INFLAMMATION

An anti-inflammatory eicosanoid switch mediates the suppression of type-2 inflammation by helminth larval products

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Eicosanoids are key mediators of type-2 inflammation, e.g., in allergy and asthma. Helminth products have been suggested as remedies against inflammatory diseases, but their effects on eicosanoids are unknown. Here, we show that larval products of the helminth *Heligmosomoides polygyrus bakeri* (*HpbE*), known to modulate type-2 responses, trigger a broad anti-inflammatory eicosanoid shift by suppressing the 5-lipoxygenase pathway, but inducing the cyclooxygenase (COX) pathway. In human macrophages and granulocytes, the *HpbE*-driven induction of the COX pathway resulted in the production of anti-inflammatory mediators [e.g., prostaglandin E_2 (PGE_2) and IL-10] and suppressed chemotaxis. *HpbE* also abrogated the chemotaxis of granulocytes from patients suffering from aspirin-exacerbated respiratory disease (AERD), a severe type-2 inflammatory condition. Intranasal treatment with *HpbE* extract attenuated allergic airway inflammation in mice, and intranasal transfer of *HpbE*-conditioned macrophages led to reduced airway eosinophilia in a COX/PGE_2-dependent fashion. The induction of regulatory mediators in macrophages depended on p38 mitogen-activated protein kinase (MAPK), hypoxia-inducible factor-1α (HIF-1α), and *Hpb* glutamate dehydrogenase (GDH), which we identify as a major immunoregulatory protein in *HpbE*. *Hpb* GDH activity was required for anti-inflammatory effects of *HpbE* in macrophages, and local administration of recombinant *Hpb* GDH to the airways abrogated allergic airway inflammation in mice. Thus, a metabolic enzyme present in helminth larvae can suppress type-2 inflammation by inducing an anti-inflammatory eicosanoid switch, which has important implications for the therapy of allergy and asthma.

INTRODUCTION

Severe type-2 inflammation, such as in asthma or nasal polyposis, represents a major clinical need, which is insufficiently targeted by current treatments (*1*–*3*). Eicosanoids are bioactive metabolites of the polyunsaturated fatty acid (PUFA) arachidonic acid (AA), which play major roles in severe and therapy-resistant forms of type-2 inflammation (*4*, *5*). However, current therapeutics fail to sufficiently modulate eicosanoid pathways (*6*), resulting in poor clinical efficacy against asthma and nasal polyps (*3*, *7*).

Among the eicosanoids, leukotrienes (LTs), formed via the 5-lipoxygenase (5-LOX) pathway, as well as the cyclooxygenase (COX) metabolite prostaglandin D_2 (PGD_2) are the key drivers of type-2 inflammation (*8*–*10*). In contrast, prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) can suppress allergic inflammation and asthma symptoms (*11*–*14*). Recently, eicosanoids have also been suggested to participate in the type-2 immune response to helminth parasites (*15*–*17*).

Infection with helminths, tissue damage, or exposure to allergens can trigger type-2 immune responses, which, if not properly controlled, can result in chronic type-2 inflammation (*18*). However, helminths can also counter-regulate type-2 immune responses, e.g., by inducing regulatory T cells or by targeting innate effector mechanisms, such as interleukin-33 (IL-33), type-2 innate lymphoid cells (ILC2s), or M2 macrophages (*19*–*22*). *Heligmosomoides polygyrus bakeri* (*Hpb*) is a natural parasite of mice with particularly potent regulatory effects on type-2 immune responses. Products of adult *Hpb* worms can suppress allergic airway inflammation in mice (*21*, *22*), and *Hpb* larvae can interfere with the innate type-2 immune response that is initiated early after infection (*23*). On the basis of their potent immunoregulatory potential, helminth products have been suggested as remedies for inflammatory diseases, including allergy and asthma (*20*, *24*). However, whether helminth products can modulate

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eicosanoid pathways and thus interfere with type-2 inflammation had not been studied.

RESULTS

Treatment with *Hpb* larval extract suppresses allergic airway inflammation in mice

Hpb larvae modulate innate type-2 immunity (*23*), and eicosanoids are a critical innate component of the type-2 immune response to house dust mite (HDM) (*8*, *13*, *25*). Thus, we hypothesized that *Hpb* larvae may produce factors that modulate eicosanoid-driven type-2 inflammation. To mimic a desirable therapeutic application, we administered an extract of infective *Hpb* L3 larvae (*HpbE*) topically (intranasally) during HDM-induced allergic airway inflammation in mice (Fig. 1A). Intranasal treatment with *HpbE* together with HDM reduced hallmarks of type-2 inflammation, including airway eosinophilia, goblet cell hyperplasia, and mucin maturation (Fig. 1, B and C). Consistent with increased eosinophil numbers, 15-HETE, a major AA metabolite of eosinophils, was increased in bronchoalveolar lavage fluid (BALF) of HDM-sensitized mice, and treatment with *HpbE* decreased 15-HETE as well as pro-inflammatory cytokines and chemokines (IL-5, IL-6, eotaxin, and RANTES) (Fig. 1D). Hence, topical treatment with *HpbE* could suppress the inflammatory response to HDM in the airways.

HpbE-treated macrophages produce reduced LTs and modulate allergic airway inflammation via PGE_2

Genetic ablation or pharmacological inhibition of eicosanoid pathways affects the development of allergic airway inflammation itself (*8*, *13*, *26*), thus impeding the assessment of *HpbE* effects in such models. However, macrophage transfer experiments can provide valuable insights into the role of eicosanoids in allergic airway inflammation (*12*) because macrophages represent particularly plastic eicosanoid-producing cells that determine the inflammatory response to HDM in the airways (*25*, *27*).

First, we characterized the eicosanoid profile of *HpbE*-treated bone marrow–derived macrophages (BMDMs) by liquid chromatography–tandem mass spectrometry (LC-MS/MS). To elicit AA release and analyze the full capacity of eicosanoid formation, cells were stimulated with ionophore (A23187). Consistent with the anti-inflammatory potential of *HpbE*, we observed a shift from type-2–inducing metabolites (PGD_2 and LTs) to regulatory metabolites (PGE_2) after treatment with *HpbE* (Fig. 2A). This shift was most likely a result of transcriptional changes in AA-metabolizing enzymes as *HpbE* induced

