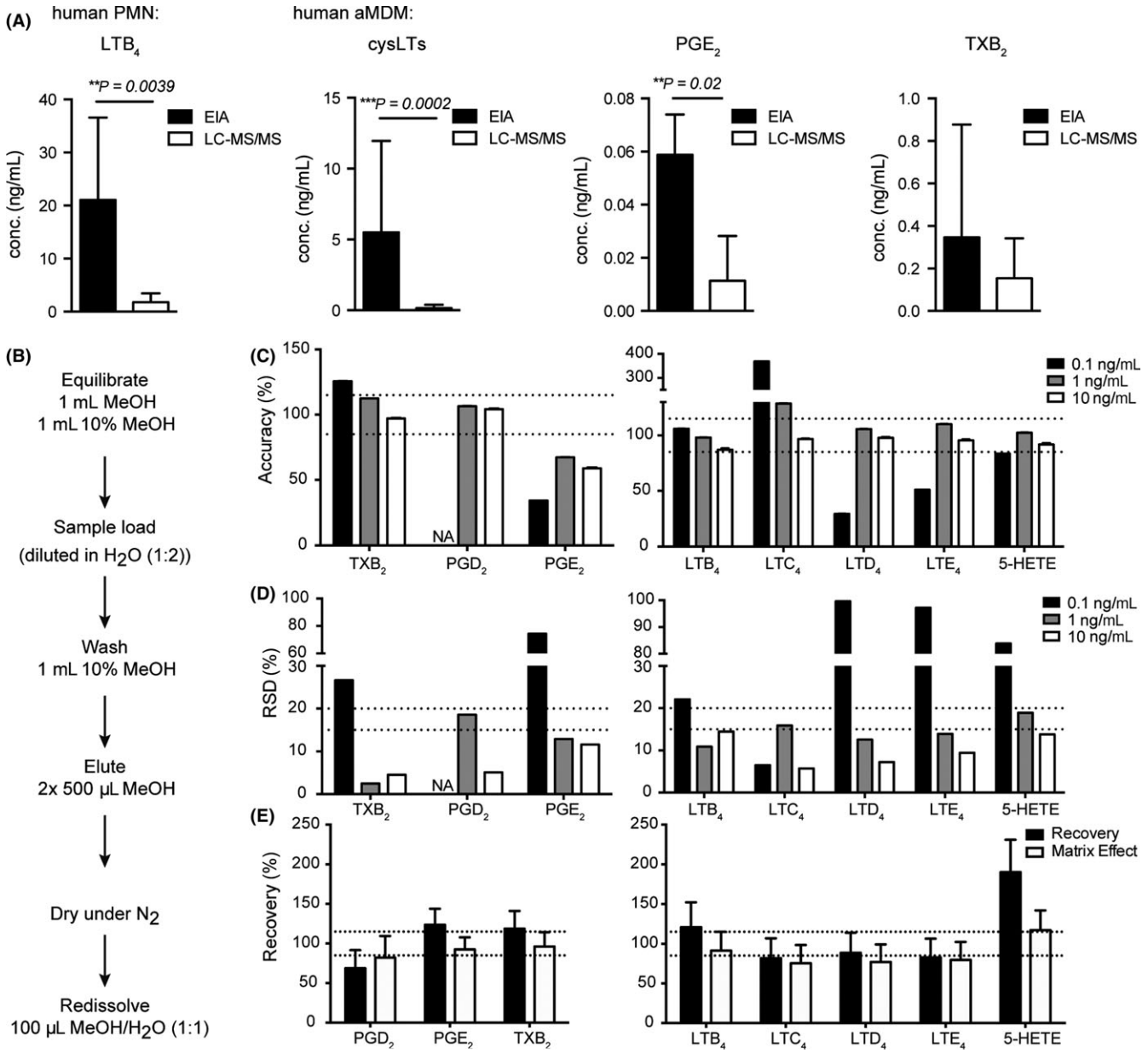


FIGURE 1 Lipid mediators involved in type 2 immune responses can be detected with high accuracy, precision, and recovery by LC-MS/MS. (A) Levels of major bioactive eicosanoids (mean + SD) in supernatants from PMN (n = 5) or MDM (n = 11-30) quantified by EIA or LC-MS/MS; (B) Sample preparation workflow; (C) Accuracy (%) at three different concentrations for key eicosanoids, shown as mean + SD. Dotted lines: ± 15% range; (D) Precision calculated as relative standard deviation (RSD) (%), shown as mean. Dotted lines: 15% and 20% RSD; (E) Recovery at 1 ng/mL. Dotted lines: ± 15% range. Samples in (C-E) were extracted and measured in triplicates on the same day. Statistical significance was determined using Wilcoxon test

resist IL-4-driven induction of 15-LOX as well as suppression of COX and 5-LOX. Indeed, *PTGS2*, *PTGES*, *ALOX5*, and *ALOX15* mRNA levels remained unaffected by IL-4 (Figure 4A).

3.4 | HDM exposure decreases 5-LOX metabolism but increases COX metabolism in human alveolar-like macrophages via p38 MAPK

Next, we assessed the eicosanoid profile of aMDM during 24-96 h exposure to IL-4 and HDM. After 24 h of HDM+IL-4 exposure, formation of 5-LOX products (LTB_4 and 5-HETE) was reduced (Figure 3B). Contrary to the effect on 5-LOX, HDM+IL-4 stimulation resulted in an increase of prostanoids (Figure 3A, Figure S3A). In line with the LC-MS/MS data, 5-LOX mRNA levels were reduced by IL-4 + HDM, while COX-2 was induced (Figure 4A). At later time points, we observed only minor changes in eicosanoid concentrations with the exception of PGD_2, which decreased after prolonged HDM exposure (Figure S3A), and 5-HETE and LTB_4, which initially decreased but increased back to control levels at 96 h (Figure S3B,C).