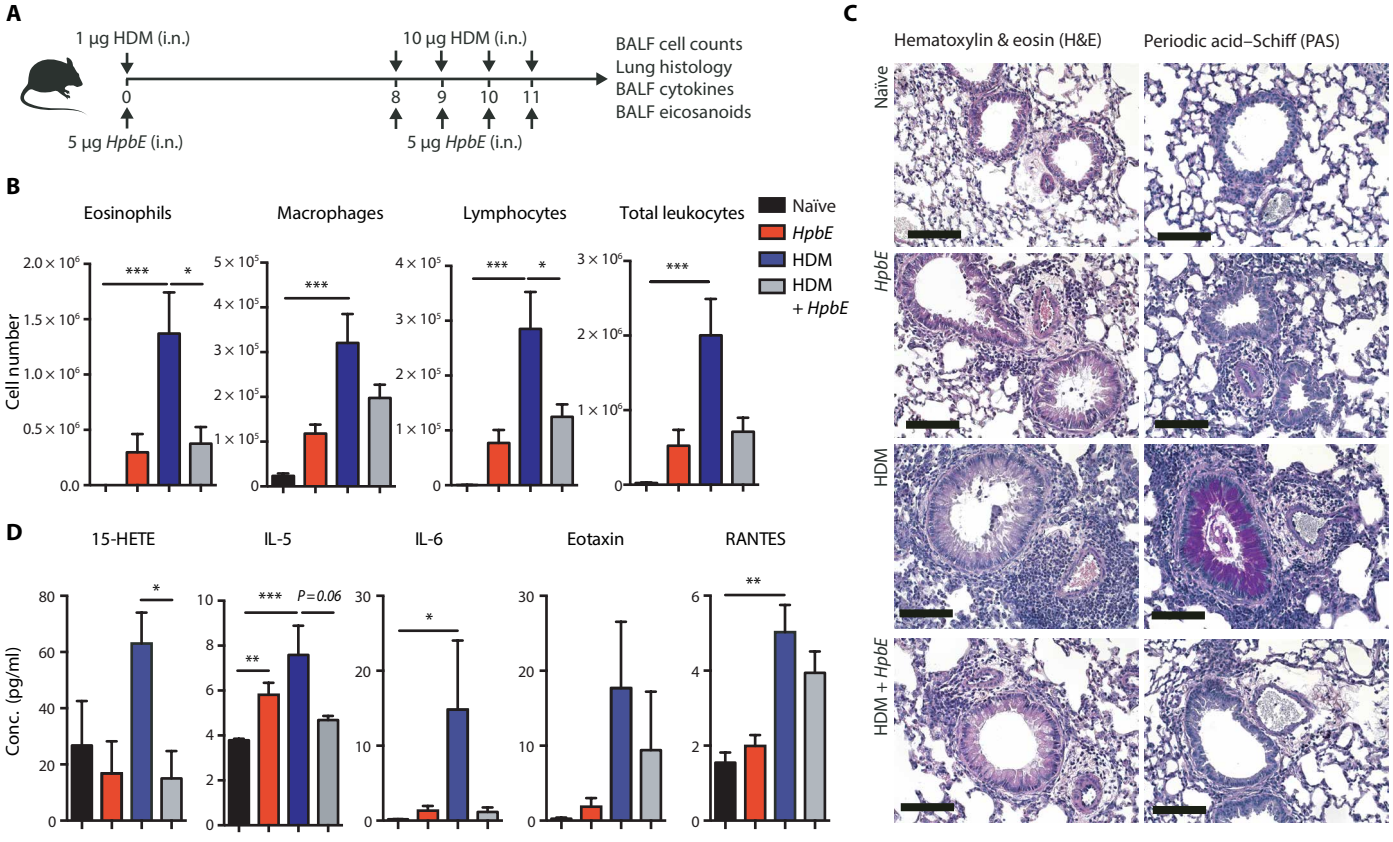

Fig. 1. Topical treatment with *Hpb* larval extract (*HpbE*) modulates type-2 airway inflammation in mice. (**A**) Experimental model of house dust mite (HDM)–induced allergic airway inflammation and intranasal (i.n.) treatment with *HpbE*. (**B**) BALF cell counts in mice sensitized (1 µg) and challenged (10 µg) with HDM ± intranasal treatment with *HpbE* (5 µg), 48 hours after the last challenge and treatment. (**C**) Representative hematoxylin and eosin (H&E)– or periodic acid–Schiff (PAS)–stained lung tissue from mice sensitized to HDM ± treatment with *HpbE*. Scale bars, 100 µm. (**D**) Concentrations of 15-HETE (LC-MS/MS) or IL-5, IL-6, eotaxin, and RANTES (Bioplex) in BALF from mice sensitized to HDM ± treatment with *HpbE*. Results are pooled from two independent experiments (B and D) or representative of stainings performed for two independent experiments (C). Results are presented as means ± SEM; $n = 3$ to 9 (naïve) or $n = 5$ to 17 (treated) per group. Statistical significance was determined by Kruskal-Wallis test followed by Dunn's multiple comparison test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

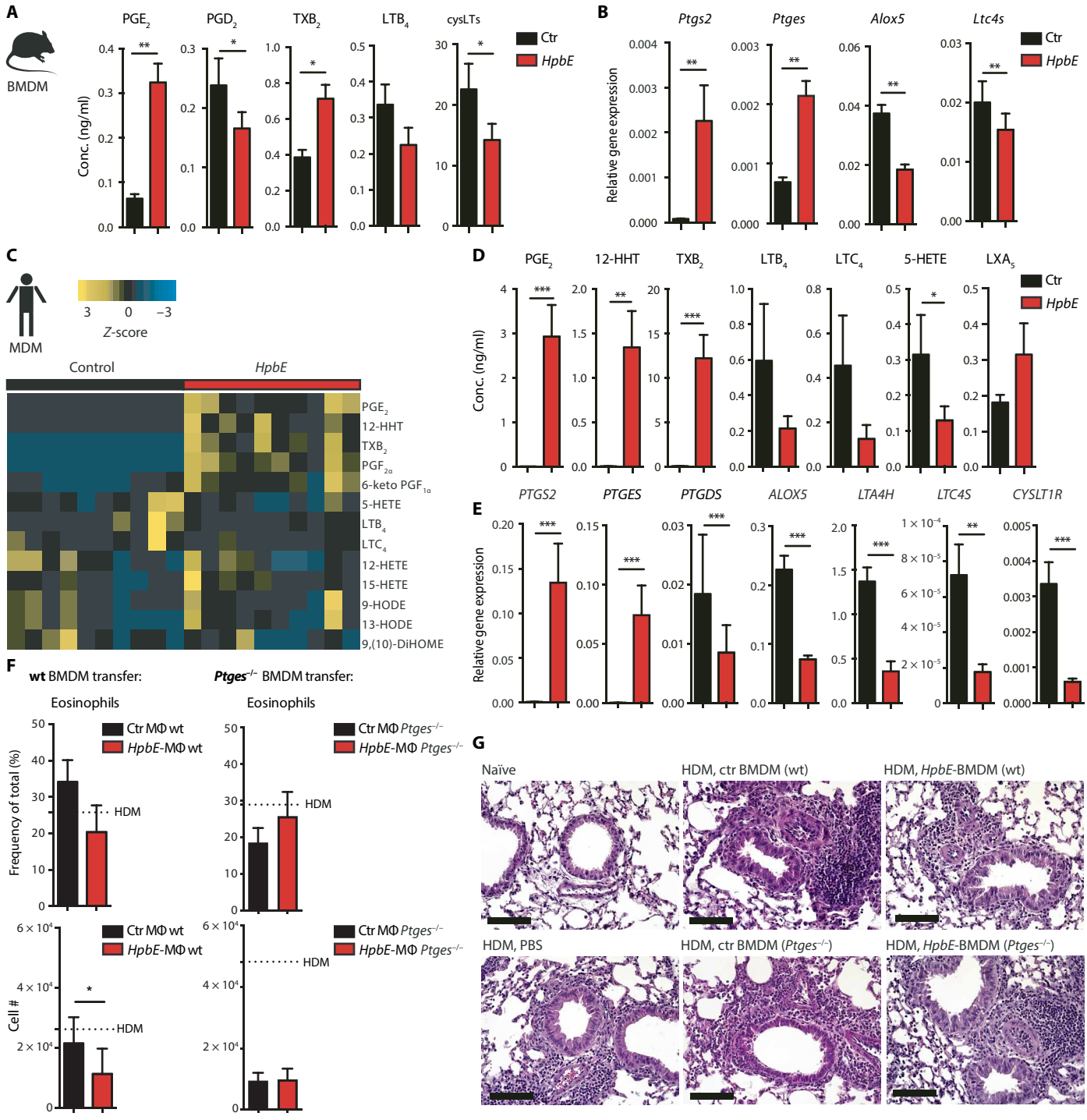

Fig. 2. *HpbE*-treated macrophages produce less LTs and modulate allergic airway inflammation via PGE_2. (**A**) Eicosanoids (LC-MS/MS) produced by mouse bone marrow–derived macrophages (BMDMs) after treatment with *Hpb* larval extract (*HpbE*) (24 hours) and stimulation with A23187 (10 min). (**B**) Relative gene expression of AA-metabolizing enzymes [quantitative polymerase chain reaction (qPCR)] in mouse BMDM treated with *HpbE*. (**C**) Heat map showing major PUFA metabolites (LC-MS/MS) produced by human monocyte-derived macrophages (MDMs) ± treatment with *HpbE* followed by A23187 (10 min). (**D**) Amounts of major bioactive AA metabolites (LC-MS/MS) produced by human MDM ± treatment with *HpbE* +A23187 (10 min). (**E**) Relative gene expression of eicosanoid pathway proteins (qPCR) in human MDM ± treatment with *HpbE*. (**F**) Frequencies of total leukocytes and total numbers of eosinophils in BALF of mice sensitized to HDM + intranasal transfer of *HpbE*-conditioned or untreated BMDM (MΦ) [wild type (wt) or *Ptges*$^{-/-}$], 18 hours after the last challenge and transfer. Dashed lines indicate eosinophil prevalence in HDM-sensitized mice. (**G**) Representative H&E-stained lung tissue from mice sensitized to HDM ± intranasal transfer of untreated or *HpbE*-conditioned BMDM (wt or *Ptges*$^{-/-}$). Scale bars, 100 μm. Data are presented as means ± SEM; $n = 8$ BMDM from C57BL/6 mice (A and B); $n = 10$ to 15 MDM from healthy human blood donors (C to E); $n = 7$ to 9 mice per group (F and G). Statistical significance was determined by Wilcoxon test (A to E) or Mann-Whitney test (F). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

COX-2 (gene: *Ptgs2*) and microsomal PGE synthase (mPGES-1, gene: *Ptges*) while suppressing 5-LOX (*Alox5*) and leukotriene C_4 synthase (*Ltc4s*) gene expression (Fig. 2B).

To investigate whether the eicosanoid-modulatory effects of *HpbE* could be translated to human macrophages, we treated human monocyte-derived macrophages (MDMs) with *HpbE* and assessed their lipid mediator profile after stimulation with A23187. Using an LC-MS/MS eicosanoid screen [including 200 different eicosanoids and PUFAs (table S1)], we confirmed that *HpbE* treatment resulted in fundamental changes in PUFA metabolites, with the largest changes observed for COX metabolites (Fig. 2C and fig. S1, A and B). Consistent with mediator profiles of murine BMDM, *HpbE* triggered a shift from pro-inflammatory 5-LOX metabolites (LTB_4, LTC_4, and 5-HETE) to PGE_2, TXB_2, and 12-hydroxyheptadecatrenoic acid (12-HHT) (Fig. 2D). It also enhanced the synthesis of the pro-resolving mediator lipoxin A_5 (LXA_5) (Fig. 2D). Thus, *HpbE* induced a broad and potentially anti-inflammatory eicosanoid switch.

In line with *HpbE*-induced transcriptional changes in BMDM, human macrophages responded to *HpbE* by inducing the expression of enzymes involved in the biosynthesis of PGE_2: COX-2 (*PTGS2*) and mPGES-1 (*PTGES*) (Fig. 2E). In contrast, *HpbE* reduced the expression of PGD_2 synthase (*PTGDS*) as well as of LT biosynthetic enzymes *ALOX5*, leukotriene A_4 hydrolase (*LTA4H*), and *LTC4S* and the high-affinity receptor for cysLTs [cysteinyl leukotriene receptor-1 (*CYSLTR1*)] (Fig. 2E). Together, *HpbE* triggered a switch from a pro-inflammatory 5-LOX–dominated to a type-2–suppressive COX-dominated eicosanoid profile.

To define the in vivo relevance of the *HpbE*-driven induction of COX metabolites in macrophages, we intranasally transferred BMDM from wild-type (wt), COX-2–deficient ($Ptgs2^{-/-}$), or mPGES-1–deficient ($Ptges^{-/-}$) mice during HDM-induced airway inflammation.

Mice that received *HpbE*-treated wt BMDM during challenges with HDM showed reduced eosinophil numbers and airway inflammation as compared to mice that received untreated wt BMDM (Fig. 2, F and G). In contrast, transfer of *HpbE*-treated $Ptges^{-/-}$ BMDM failed to reduce eosinophil numbers and eosinophilic airway inflammation compared to transfer of untreated $Ptges^{-/-}$ BMDM (Fig. 2, F and G). Last, *HpbE*-treated $Ptgs2^{-/-}$ BMDM transfer led to exaggerated HDM-induced airway inflammation (fig. S1C). This suggested that macrophage-derived COX metabolites, particularly mPGES-1–derived PGE_2, can contribute to the anti-inflammatory effects of *HpbE* in vivo.

HpbE induces type-2–suppressive cytokines and prevents M2 polarization

To investigate whether treatment with *HpbE* also modified cytokine profiles and the polarization of macrophages, we quantified cytokines implicated in macrophage polarization and the regulation of type-2 inflammation. Treatment of human MDM with *HpbE* resulted in the induction of IL-10, IL-1β, IL-12, IL-18, IL-27, and tumor necrosis factor–α (TNF-α) (Fig. 3, A and B), all known to modulate M2 polarization and type-2 immune responses (*23*, *28*–*32*). However, *HpbE* hardly affected the production of mediators of type-2 inflammation (IL-33 or CCL17) by macrophages (Fig. 3B). The *HpbE*-triggered induction of IL-10 and IL-1β also occurred in murine BMDM, albeit at 10- to 100-fold lower amplitude as compared to human MDM (Fig. 3C).

In addition, *HpbE* down-regulated the expression of M2 markers {*ALOX15* [15-lipoxygenase (15-LOX)] and *MRC1* (mannose receptor C-type 1, MR/CD206)}, but not transglutaminase-2 (*TGM2*) in human MDM, suggesting that it could partially counteract M2 polarization (Fig. 3D). Because human and mouse M2 macrophages are defined by distinct sets of markers (*33*), we also investigated the effect of *HpbE* on murine M2 polarization. However, *HpbE* did not significantly affect M2 marker genes (*Tgm2*, *Arg1*, *Mrc1*, *Tmed1*/*St2l*, or *Retnla*/*Fizz1*) in mouse BMDM (Fig. 3E). Together, these data suggest that *HpbE* can broadly modulate the polarization and mediator output of human macrophages to induce a regulatory, type-2–suppressive phenotype.

HpbE has a unique potential to induce type-2–suppressive mediators

For the treatment of complex type-2 inflammatory diseases such as allergy, asthma, and nasal polyps, regulation of multiple pathways is often superior to targeting single mechanisms. Glucocorticoids (GCs), which regulate a broad array of inflammatory pathways, are widely used in the treatment of these diseases and still represent the first-line therapy for most patients. Thus, we compared the immunoregulatory effects of *HpbE* to those of GCs [dexamethasone (Dex) and fluticasone propionate (FP)] with a focus on eicosanoid pathways and the anti-inflammatory cytokine IL-10. While *HpbE* triggered a shift from pro-inflammatory genes (*ALOX5*, *LTC4S*, and *PTGDS*) and 5-LOX metabolites to anti-inflammatory pathways (*PTGS2*, *PTGES*, PGE_2, and IL-10), GCs failed to significantly regulate these pathways (fig. S2, A to C).

In human granulocytes [polymorphonuclear leukocytes (PMNs)], which also represent an important source of eicosanoids, *HpbE* and FP, but not Dex, induced a similar shift from pro- to- anti-inflammatory metabolites of AA or linoleic acid (fig. S2D) (*34*). Together, this suggested that compared to GCs, *HpbE* is more efficient in inducing a regulatory mediator profile that can counteract type-2 inflammation.

During their life cycles, most helminths develop from free-living infective larval stages (L1 to L3) via tissue-migratory stages (L4) to an adult or juvenile stage (L5). Because larval stages of *Schistosoma mansoni* as well as excretory secretory products of adult (L5) *Hpb* (HES) can induce type-2–suppressive mediators (*35*, *36*), we compared *S. mansoni*– or HES-elicited effects on eicosanoids and IL-10 to those of *HpbE*. An extract of *S. mansoni* larvae (*SmE*) failed to induce a shift from 5-LOX to COX metabolism and was less potent in triggering IL-10 production as compared to *HpbE* (fig. S3, A and B). Similarly, adult-stage HES failed to induce the COX pathway or IL-10 (fig. S3, C and D). In addition, extracts of L4 and L5 stages of *Hpb* did not show any induction of PGE_2 and exhibited only minor suppressive effects on cysLTs as compared to L3 stage extract (*HpbE*) (fig. S3E). In contrast to L3 extract, L4 and L5 extracts did not induce type-2–suppressive cytokines (IL-1β and IL-10; fig. S3E).

As changes in the microbiota contribute to the suppression of type-2 inflammation by *Hpb* infection (*37*), we further identified *HpbE*-associated bacteria by aerobic or anaerobic culture of plated *HpbE* followed by MS and assessed whether a mix of *HpbE*-associated bacteria would exert similar effects as *HpbE*. However, COX metabolites, IL-10, and COX pathway genes remained unaffected by treatment with *HpbE*-associated bacteria (fig. S3, F and G). To further exclude that the *HpbE*-triggered induction of regulatory mediators was due to lipopolysaccharide (LPS) contamination, we additionally quantified mediator profiles of macrophages treated with LPS at the concentration present in *HpbE* (60 ng/ml). However, LPS alone failed to significantly induce COX metabolites (fig. S3H). Together, this suggested that *HpbE* has a unique immunoregulatory profile, which is distinct

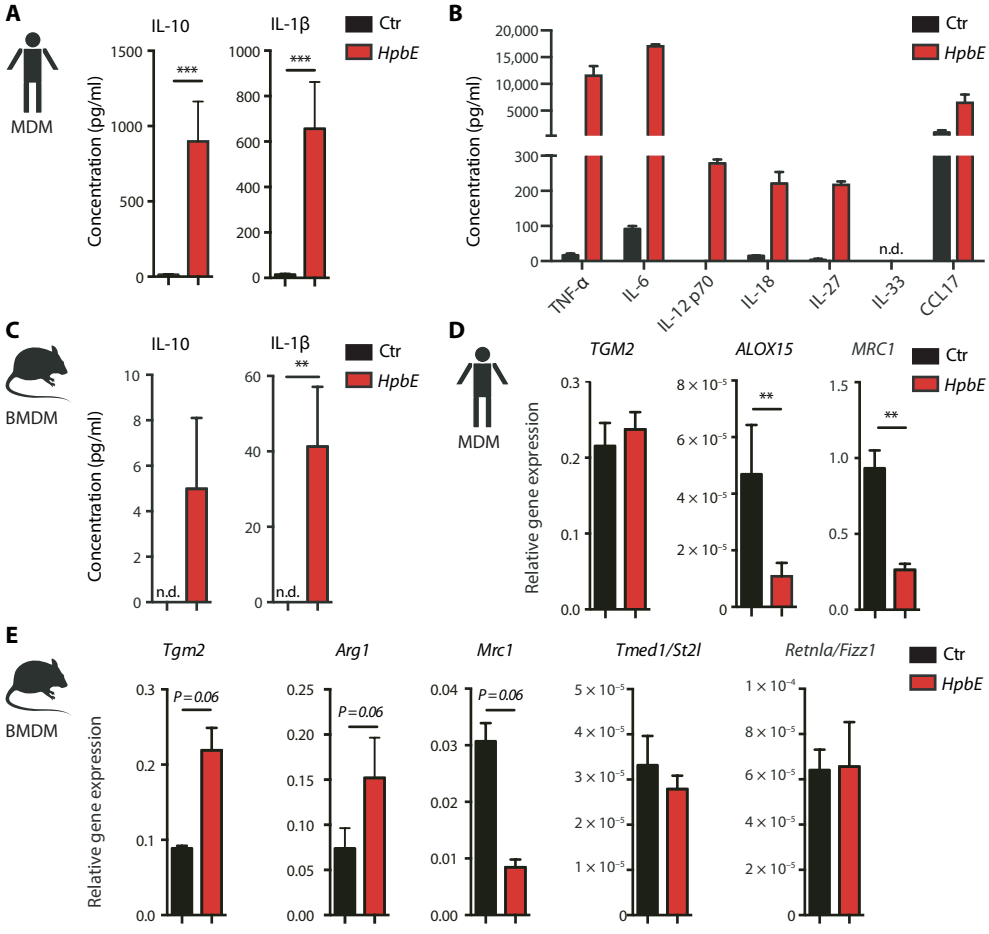

Fig. 3. *HpbE* induces type-2–suppressive cytokines and prevents M2 polarization. (**A**) Amounts of IL-10 and IL-1β (ELISA) produced by human MDM ($n = 14$ to 15) ± treatment with *HpbE*. (**B**) Amounts of TNF-α, IL-6, IL-12p70, IL-18, IL-27, IL-33, and CCL17/TARC (Bioplex) produced by human MDM ($n = 3$) after treatment with *HpbE*. (**C**) Amounts of IL-10 and IL-1β (Bioplex) produced by mouse BMDM ($n = 8$) ± treatment with *HpbE*. (**D**) Gene expression of M2 markers (qPCR) in human MDM ($n = 9$ to 10) ± treatment with *HpbE*. (**E**) Gene expression of M2 markers (qPCR) in mouse BMDM ($n = 5$) ± treatment with *HpbE*. Data are presented as means ± SEM. Statistical significance was determined by Wilcoxon test. $^{**}P < 0.01$; $^{***}P < 0.001$. n.d., not detectable.

from GCs and somatic extracts from *S. mansoni* larvae or more mature stages of *Hpb* as well as from *Hpb*-associated bacteria.