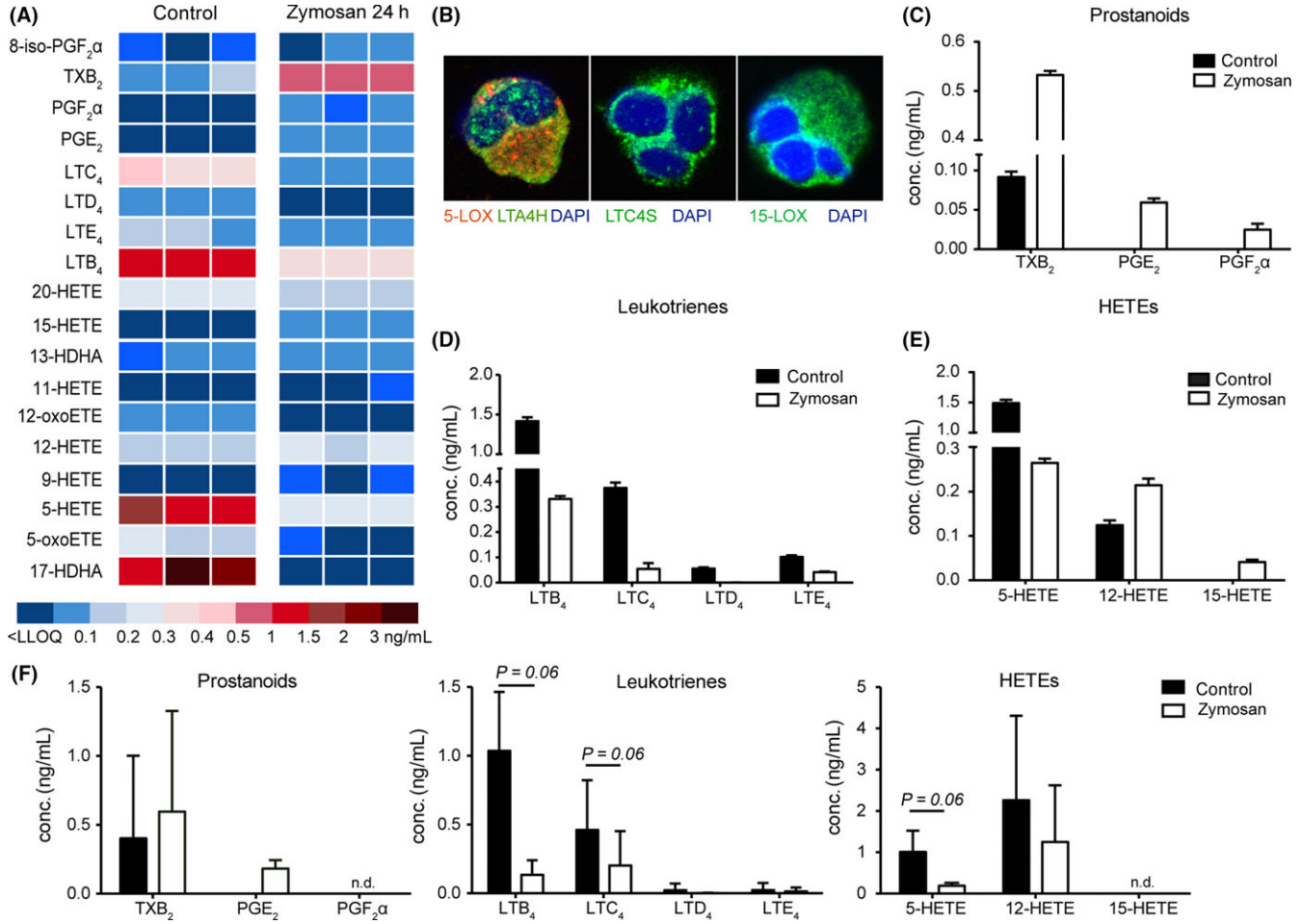


FIGURE 2 Zymosan triggers eicosanoid reprogramming in human granulocytes. (A) Heatmap of LC-MS/MS data for human PMN (pool of n = 6 donors) ± zymosan, analyzed as three technical replicates. (B) Neutrophil (left) or eosinophils (right) stained for 5-LOX and LTA4H or LTC4S and 15-LOX, respectively. Blue: DAPI (nuclei). (C-E) Levels of COX metabolites (C), leukotrienes (D), and HETEs (E) produced by PMN, presented as mean + SD (pool of n = 6, measured in triplicates). (F) Levels of prostaglandins, leukotrienes, and HETEs produced after 24 h ± zymosan (n = 5). Statistical significance was determined using Wilcoxon test

In the absence of IL-4, HDM also triggered prostanoid production, while 5-LOX products were reduced in aMDM from five out of seven donors (Figure 3D,E).

To identify the mechanisms underlying this eicosanoid reprogramming, we first neutralized dectin-2, which has been described as the major C-type lectin receptor recognizing HDM.[7] However, blocking dectin-2 did not interfere with HDM-triggered changes in either COX or LOX metabolites (Figure 3F). Similarly, blockade of TLR-2 or TLR-4 or addition of polymyxin B to inactivate LPS did not affect mediator reprogramming by HDM (Figure S4). As the MAP kinase p38 has been implicated in the regulation of eicosanoid pathways,[29] we assessed p38 phosphorylation in response to HDM. Levels of phosphorylated p38 were increased in HDM-exposed as compared to unstimulated aMDM and prostanoid formation was significantly reduced when macrophages were co-incubated with HDM and a p38 inhibitor (Figure 3F,G). In addition, p38 inhibition during HDM exposure restored 5-LOX product formation (Figure 3F, Figure S5A).