HpbE modulates eicosanoid production and chemotaxis of human granulocytes

Next, we determined whether *HpbE* would also affect the PUFA metabolism of human granulocytes by LC-MS/MS analysis. To limit apoptosis and mimic an inflammatory setting, granulocytes were cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), which resulted in 40 to 50% viability after 24 hours. Treatment with *HpbE* reduced neutrophil viability but had no effect on the viability of eosinophils (fig. S4A). We confirmed that neutrophils and eosinophils show abundant expression of transcripts for LT biosynthetic enzymes (*ALOX5*, *LTA4H*, and *LTC4S*) at baseline (*38*), allowing them to synthesize high amounts of LTs upon ionophore stimulation (Fig. 4, A to E, and fig. S4B). In line with the profiles observed for macrophages, granulocytes showed an induction of COX metabolites (particularly 12-HHT and TXB_2) after treatment with *HpbE* (Fig. 4, A and B, and fig. S4C). Furthermore, the abundance of 5-LOX metabolites was reduced by *HpbE* treatment in ionophore-stimulated mixed human granulocytes as well as in purified eosinophils (Fig. 4, A and C). Similar to *HpbE*-driven changes in AA metabolism genes in macrophages, transcripts for enzymes involved in the synthesis of pro-inflammatory mediators (*ALOX5*, *LTA4H*, and *PTGDS*) were down-regulated, whereas *PTGS2* and *PTGES* were induced by *HpbE* in human granulocytes (Fig. 4, D and E).

Eicosanoid-driven granulocyte recruitment represents a key event in type-2 inflammation (*5*, *8*). Thus, we studied how *HpbE* would affect granulocyte recruitment in a clinically relevant setting of type-2 inflammation, in which AA metabolites play a major role. We collected granulocytes and nasal polyp secretions from patients suffering from aspirin-exacerbated respiratory disease (AERD) and assessed the effects of *HpbE* on the migration of patient granulocytes toward nasal polyp secretions ex vivo. Pretreatment of AERD granulocytes with *HpbE* resulted in a marked reduction in cell recruitment, an effect not achieved by FP or the cysLT1R antagonist montelukast (MK), which is commonly used to treat AERD symptoms (Fig. 4F). In keeping with the suppression of granulocyte chemotaxis, *HpbE* reduced surface expression of chemotactic receptors [C-C chemokine receptor type 3 (CCR3) and PGD_2 receptor 2 ($CRTH_2$)] on human eosinophils (Fig. 4G). To investigate whether COX metabolites released by *HpbE*-treated human macrophages could affect granulocyte

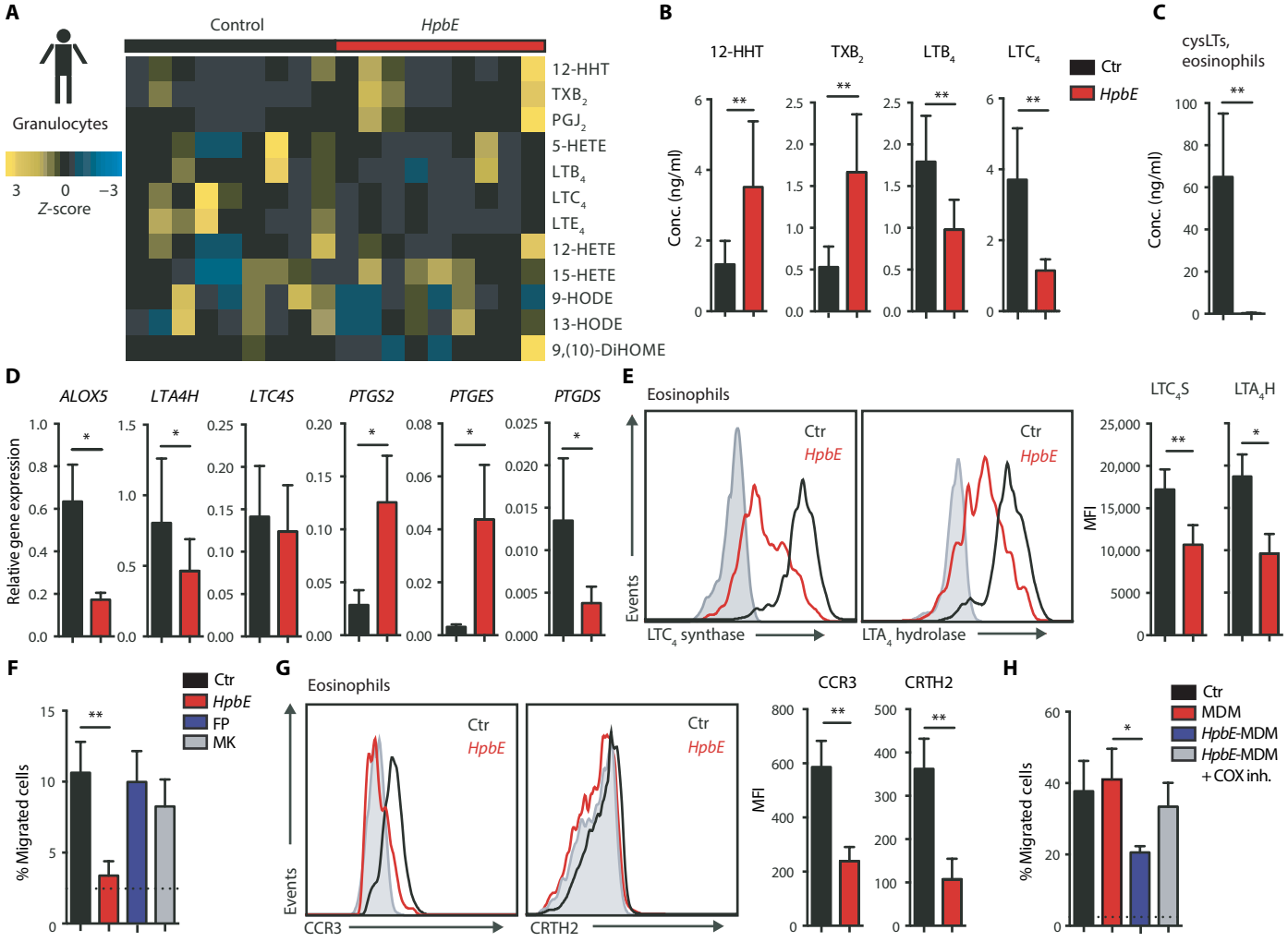

Fig. 4. *HpbE* modulates eicosanoid profiles and chemotaxis of granulocytes in a human setting of type-2 inflammation. (**A**) Heat map showing major PUFA metabolites (LC-MS/MS) produced by mixed human granulocytes ± treatment with *HpbE* (24 hours) followed by A23187 (10 min). (**B**) Amounts of major eicosanoids (LC-MS/MS) produced by mixed human granulocytes ± treatment with *HpbE* (24 hours) + A23187 (10 min). (**C**) Amounts of cysLTs [enzyme immunoassay (EIA), validated by LC-MS/MS] produced by purified human eosinophils ± treatment with *HpbE* (24 hours) + A23187 (10 min). (**D**) Relative gene expression of AA-metabolizing enzymes (qPCR) in mixed human granulocytes ± treatment with *HpbE*. (**E**) Expression of LT synthetic enzymes (LTC4S and LTA4H) (flow cytometry) in human eosinophils ± treatment with *HpbE*. (**F**) Chemotaxis of granulocytes from patients with AERD toward nasal polyp secretions ± treatment with *HpbE*/fluticasone propionate (FP)/montelukast (MK). Dashed line depicts basal migration. (**G**) Surface expression of chemotactic receptors (CCR3 and CRTH2) (flow cytometry) in human eosinophils ± treatment with *HpbE*. (**H**) Chemotaxis of mixed human granulocytes ± pretreatment with conditioned medium from MDM (±*HpbE*, ±COX inhibitor indomethacin). Dashed line depicts basal migration. Data are pooled from at least three independent experiments and presented as means ± SEM; $n = 6$ to 9 mixed granulocytes or purified eosinophils from human blood donors. Statistical significance was determined by Wilcoxon test (two groups) or Friedman test (four groups). $^*P < 0.05$; $^{**}P < 0.01$.

recruitment, we performed chemotaxis assays in the presence of conditioned medium from MDM treated with *HpbE* and the non-selective COX inhibitor indomethacin. Conditioned medium from *HpbE*-treated human macrophages reduced granulocyte chemotaxis, and indomethacin partially restored cell recruitment (Fig. 4H). Thus, either directly or by acting on macrophages, *HpbE* can suppress the chemotaxis of granulocytes, including those from patients experiencing severe type-2 inflammation.