We further examined the effect of HDM on mRNA and protein levels of COX and LOX pathway enzymes. *PTGES* (mPGES1) and *PTGS2* (COX-2) were increased, while *ALOX5* (5-LOX) expression was down-regulated by HDM on both transcript and protein level (Figure 4B,C). M2 polarization markers were either significantly reduced (*ALOX15*) or unaffected by HDM exposure (*TGM2*) (Figure 4A-C). Altogether, HDM-induced eicosanoid reprogramming likely occurred as a result of profound changes in the expression of eicosanoid pathway genes.

3.5 | HDM exposure triggers the production of proinflammatory cytokines and chemokines

To study whether allergen-driven eicosanoid reprogramming was associated with an altered cytokine profile, we performed multiplex bead assays of supernatants from aMDM. We observed a significant increase in proinflammatory cytokines (IL-6, IL-1β, TNF-α, and IL-12 p70) after 24 h HDM exposure (Figure 5A,B). HDM also triggered the release of chemokines (CXCL9/10, IL-8) involved in granulocyte recruitment (Figure 5A). Similar to the effects on eicosanoid reprogramming, p38 inhibition reduced the HDM-induced production of

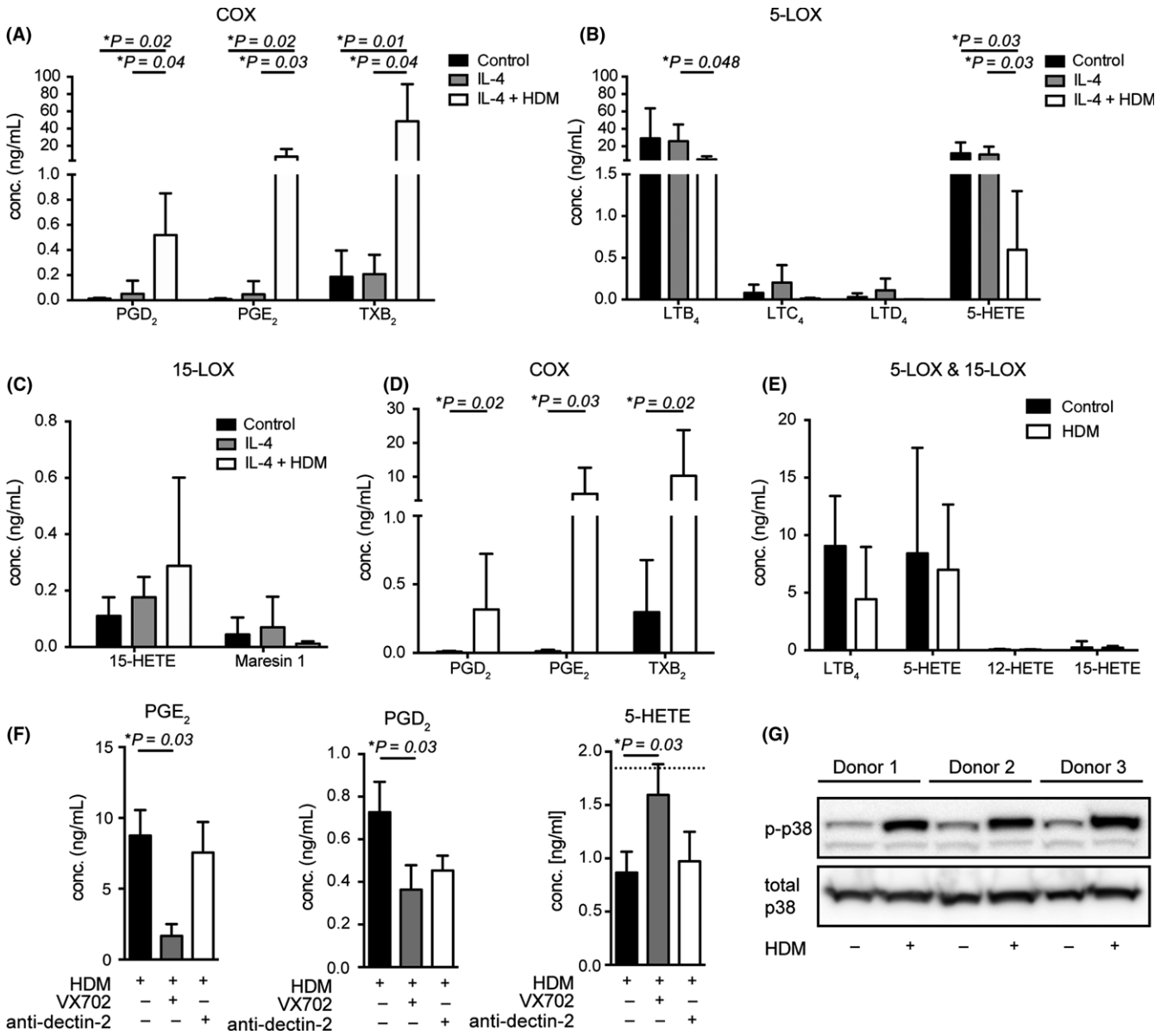


FIGURE 3 House dust mite extract triggers COX metabolism but suppresses 5-LOX metabolism in human alveolar-like macrophages via p38 MAPK. (A-C) LC-MS/MS data for 5-LOX (A), COX (B), or 15-LOX (C) metabolites of aMDM, stimulated or not with 10 ng/mL IL-4 ± 10 μg/mL HDM (n = 4); (D-F) LC-MS/MS data for COX (D) or 5/15-LOX metabolites (E), (n = 7) and of aMDM pre-incubated with p38 inhibitor or dectin-2 neutralizing antibody (F) before HDM exposure (n = 5). (G) Representative WB for total and phosphorylated p38 in aMDM (n = 3). Data are shown as mean + SD; statistical significance was determined using Kruskal-Wallis test with Dunn's correction (A-C, F) or Wilcoxon test (D)

IL-6 and TNF-α, while dectin-2 neutralization did not affect the expression of these cytokines (Figure 5C,D, Figure S5B).