HpbE induces IFN-γ, IL-10, and an anti-inflammatory eicosanoid switch in human PBMCs

To test whether the regulatory potential of *HpbE* extended to type-2 cytokines, we analyzed IL-4, IL-5, and IL-13 expression in human peripheral blood mononuclear cells (PBMCs) after treatment with *HpbE*. Type-2 cytokines were hardly affected by *HpbE*, which instead triggered a marked induction of interferon-γ (IFN-γ) and IL-10 (fig. S5, A and B). In line with eicosanoid modulation in macrophages and granulocytes, *HpbE* treatment of PBMCs also triggered the synthesis of prostanoids (PGE_2 and TXB_2) while decreasing 5-LOX metabolites (5-HETE and LTB_4) (fig. S5C). However, in contrast to macrophages and granulocytes, *HpbE*-treated PBMCs produced high amounts of 12-/15-LOX metabolites (fig. S5C), which can be metabolized into pro-resolving mediators (*39*). The modulation of IL-10 and eicosanoids in PBMCs was entirely dependent on $CD14^+$ monocytes, as $CD14^-$ PBMCs produced low amounts of these mediators with no apparent regulation by *HpbE* (fig. S5D). Thus, *HpbE* acted predominantly on monocytes/macrophages to induce a regulatory and potentially pro-resolving eicosanoid profile.

The *HpbE*-induced regulatory eicosanoid switch depends on HIF-1α and the COX pathway

To identify mechanisms by which *HpbE* could trigger the production of type-2–suppressive mediators, we genetically or pharmacologically targeted regulatory pathways and studied eicosanoid profiles and macrophage polarization. Because hypoxia-inducible factor-1α (HIF-1α) is a positive regulator of the COX pathway (*40*), we first assessed the effect of *HpbE* on HIF-1α activation and COX-2 expression in BMDM. After treatment with *HpbE*, nuclear translocation of HIF-1α and expression of COX-2 were increased (Fig. 5A). In contrast to wt BMDM, HIF-1α–deficient BMDM (HIF-1α$^{fl/fl}$xLysMCre) failed to up-regulate TXB_2 and PGE_2 in response to *HpbE*, whereas the suppression of (PGD_2 and LTB_4) remained intact (Fig. 5B). In addition, HIF-1α–deficient BMDM showed a reduced *HpbE*-driven induction of IL-6, TNF-α, and IL-10 as well as of the M2 markers *Tgm2* and *Arg1* (Fig. 5, C and D). Expression of *Mrc1* and *Retnla* was generally higher in BMDM lacking HIF-1α, but *HpbE* down-regulated *Mrc1* expression regardless of HIF-1α (Fig. 5D). Thus, the induction of type-2–suppressive mediators in BMDM was largely dependent on HIF-1α.

Because HIF-1α is positively regulated by the mitogen-activated protein kinase (MAPK) p38, we studied the involvement of p38 signaling in the induction of type-2–suppressive mediators by *HpbE*. In human MDM, p38 was phosphorylated upon treatment with *HpbE*, correlating with the induction of COX-2 (Fig. 5E). A p38 inhibitor (VX-702) abrogated the induction of IL-10, IL-1β, and PGE_2 synthetic enzyme transcripts (*PTGS2* and *PTGES*) (Fig. 5, F to H). In line with HIF-1α–dependent regulation in murine BMDM, a pharmacological inhibitor of HIF-1α (acriflavine) attenuated the *HpbE*-induced expression of IL-10, IL-1β, and COX pathway enzymes in human MDM (Fig. 5, F to H). However, p38 and HIF-1α were not responsible for the modulation of the 5-LOX pathway, which was still active in the presence of inhibitors (Fig. 5H).

To investigate whether the *HpbE*-triggered production of IL-10 and IL-1β occurred downstream of the COX pathway, we studied whether COX inhibitors could modify the induction of these cytokines in MDM. A nonselective COX inhibitor (indomethacin), but not a selective COX-2 inhibitor (CAY10404), reduced the induction of IL-10, IL-1β, and *PTGES* (Fig. 5, F to H, and fig. S6, A and B). In contrast, the *HpbE*-triggered expression of COX-2 was reduced both by indomethacin and by selective inhibition of COX-2, suggesting that COX-2 metabolites could drive an autocrine feedback loop to promote COX-2 expression (Fig. 5H and fig. S6B).

Because the transcription factor nuclear factor κB (NF-κB) and the kinases phosphatidylinositol 3-kinase (PI3K), protein kinase A, and PTEN can regulate eicosanoid pathways, we additionally assessed the contribution of these mechanisms to the induction of type-2–suppressive mediators by *HpbE*. Inhibition of NF-κB (by BAY 11-7085) reduced PGE_2, IL-10, and IL-1β production as well as gene expression of PGE_2 synthetic enzymes and IL-10 in *HpbE*-treated human MDM (fig. S6, C and D). In contrast, inhibitors of PI3K, protein kinase A, or PTEN did not interfere with the induction of PGE_2, IL-10, or IL-1β (fig. S6E). Thus, the *HpbE*-driven induction of type-2–suppressive mediators largely depended on the activation of p38 MAPK, HIF-1α, NF-κB, and the COX pathway.

TLR2, dectin-1, and dectin-2 contribute to the induction of the COX pathway by *HpbE*

To further elucidate the upstream mechanisms underlying prostanoid and cytokine modulation by *HpbE*, we blocked IL-1β or pattern recognition receptors [PRRs; Toll-like receptor 2 (TLR2), dectin-1, and dectin-2], which had all been previously linked to helminth-driven immunoregulation (*15*, *23*, *41*, *42*). Blockade of IL-1β in MDM affected the *HpbE*-driven modulation neither of IL-10 nor of eicosanoid pathways (fig. S7A). However, neutralizing antibodies against TLR2, dectin-1, or dectin-2 reduced the induction of PGE_2 synthetic enzymes by *HpbE*, whereas the modulation of IL-10 or 5-LOX was not affected (fig. S7, A and B). This suggested that TLR2, dectin-1, and dectin-2 contributed to the induction of the COX pathway but not to other immunoregulatory effects by *HpbE*.

Glutamate dehydrogenase is a major immunoregulatory protein in *HpbE*

As the above-defined mechanisms did not provide a molecular explanation for the immunoregulatory effects of *HpbE*, we further characterized its active components. Heat inactivation of *HpbE* attenuated the induction of prostanoids, IL-10, and IL-1β in MDM as well as the *HpbE*-driven suppression of granulocyte recruitment (Fig. 6, A and B). In addition, the induction of IL-10 by *HpbE* was abrogated if the extract was pretreated with proteinase K (Fig. 6C). This suggested that the induction of type-2–suppressive mediators by *HpbE* was largely dependent on heat-labile and proteinase K–digestible molecules, most likely proteins.

To identify immunoregulatory proteins present in *HpbE*, we fractionated the extract by size exclusion chromatography and identified active fractions based on the capacity to induce the COX metabolite TXB_2 as well as IL-10 (Fig. 6, D and E). We then identified proteins present in active and non-active fractions by MS. *Hpb* glutamate dehydrogenase (GDH) was uniquely present in active fractions of *HpbE*, qualifying it as an immunoregulatory candidate (Fig. 6F and table S2). An inhibitor of GDH (bithionol), which is also used as an anti-helminthic, reduced the *HpbE*-triggered induction of prostanoids and IL-10 in a dose-dependent manner without affecting cell viability (Fig. 6, G and H, and fig. S8A). In line with the unique anti-inflammatory properties of L3 (*HpbE*) versus L4 and L5 extracts (fig. S3E), *HpbE* showed higher GDH activity as compared to L4 and L5 preparations (fig. S8B).

To further validate *Hpb* GDH as a major immunoregulatory component of *HpbE*, we generated monoclonal antibodies specific for *Hpb* GDH [i.e., not cross-reactive with mammalian (human/mouse) GDH] (fig. S9A). Administration of an anti-*Hpb* GDH antibody (clone 4F8), but not an isotype control antibody, resulted in a dose-dependent reduction of the *HpbE*-induced production of IL-10 and PGE_2 in human MDM (Fig. 6I and fig. S9B).

Recombinant *Hpb* GDH reduces allergic airway inflammation in mice

Last, we developed a strategy for the recombinant production of *Hpb* GDH, allowing us to directly assess immunoregulatory effects of the protein in vitro and in vivo. Recombinant *Hpb* GDH was active, and its activity was partially inhibited by bithionol (fig. S9C). In human MDM, treatment with *Hpb* GDH induced PGE_2 and IL-10, but reduced cysLT production, thus recapitulating key anti-inflammatory effects of total *HpbE* (Fig. 7A).

To validate the use as a potential therapeutic enzyme in vivo, mice were treated intranasally with *Hpb* GDH during HDM-induced allergic airway inflammation. Administration of *Hpb* GDH attenuated the HDM-triggered eosinophil infiltration and airway inflammation as well as goblet cell hyperplasia and mucin maturation (Fig. 7, B and C).