3.6 | HDM-exposed macrophages have a reduced capacity to recruit granulocytes

The recruitment of inflammatory neutrophils and eosinophils is a hallmark response of asthma. Thus, we addressed the functional consequence of HDM-driven mediator reprogramming by performing chemotaxis assays with human PMN. Migration of PMN toward a chemoattractant mixture was diminished if PMN were exposed to supernatant from aMDM stimulated with HDM as compared to supernatant from unstimulated aMDM (Figure 5E). Addition of the COX inhibitor indomethacin during HDM stimulation did not affect the HDM-triggered reduction in chemotaxis, suggesting that prostanoids were not responsible for this effect. In contrast, p38 inhibition could restore PMN chemotaxis, correlating with increased LTB_4 levels (Figure S5A,C) and reduced IL-6 and TNF-α concentrations (Figure S5B).

Taken together, HDM exposure induced a proinflammatory macrophage phenotype characterized by abundant production of bronchoconstrictive thromboxane and TNF-α, but low production of LTB_4 and impaired chemotactic potential.

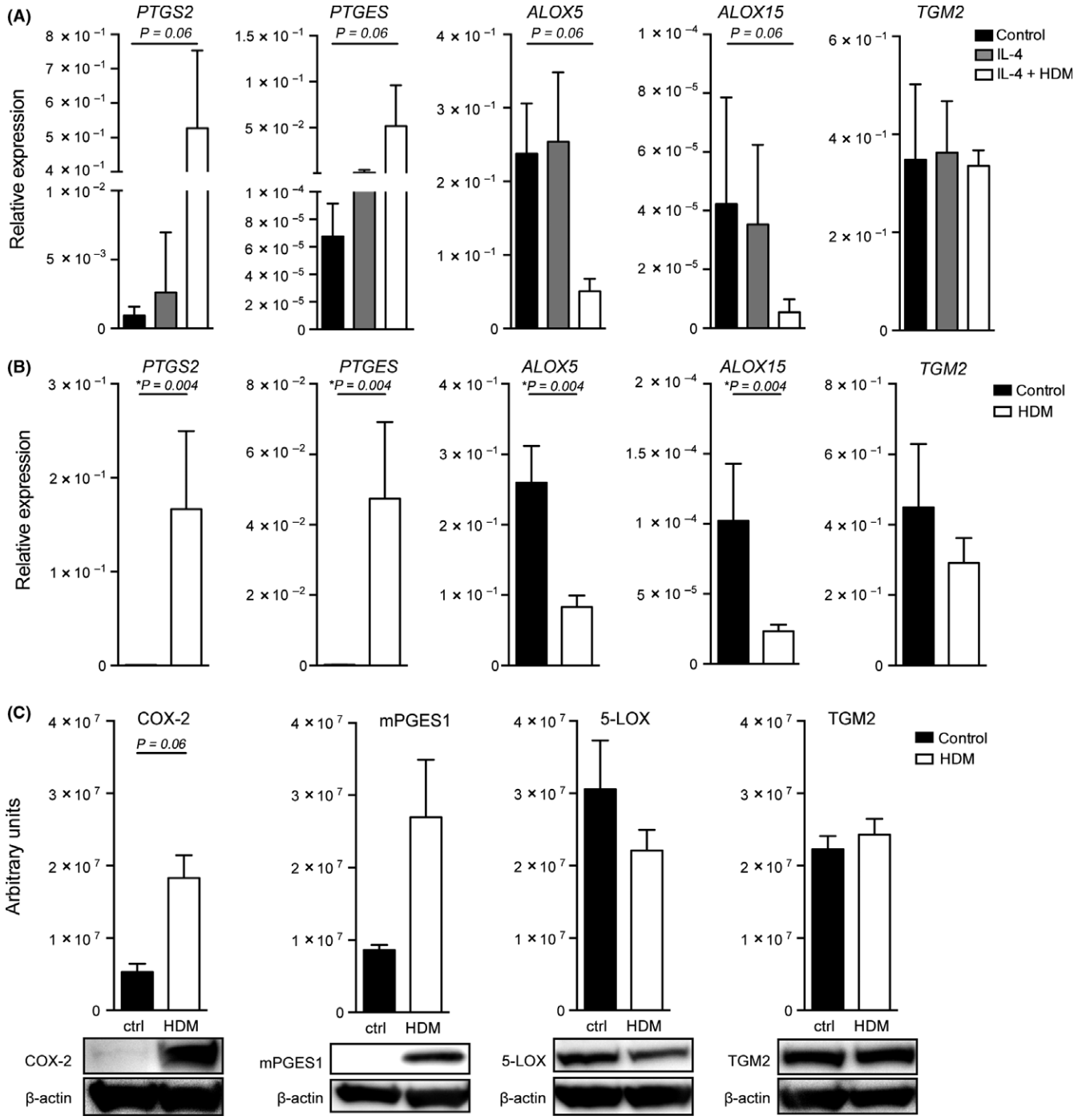


FIGURE 4 HDM-driven eicosanoid reprogramming occurs on the mRNA and protein level. (A and B) relative gene expression of aMDM stimulated or not with 10 ng/mL IL-4 ± 10 μg/mL HDM, (n = 7) (A) or with 10 μg/mL HDM (n = 9) (B) for 24 h; (C) protein levels normalized to β-actin (upper panels) and representative WB images (lower panels) of aMDM ± 10 μg/mL HDM for 24 h (n = 5-7). Data are shown as mean + SD; statistical significance was determined using Wilcoxon test

3.7 | Distinct eicosanoid profiles are induced during the type 2 immune response to HDM or nematode infection in the airways

To assess whether eicosanoid reprogramming is a general feature of type 2 immune responses, we characterized lipid mediator profiles in the airways of HDM-sensitized or nematode-infected mice. When comparing eicosanoid profiles after sensitization and challenge with HDM or infection with the lung-migrating nematode *Nippostrongylus brasiliensis* (*Nb*), we observed an abundant formation of prostanoids in BALF from *Nb*-infected, but not from HDM-sensitized mice ($P < 0.05$ for all COX metabolites) (Figure 6A). In addition, no prostanoids could be detected in the BALF of naïve mice (Figure 6A). In contrast, cysLTs were detectable in the airways of *Nb*-infected as well as of HDM-sensitized mice (Figure 6B). Moreover, high levels of 12-/15-LOX metabolites (particularly 12-HETE and 13-HODE) were

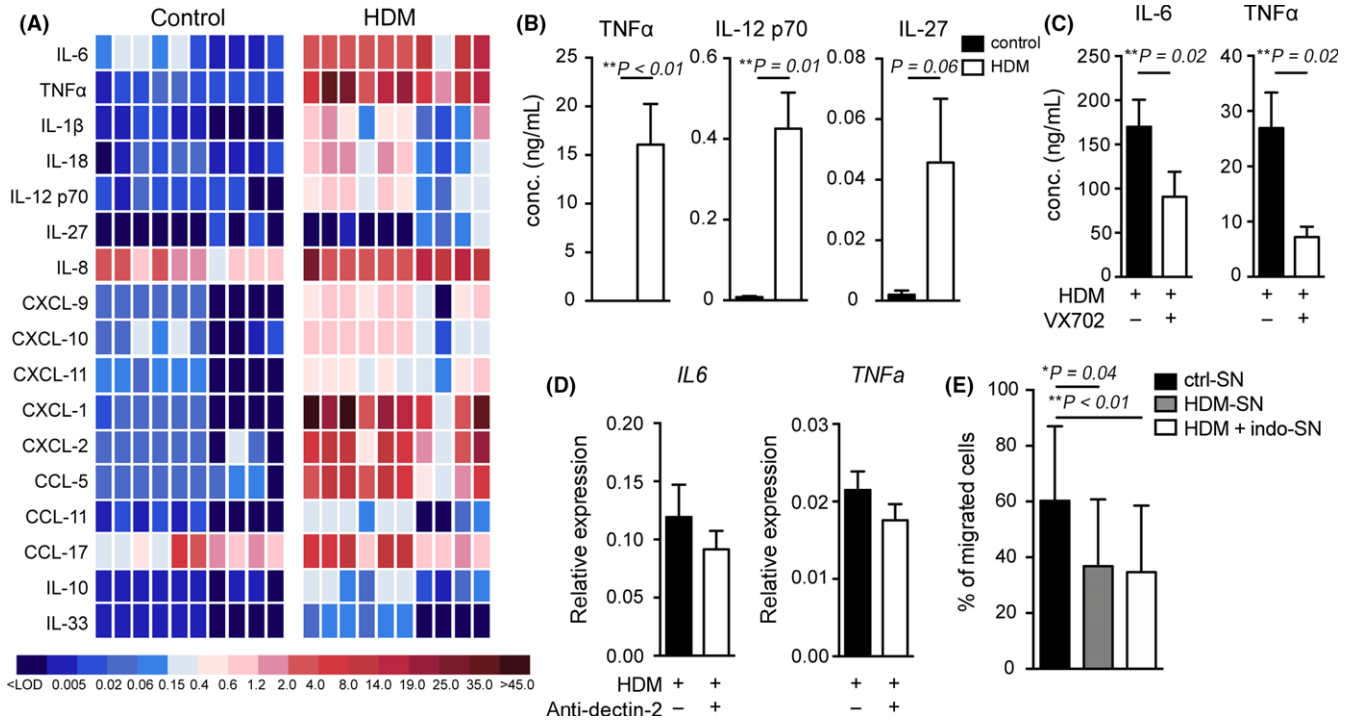


FIGURE 5 HDM exposure triggers the production of proinflammatory cytokines and chemokines, but reduces the granulocyte-chemotactic potential of human macrophages. (A) Overview of cytokine levels [ng/mL], (B) TNF-α, IL-12 p70, and IL-27 (mean + SD) for aMDM from 10 different blood donors ± 10 μg/mL HDM for 24 h; (C) concentration (n = 10) and (D) gene expression (n = 6) of IL-6 and TNF-α in aMDM pre-incubated with p38 inhibitor VX702 or dectin-2 neutralizing antibody before HDM exposure; (E) Percentage of granulocytes migrating toward supernatants (SN) of aMDM ± 10 μg/mL HDM ± 100 μM indomethacin (n = 7). Statistical significance was determined using Wilcoxon test (B-D) or Friedman test with Dunn's correction (E)