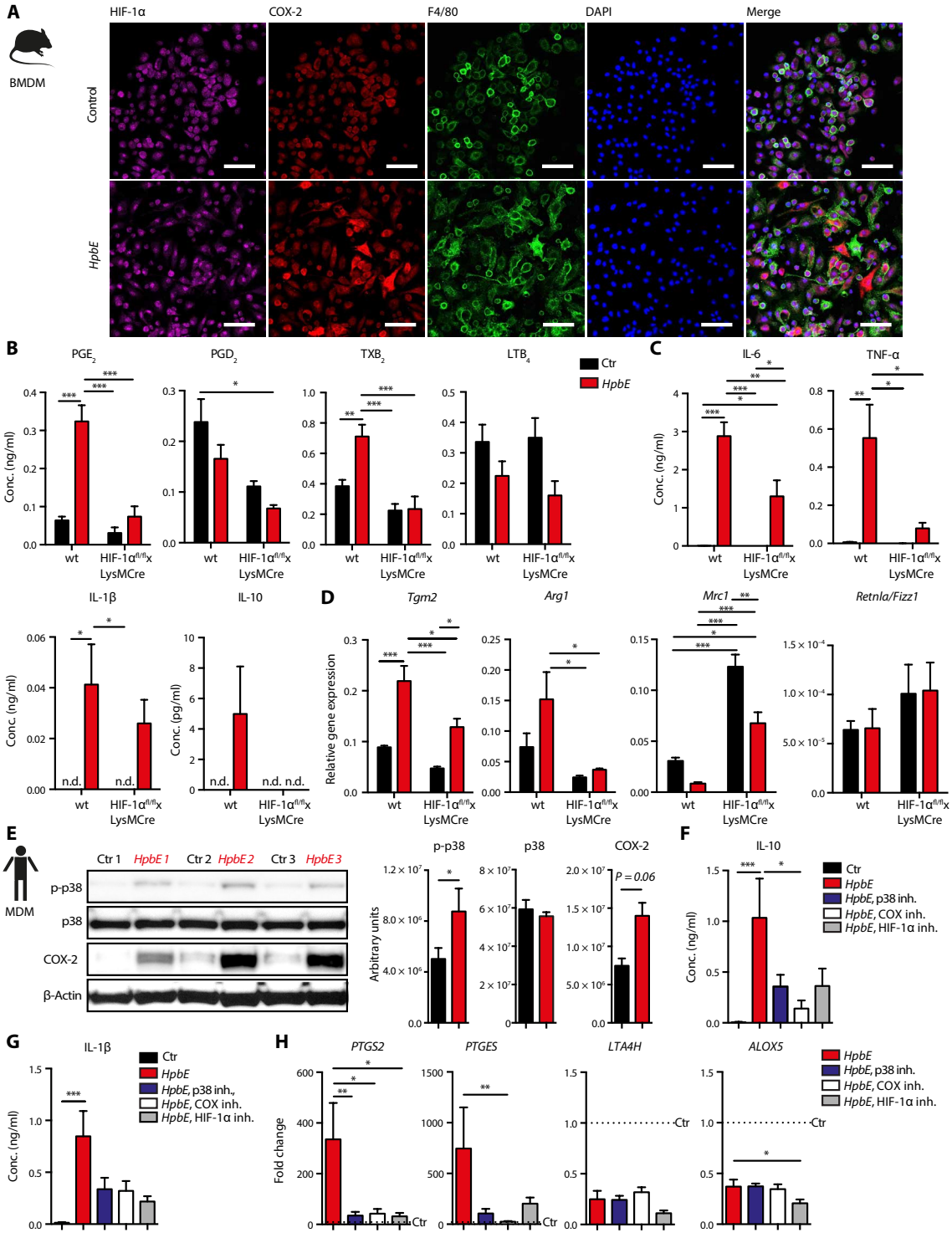

Fig. 5. Induction of type-2–suppressive macrophages by *HpbE* is mediated via HIF-1α, p38 MAPK, and COX. (**A**) Representative immunofluorescence staining of HIF-1α, COX-2, 4′,6-diamidino-2-phenylindole (DAPI) (cell nuclei), and F4/80 in BMDM ± treatment with *HpbE*. (**B**) Eicosanoid production (LC-MS/MS) in BMDM (wt or HIF-1α$^{floxed/floxed}$xLysMCre) ± treatment with *HpbE* (24 hours) + A23187 (10 min). (**C**) Amounts of IL-6, TNF-α, IL-1β, or IL-10 (Bioplex) in BMDM (wt or HIF-1α$^{floxed/floxed}$xLysMCre) ± treatment with *HpbE*. (**D**) Gene expression of M2 markers (qPCR) in BMDM (wt or HIF-1α$^{floxed/floxed}$xLysMCre) ± treatment with *HpbE*. (**E**) Protein amounts of phospho-p38, total p38, COX-2, or β-actin (Western blot) in MDM ± treatment with *HpbE*. Left, representative blots for $n = 3$ blood donors; right, quantification for $n = 5$ to 9 donors. (**F** and **G**) Amounts of IL-10 or IL-1β (ELISA) in MDM ± treatment with *HpbE* ± inhibitors of p38 (VX-702), COX (indomethacin), or HIF-1α (acriflavine). (**H**) Fold change of eicosanoid enzymes in MDM treated with *HpbE* ± inhibitors of p38 (VX-702), COX (indomethacin), or HIF-1α (acriflavine). Dotted lines indicate expression in untreated cells. Data are pooled from at least two independent experiments and presented as means ± SEM; $n = 5$ to 9. Statistical significance was determined by two-way ANOVA (A to D), Wilcoxon test (E), or Friedman test (F to H). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

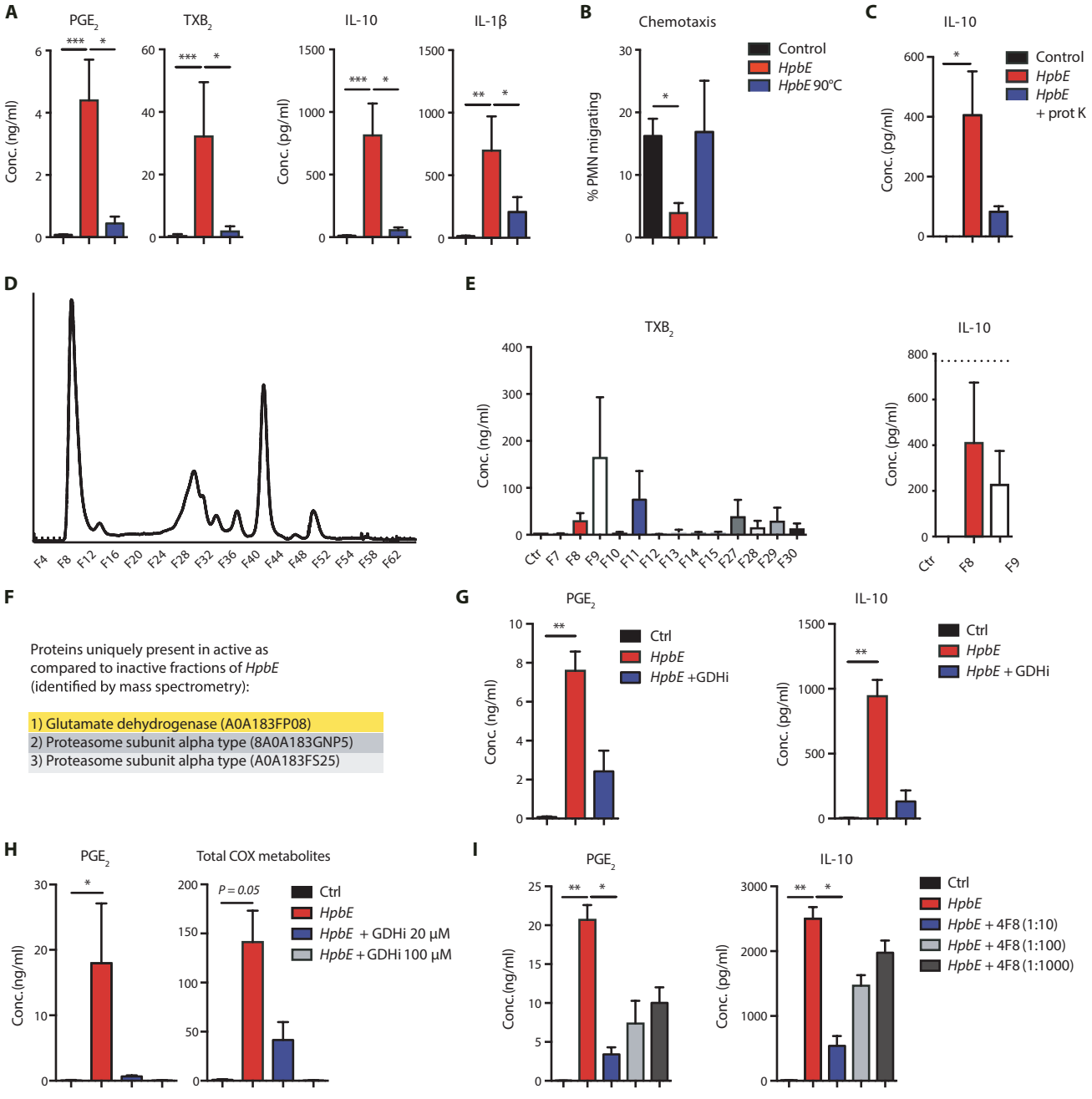

Fig. 6. GDH is a major immunoregulatory factor in *HpbE*. (**A** and **B**) Amounts of prostanoids (EIA) or IL-10 and IL-1β (ELISA) in human MDM (A) or chemotaxis of human PMNs (B) ± treatment with *HpbE* or heat-inactivated *HpbE* (*HpbE* 90°C). (**C**) Amounts of IL-10 (ELISA) in human MDM ± treatment with *HpbE* ± pretreatment with proteinase K (prot K). (**D**) Size exclusion chromatogram for fractionation of *HpbE*. (**E**) Amounts of TXB_2 (EIA) or IL-10 (ELISA) in MDM ± treatment with *HpbE* fractions. (**F**) Summary of results from MS identification of proteins in active fractions of *HpbE*. (**G**) Amounts of PGE_2 (EIA) or IL-10 (ELISA) in MDM ± treatment with *HpbE* ± inhibitor of GDH (GDHi; bithionol, 20 μM). (**H**) Amounts of PGE_2 or total COX metabolites (LC-MS/MS) in MDM ± treatment with *HpbE* ± inhibitor of GDH (bithionol, 20 or 100 μM). (**I**) Amounts of PGE_2 (EIA) or IL-10 (ELISA) in MDM ± treatment with *HpbE* ± different dilutions of a monoclonal antibody (clone 4F8) against *Hpb* GDH. Data are pooled from at least two independent experiments and presented as means ± SEM for MDM from $n = 2$ to 10 healthy human blood donors. Statistical significance was determined by Friedman test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