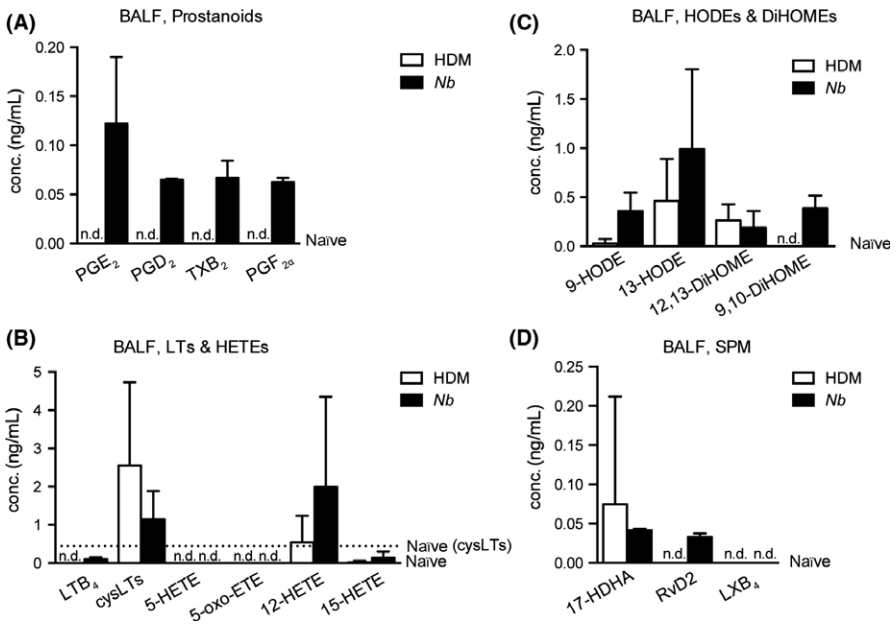


FIGURE 6 Distinct eicosanoid profiles are induced during the type 2 immune response to HDM or nematode infection in the airways. (A-D) LC-MS/MS analysis of prostanoids (A), LOX metabolites of AA (B), LA metabolites (C), and SPMs (D) in BALF from HDM-sensitized or *Nb*-infected mice (n = 3-6), representative data from two independent experiments are presented as mean + SD. Dotted lines represent levels for naïve mice. Statistical significance was determined using Kruskal-Wallis test with Dunn's correction

produced in the airways of *Nb*-infected and HDM-sensitized mice (Figure 6B,C). LA-derived metabolites (9-/13-HODE, 9,10-/11,13-DiHOME) were synthesized in similar quantities as compared to AA metabolites in the airways after challenge with HDM or infection with *Nb* with a tendency for higher levels in *Nb*-infected mice ($P = 0.025$ for 9,10 DiHOME, $P = 0.124$ for 9-HODE). Finally, BALF from *Nb*-infected mice also contained detectable levels of SPMs (17-HDHA and RvD2) (Figure 6D). Thus, lipid mediator reprogramming occurs during the type 2 immune response to HDM or nematode parasites in the airways in vivo with partially distinct profiles.

The induction of the COX pathway and simultaneous suppression of the 5-LOX pathway may represent an early response of macrophages in type 2 immune settings, which then governs the ensuing type 2 immune response to allergens or helminth infection.

4 | DISCUSSION

Eicosanoid lipid mediators play central roles in type 2 immune responses, particularly in allergic inflammation. Thus, the comprehensive assessment of eicosanoid profiles in settings of type 2 inflammation can provide important information about the ensuing immune response and the functional plasticity of the cell types involved. Here, we describe an LC-MS/MS workflow, which allowed us to characterize eicosanoid reprogramming in two distinct settings of type 2 inflammation. First, we show that the lipid mediator metabolism of human alveolar-like macrophages (aMDM) is highly responsive to allergen-driven reprogramming. Second, we describe profound changes in lipid mediator profiles during the type 2 immune response to HDM or nematode infection in vivo.

Using a newly developed LC-MS/MS workflow, up to 52 oxylipins could be quantified in cell culture supernatants and biological samples from the airways. To our knowledge, this represents one of the largest oxylipin panels that has been validated and applied in the context of type 2 immune responses. This workflow allowed for the sensitive and reliable quantification of central eicosanoid mediators of type 2 inflammation (eg, LTs, TXB_2, PGD_2), while the accuracy should be improved for other mediators (eg, PGE_2). To initially validate the LC-MS/MS workflow, we studied zymosan-triggered eicosanoid reprogramming in human PMN. At baseline, stimulation with Ca^{2+} ionophore resulted in the release of 5-HETE and LTs, which is consistent with previous studies.[30,31] In keeping with the literature, zymosan induced a shift in the eicosanoid metabolism, characterized by higher amounts of prostanoids.[32] Previous studies largely focused on the acute effects of zymosan or HDM and showed that both stimuli could trigger LT production by myeloid cells, when applied for short times (2-60 min).[7,33] Here, we focused on the prolonged exposure to TLR2/dectin ligands (zymosan and HDM) as they are involved in the initiation of type 2 inflammation.[2,8,34] Lipid mediator class switching from 5-LOX to COX metabolites occurred for both stimuli, thus suggesting that lipid mediator reprogramming during type 2 inflammation happens analogous to settings of type 1 inflammation.[16]

The induction of prostanoids and suppression of 5-LOX metabolites appears to be a common feature of macrophages in type 2 immune settings in response to allergens, IL-4 or nematode infection. Indeed, the reduced production of 5-LOX metabolites could be a result of high levels of IL-4 produced by T_H2 cells, ILC2s and/ or basophils [35] as IL-4 is known to suppress 5-LOX expression in various cell types, including macrophages.[17,36] In a model of filarial nematode infection, eicosanoid reprogramming in nematode-elicited macrophages was shown to depend on IL-4 receptor signaling.[6] In line with this study, we confirmed the induction of prostanoids for a different nematode parasite, thus suggesting that activation of the COX pathway is a general feature of the immune response to nematodes. Recently, soluble egg antigen of a distinct helminth species (the trematode *Schistosoma mansoni*) was reported to induce PGE_2, which contributed to T_H2 polarization.[37] This suggests an important functional role of prostanoids during the type 2 immune response to helminth infection.

The plasticity of macrophages and their extraordinary capacity to produce lipid mediators suggests that these cells are key drivers of eicosanoid reprogramming in type 2 immunity. During allergen-triggered type 2 immune responses in the airways, the macrophage pool consists of resident alveolar macrophages (AMs) and macrophages derived from recruited monocytes.[38] We used aMDM (differentiated in the presence of GM-CSF and TGFβ1) as a cellular model to mimic this mixed macrophage population. Although aMDM may not fully recapitulate macrophages in the lung, these cells showed several typical features of AMs, including high baseline expression of LT-biosynthetic enzymes and of the proinflammatory cytokine IL-1β.[27,28]

We particularly focused on HDM extract as a trigger of type 2 inflammation with well-established functional roles for lipid mediators.[2,8,39] Exposure of aMDM to HDM for 24-96 h resulted in a dynamic mediator class switching of LOX and COX metabolites. While the production of regulatory mediators (eg, PGE_2) peaked after 48 h of HDM exposure, proinflammatory 5-LOX metabolites were initially suppressed, but increased back to baseline over time. This may explain why cysLTs were formed in the airways of HDM-sensitized mice during a two-week model of allergic airway inflammation. However, in addition to macrophages, other cell types including eosinophils and airway epithelial cells can contribute to the formation of LOX metabolites (including 5-LOX-derived cysLTs and 12/15-LOX-derived HETEs and HODEs) during HDM-triggered airway inflammation in vivo.[18] Nevertheless, macrophages likely represent a major source of lipid mediators during the initial exposure to HDM as they are abundant in the airways and highly express or readily upregulate LOX and COX enzymes.

Given the production of several neutrophil-chemotactic factors by HDM-exposed aMDM, we hypothesized that aMDM would show an increased potential to trigger granulocyte chemotaxis after HDM exposure. However, in line with previous in vivo studies, secretions from HDM-exposed aMDM rather tended to decrease granulocyte chemotaxis.[40] This may result in impaired host defense and thus increased susceptibility to infections, which is a common complication in asthmatic patients.[41] To address the functional contribution of COX metabolites, we studied granulocyte chemotaxis in the presence of secretions from HDM-exposed aMDM, which had been treated with the COX inhibitor indomethacin. However, COX inhibition did not affect the chemotactic potential of HDM-exposed aMDM. Instead, a p38 inhibitor restored LTB_4 production and neutrophil chemotaxis, suggesting that the reduced production of the neutrophil chemoattractant LTB_4 by HDM-exposed aMDM may contribute to the impaired chemotactic potential.

Contrary to previous reports of dectin-2 as an essential HDM receptor,[7,8] we did not observe a reduction of HDM-triggered prostanoid or cytokine production when neutralizing dectin-2. However, this may be due to the timing of dectin-2 ligation as previous studies

were focused on acute responses (20-60 min) after HDM exposure. Indeed, while the initial response might depend on dectin-2, other mechanisms likely drive mediator reprogramming during longer exposure. Our results suggest that p38 activation by a dectin-2 and TLR-2/-4-independent mechanism contributed to eicosanoid and cytokine reprogramming in macrophages.

Taken together, HDM exposure induced a potentially pathogenic macrophage phenotype, characterized by abundant production of prostanoids (particularly TXB_2) and proinflammatory cytokines (particularly TNF-α). Given that several of the HDM-triggered macrophage mediators are implicated in severe, steroid-resistant airway inflammation, mediator reprogramming in macrophages should be explored as a therapeutic target in therapy-resistant allergy and asthma.

ACKNOWLEDGMENTS

The authors wish to thank the animal caretakers of the University of Lausanne for animal husbandry and Manuel Kulagin and Beatrice Volpe for technical assistance, as well as Carlo Angioni and Yannick Schreiber of University of Frankfurt for help with LC-MS/MS analysis. We would also like to thank Werner Römisch-Margl at the Helmholtz Zentrum München for setting up the Hamilton robot extraction method.

CONFLICTS OF INTEREST

CSW received grant support from Allergopharma, PLS Design as well as Zeller AG and received speaker honoraria from Allergopharma. All authors have no conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

FDRH, AF, MH, DT, TB, PH, MRJ, and FA performed experiments. FDRH, AF, MH, DT, and JEvB analyzed data. JEvB, JA, NLH, and CBSW designed the study; JEvB, FDRH, and AF wrote the manuscript.

ORCID

Julia Esser-von Bieren https://orcid.org/0000-0003-1898-9382

REFERENCES

1. Esser-von BJ. Immune-regulation and -functions of eicosanoid lipid mediators. *Biol Chem*. 2017;398:1177-1191.
2. Barrett NA, Rahman OM, Fernandez JM, et al. Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. *J Exp Med*. 2011;208:593-604.
3. Hirai H, Tanaka K, Yoshie O, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med*. 2001;193:255-261.
4. Machado ER, Ueta MT, Lourenço EV, et al. Leukotrienes play a role in the control of parasite burden in murine strongyloidiasis. *J Immunol Baltim Md*. 1950;2005(175):3892-3899.
5. Patnode ML, Bando JK, Krummel MF, Locksley RM, Rosen SD. Leukotriene B4 amplifies eosinophil accumulation in response to nematodes. *J Exp Med*. 2014;211:1281-1288.
6. Thomas GD, Rückerl D, Maskrey BH, Whitfield PD, Blaxter ML, Allen JE. The biology of nematode- and IL4Rα-dependent murine macrophage polarization in vivo as defined by RNA-Seq and targeted lipidomics. *Blood*. 2012;120:93-104.
7. Barrett NA, Maekawa A, Rahman OM, Austen KF, Kanaoka Y. Dectin-2 recognition of house dust mite triggers cysteinyl leukotriene generation by dendritic cells. *J Immunol*. 2009;182:1119-1128.
8. Clarke DL, Davis NHE, Campion CL, et al. Dectin-2 sensing of house dust mite is critical for the initiation of airway inflammation. *Mucosal Immunol*. 2014;7:558-567.
9. Balgoma D, Larsson J, Rokach J, et al. Quantification of lipid mediator metabolites in human urine from asthma patients by electrospray ionization mass spectrometry: controlling matrix effects. *Anal Chem*. 2013;85:7866-7874.
10. Kowal K, Gielicz A, Sanak M. The effect of allergen-induced bronchoconstriction on concentration of 5-oxo-ETE in exhaled breath condensate of house dust mite-allergic patients. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2017;47:1253-1262.
11. Lundström SL, Yang J, Källberg HJ, et al. Allergic asthmatics show divergent lipid mediator profiles from healthy controls both at baseline and following birch pollen provocation. *PLoS One*. 2012;7:e33780.
12. Mastalerz L, Celejewska-Wójcik N, Wójcik K, et al. Induced sputum supernatant bioactive lipid mediators can identify subtypes of asthma. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2015;45:1779-1789.
13. Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA. High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acylethanolamines. *Biochim Biophys Acta*. 2011;1811:724-736.
14. Norris PC, Dennis EA. A lipidomic perspective on inflammatory macrophage eicosanoid signaling. *Adv Biol Regul*. 2014;54:99-110.
15. von Moltke J, Trinidad NJ, Moayeri M, et al. Rapid induction of inflammatory lipid mediators by the inflammasome in vivo. *Nature*. 2012;490:107-111.
16. Norris PC, Gosselin D, Reichart D, Glass CK, Dennis EA. Phospholipase A2 regulates eicosanoid class switching during inflammasome activation. *Proc Natl Acad Sci USA*. 2014;111:12746-12751.
17. Esser J, Gehrmann U, D'Alexandri FL, et al. Exosomes from human macrophages and dendritic cells contain enzymes for leukotriene biosynthesis and promote granulocyte migration. *J Allergy Clin Immunol*. 2010;126:1032-1040.
18. Dietz K, de LRJM, Gollwitzer ES, et al. Age dictates a steroid-resistant cascade of Wnt5a, transglutaminase 2, and leukotrienes in inflamed airways. *J Allergy Clin Immunol*. 2017;139:1343-1354.
19. Bouchery T, Kyle R, Camberis M, et al. ILC2s and T cells cooperate to ensure maintenance of M2 macrophages for lung immunity against hookworms. *Nat Commun*. 2015;6:6970.
20. Camberis M, Le Gros G, Urban J Jr. Animal model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. *Curr Protoc Immunol*. 2003;Unit 19.12.
21. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing 2017 https://www.R-project.org/. Accessed March 3, 2017.
22. Borgeat P, Fruteau de Laclos B, Rabinovitch H, et al. Eosinophil-rich human polymorphonuclear leukocyte preparations characteristically release leukotriene C4 on ionophore A23187 challenge. *J Allergy Clin Immunol*. 1984;74:310-315.
23. Pouliot M, Gilbert C, Borgeat P, et al. Expression and activity of prostaglandin endoperoxide synthase-2 in agonist-activated human neutrophils. *FASEB J*. 1998;12:1109-1123.