Searching for new approaches to target eicosanoids in type-2 inflammation, we identified an extract of a parasitic nematode (*HpbE*) and its active component *Hpb* GDH. *HpbE* showed broad immunoregulatory effects in various myeloid cell types and when administered topically to the airways of allergen-sensitized mice. In a human ex vivo setting of type-2 inflammation, *HpbE* effectively reduced the chemotaxis of granulocytes toward nasal polyp secretions. The effects of *HpbE* on the chemotaxis of granulocytes from patients with AERD were particularly notable because this disease is characterized by an

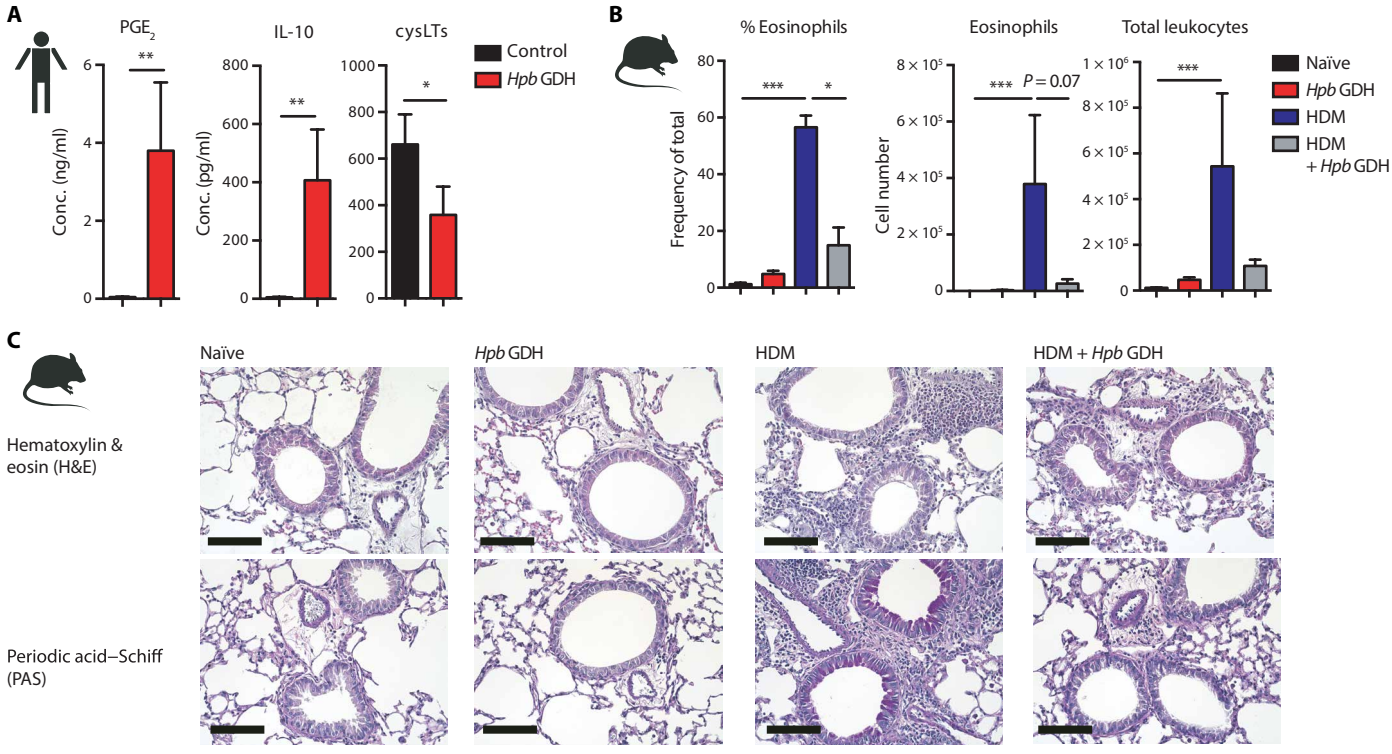

Fig. 7. Recombinant *Hpb* GDH induces anti-inflammatory mediators in human macrophages and suppresses allergic airway inflammation in mice. (**A**) Amounts of eicosanoids (LC-MS/MS/EIA) or IL-10 (ELISA) in human MDM ± treatment with recombinant *Hpb* GDH (5 μg/ml) (24 hours) ± A23187 (10 min). (**B**) BALF cell counts in mice sensitized (1 μg) and challenged with HDM (10 μg) ± intranasal treatment with *Hpb* GDH (10 μg), 48 hours after the last challenge and treatment. (**C**) Representative H&E- or PAS-stained lung tissue from mice sensitized to HDM ± treatment with *Hpb* GDH. Scale bars, 100 μm. Results are pooled from at least two independent experiments (A and B) or representative of stainings performed for two independent experiments (C). Results are presented as means ± SEM for MDM from $n = 7$ to 9 healthy human blood donors or $n = 9$ to 10 mice per group. Statistical significance was determined by Friedman test (A) or Kruskal-Wallis test followed by Dunn's multiple comparison test (B). $^*P = 0.05$; $^{**}P = 0.01$; $^{***}P < 0.001$.

aberrant AA metabolism and AA signaling as well as GC-resistant type-2 inflammation (*5*).

Of note, although *HpbE* is derived from a murine parasite, it showed largely overlapping anti-inflammatory effects on eicosanoids and granulocyte activation in human cells ex vivo and in mice in vivo. *HpbE* likely affected granulocytes directly (e.g., by modulating eicosanoids and chemotactic receptors) as well as indirectly by inducing granulocyte-regulatory factors such as PGE_2 in macrophages. Although other cell types can also contribute to eicosanoid production during allergic airway inflammation, we focused on macrophages as a particularly plastic, long-lived, and thus therapeutically accessible source of these mediators. The macrophage pool in inflamed airways consists of resident and infiltrating monocyte-derived cells, which develop into alveolar macrophages under the control of GM-CSF and transforming growth factor–β1 (TGF-β1) in the lung microenvironment (*43*, *44*). Thus, although the BMDM and MDM used in this study may not entirely reflect airway macrophages, they likely represent a relevant model for infiltrating, inflammatory monocytes, which crucially contribute to allergic airway inflammation (*27*).

When treated with *HpbE* or *Hpb* GDH, monocytes and macrophages produced high amounts of regulatory prostanoids, which can modulate type-2 immune responses by activating a regulatory macrophage phenotype and limiting type-2 cytokine production (*12*, *45*, *46*). In contrast, eicosanoid pathways that typically drive type-2 inflammation (5-LOX/LTs and PTGDS/PGD_2) were suppressed by treatment with *HpbE* or *Hpb* GDH. *HpbE* also triggered the production of TXB_2, a mediator involved in platelet and vascular function as well as tissue repair. Thus, in addition to modulating type-2 inflammation, helminth-induced eicosanoids may limit bleeding and tissue damage. Given the broad eicosanoid-modulatory potential of *HpbE* and *Hpb* GDH, it appears unlikely that only one metabolite or receptor is responsible for their anti-inflammatory effects. Transfer of *HpbE*-conditioned wt, but not *Ptges*$^{-/-}$, BMDM reduced eosinophilic airway inflammation as compared to transfer of untreated BMDM of the same genotype, suggesting that macrophage-derived PGE_2 may contribute to *HpbE*-driven immunoregulation. In addition, the exaggerated allergic airway inflammation observed after transfer of *HpbE*-conditioned *Ptgs2*$^{-/-}$ BMDM supports a role for macrophage-derived COX metabolites, including PGE_2, in the anti-inflammatory effects of *HpbE*. However, because we only obtained limited numbers of *Ptgs2*$^{-/-}$ BMDM and could thus not transfer untreated cells of this genotype, the strong inflammation observed after transfer of *HpbE*-conditioned *Ptgs2*$^{-/-}$ BMDM may also be due to COX-2 deficiency per se. Transfer of *Ptges*$^{-/-}$ BMDM led to reduced HDM-induced airway inflammation regardless of *HpbE* conditioning. Thus, when transferred into an inflammatory environment, mPGES-1/COX-2–deficient BMDM may respond differently as compared to wt BMDM. Of note, when transferred into the airways during HDM challenge, untreated wt BMDM tended to increase eosinophilic inflammation compared to HDM-sensitized mice without BMDM transfer, possibly due to HDM-induced

chemokine production by the transferred macrophages (*47*). In combination with the COX-dependent reduction of human granulocyte chemotaxis, the BMDM transfer data support an important role for macrophage-derived COX metabolites in the modulation of granulocyte responses during type-2 inflammation.