24. Esser J, Gehrmann U, Salvado MD, et al. Zymosan suppresses leukotriene C_4 synthase activity in differentiating monocytes: antagonism by aspirin and protein kinase inhibitors. *FASEB J*. 2011;25:1417-1427.
25. Yu X, Buttgereit A, Lelios I, et al. The cytokine TGF-β promotes the development and homeostasis of alveolar macrophages. *Immunity*. 2017;47:903-912.
26. Schneider C, Nobs SP, Kurrer M, Rehrauer H, Thiele C, Kopf M. Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat Immunol*. 2014;15:1026-1037.
27. Balter MS, Toews GB, Peters-Golden M. Different patterns of arachidonate metabolism in autologous human blood monocytes and alveolar macrophages. *J Immunol Baltim Md*. 1950;1989(142):602-608.
28. Tomlinson GS, Booth H, Petit SJ, et al. Adherent human alveolar macrophages exhibit a transient proinflammatory profile that confounds responses to innate immune stimulation. *PLoS ONE*. 2012;7:e40348.
29. Sokolowska M, Chen L-Y, Eberlein M, et al. Low molecular weight hyaluronan activates cytosolic phospholipase A2α and eicosanoid production in monocytes and macrophages. *J Biol Chem*. 2014;289:4470-4488.
30. Arm JP, Horton CE, House F, Clark TJH, Spur BW, Lee TH. Enhanced generation of leukotriene B_4 by neutrophils stimulated by unopsonized zymosan and by calcium ionophore after exercise-induced asthma. *Am Rev Respir Dis*. 1988;138:47-53.
31. Gijón MA, Zarini S, Murphy RC. Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. *J Lipid Res*. 2007;48:716-725.
32. Goldstein IM, Malmsten CL, Samuelsson B, Weissmann G. Prostaglandins, thromboxanes, and polymorphonuclear leukocytes: mediation and modulation of inflammation. *Inflammation*. 1977;2:309-317.
33. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. *Blood*. 2012;120:60-72.
34. Han M, Chung Y, Young Hong J, et al. Toll-like receptor 2-expressing macrophages are required and sufficient for rhinovirus-induced airway inflammation. *J Allergy Clin Immunol*. 2016;138:1619-1630.
35. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity*. 2004;20:267-277.
36. Spanbroek R, Hildner M, Köhler A, et al. IL-4 determines eicosanoid formation in dendritic cells by down-regulation of 5-lipoxygenase and up-regulation of 15-lipoxygenase 1 expression. *Proc Natl Acad Sci USA*. 2001;98:5152-5157.
37. Kaisar MMM, Ritter M, Del Fresno C, et al. Dectin-1/2-induced autocrine PGE2 signaling licenses dendritic cells to prime Th2 responses. *PLoS Biol*. 2018;16:e2005504.
38. Zasłona Z, Przybranowski S, Wilke C, et al. Resident alveolar macrophages suppress, whereas recruited monocytes promote, allergic lung inflammation in murine models of asthma. *J Immunol Baltim Md*. 1950;2014(193):4245-4253.
39. Zasłona Z, Okunishi K, Bourdonnay E, et al. Prostaglandin E_2 suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells. *J Allergy Clin Immunol*. 2014;133:379-387.
40. Habibzay M, Saldana JI, Goulding J, Lloyd CM, Hussell T. Altered regulation of Toll-like receptor responses impairs antibacterial immunity in the allergic lung. *Mucosal Immunol*. 2012;5:524-534.
41. James KM, Peebles RS, Hartert TV. Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol*. 2012;130:343-351.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Henkel FDR, Friedl A, Haid M, et al. House dust mite drives proinflammatory eicosanoid reprogramming and macrophage effector functions. *Allergy*. 2019;00:1–12. https://doi.org/10.1111/all.13700