To achieve therapeutic efficacy in allergy, asthma, or nasal polyposis, the modulation of multiple eicosanoid pathways (e.g., cysLTs, PGD_2, and PGE_2) may be favorable, as targeting single pathways has failed to provide a substantial clinical benefit (*7*, *48*). Although the 5-LOX pathway represents a key drug target in multiple chronic diseases, there are currently no known drugs that work by simultaneously preventing LT generation and inducing anti-inflammatory eicosanoids. The regulatory eicosanoid switch triggered by *HpbE* and its active component *Hpb* GDH thus represents a key asset compared to current anti-inflammatory drugs.

Given that nematode larvae (*Caenorhabditis elegans* and *Nippostrongylus brasiliensis*) had been reported to induce LT production and eosinophil recruitment (*16*, *47*), the LT-suppressive effects of *HpbE* were unexpected. However, our findings suggest that distinct helminth species and stages may differentially affect eicosanoid pathways and thus either promote or suppress type-2 inflammation. The regulated LT response induced by *Hpb* products may explain why Hpb is not rapidly expelled by a LT-dependent type-2 immune response as observed for *N. brasiliensis* (*17*).

To potentially harness the eicosanoid-modulatory effects of *HpbE* and *Hpb* GDH, we chose a topical administration in a model of HDM-induced allergic airway inflammation, where the central role of prostanoids and LTs is well documented (*6*, *8*, *12*). Intranasal treatment with *HpbE* or *Hpb* GDH attenuated hallmarks of HDM-induced type-2 inflammation. This was in line with previous studies, showing that *Hpb* infection or treatment with excretory secretory products from the *Hpb* adult stage ("HES") can suppress allergic inflammation by modulating type-2 cytokine responses (*21*, *35*, *49*). However, in comparison to HES, *HpbE* and *Hpb* GDH showed a distinct potency to induce PGE_2 and IL-10, which can act in concert to induce regulatory macrophages and suppress type-2 inflammation (*12*). *HpbE* appeared to preferentially target early, innate immune mechanisms (e.g., granulocyte recruitment), whereas HES can modulate adaptive type-2 immune responses, e.g., by inducing regulatory T cells (*19*). *HpbE* or *Hpb* GDH may thus be particularly suited to target eicosanoids and myeloid cells as crucial innate components of type-2 inflammation.

Recently, protein components of HES (HpARI and Hp-TGM) have been identified as major immunoregulatory factors (*21*, *50*). Particularly, HpARI and the hookworm protein AIP-2 were shown to have potent allergy-suppressive effects (*20*, *21*), but their effects on eicosanoids have not been studied. Using fractionation and MS, the enzyme *Hpb* GDH was identified as a major immunoregulatory candidate in *HpbE*. Recombinant *Hpb* GDH could recapitulate all major anti-inflammatory effects of total *HpbE* in vitro and in vivo, suggesting a potential use as a therapeutic enzyme. However, because *Hpb* GDH only shows 60% identity to human GDH (fig. S10), immunogenicity may represent a limitation to its clinical development as a new biotherapeutic. Thus, future studies should assess whether treatment with *Hpb* GDH results in the generation of neutralizing antibodies and design strategies to reduce the immunogenicity of the protein.

In addition, it will be important to define the mechanism of action by which *Hpb* GDH exerts its effects on eicosanoid pathways. Several PRRs (TLR2 and dectin-1/2) participated in the induction of PGE_2 synthetic enzymes by *HpbE*. As TLR2 and dectins bind to carbohydrate structures, the full immunoregulatory potential of *HpbE* might rely on co-factors (e.g., carbohydrates or glycoproteins), which may act in concert with *Hpb* GDH or promote its activity. On the basis of its sequence, the natural, worm-derived *Hpb* GDH may also be O-glycosylated, which may result in recognition by C-type lectins and contribute to the immunoregulatory activities of *HpbE*. This would be in line with a recent study, showing dectin-dependent induction of PGE_2 in dendritic cells by *Schistosoma* egg antigens (*15*). However, recombinant *Hpb* GDH from *Escherichia coli*, which is most likely not glycosylated, was also immunologically active, suggesting that glycosylation is not the major factor determining its immunoregulatory activity.

In keeping with anti-inflammatory roles for myeloid NF-κB and HIF-1α in airway inflammation (*51*, *52*), both transcription factors contributed to the *HpbE*-triggered induction of type-2–suppressive mediators (PGE_2, IL-10, and IL-1β). In addition, p38 MAPK, which mediated the induction of COX metabolites, had previously been implicated in the modulation of macrophage activation by a protein from a filarial nematode (AvCystatin) (*53*). However, if and how *Hpb* GDH may be involved in the activation of p38 MAPK, NF-κB, or HIF-1α pathways remains to be studied.

Last, because eicosanoids represent important mediators in severe and therapy-resistant type-2 inflammatory diseases (*5*, *54*, *55*), their broad anti-inflammatory modulation by *HpbE* may represent a promising future therapeutic approach. The identification of *Hpb* GDH as a major eicosanoid-modulatory component of *HpbE* provides a basis for the development of a new helminth-based therapeutic enzyme with a unique immunoregulatory profile.

MATERIALS AND METHODS

Study design

The aim of this study was to investigate if and how helminth products could modulate eicosanoid pathways to regulate type-2 inflammation. We characterized eicosanoid profiles of multiple myeloid cell types after treatment with helminth preparations by LC-MS/MS and selected the most promising candidates (*HpbE* and its active component *Hpb GDH*) for in vivo and ex vivo testing. To define in vivo effects of *HpbE*, *Hpb GDH*, or *HpbE*-conditioned macrophages, we treated mice intranasally with *HpbE* or recombinant *Hpb GDH* or transferred *HpbE*-conditioned macrophages during experimental HDM allergy and assessed type-2 inflammation by differential cell counts, histology, and multiplex cytokine analysis. All readouts were performed by a blinded experimenter. For the human part of our study, healthy volunteers (total $n = 15$) and AERD patients ($n = 6$) (Caucasian men and women) were recruited. Sample sizes, replicates, and statistical methods are specified in the figure legends. Primary data are reported in data file S1.

Mice

C57BL/6J mice were bred and maintained under specific pathogen–free conditions at the École Polytechnique Fédérale de Lausanne (EPFL) or at the Centre Hospitalier Universitaire Vaudois (CHUV). Alternatively, BALB/c and C57BL/6J mice were obtained from Charles River Laboratories (Sulzfeld, Germany). Unless stated otherwise, 6- to 12-week-old mice of both sexes were used. All animal experiments were approved by the local authorities (Swiss Veterinary Office or Regierung von Oberbayern).

Hpb infection and preparation of larval extract

Infective stage-three larvae (L3) of *Hpb* were obtained from the eggs of *Hpb*-infected mice as previously published (*56*). For preparation of *Hpb* larval extract (*HpbE*), L3 larvae were homogenized in two cycles at 6000 rpm for 60 s in a Precellys homogenizer using Precellys tough micro-organism lysing kits VK05 (Bertin Pharma). Remaining debris were removed by centrifugation (20 min, 14,000 rpm, 4°C). When indicated, heat-inactivated *HpbE* (*HpbE* 90°C) was prepared by heating at 90°C overnight.

HDM-induced allergic airway inflammation and intranasal treatment with *HpbE* or *Hpb* GDH

Eight-week-old female C57BL/6J mice were sensitized on day 0 by bilateral intranasal instillations of HDM extract from *Dermatophagoides farinae* [1 μg of extract in 20 μl of phosphate-buffered saline (PBS); Stallergenes SA] and challenged on days 8 to 11 with 10 μg of the same extract dissolved in 20 μl of PBS. Control animals received the same amount of PBS. *HpbE* treatment (5 μg of *Hpb* extract in 20 μl of PBS) was performed intranasally before sensitization and challenges, and *Hpb* GDH treatment (10 μg of protein in 20 μl of PBS) was performed intranasally before challenges. In the absence of *HpbE* or *Hpb* GDH treatment, the mice received 20 μl of PBS. Three days after the last challenge, the airways of the mice were lavaged five times with 0.8 ml of PBS. Aliquots of cell-free BALF were frozen immediately with or without equal volumes of methanol. Viability, yield, and differential cell count of BAL cells were performed as described before (*57*).

Human blood and tissue samples

PBMCs or PMNs were isolated from the blood of healthy human donors or patients with AERD. Nasal polyp tissues were obtained during polypectomy of patients suffering from chronic rhinosinusitis with nasal polyps. Nasal polyp secretions were obtained from cultured nasal polyp tissues as described previously (*6*). All blood and tissue donors participated in the study after informed written consent. Blood and tissue sampling and experiments including human blood cells were approved by the local ethics committee at the University clinic of the Technical University of Munich (internal reference: 422/16).

Macrophage cultures

MDMs or BMDMs were generated by culture in the presence of human or murine recombinant GM-CSF (10 ng/ml) (Miltenyi Biotec) and human recombinant TGF-β1 (2 ng/ml) (PeproTech) as previously described (*6*, *58*). On day 6, cells were harvested and used for further experiments. More detailed procedures can be found in the Supplementary Materials.

Eicosanoid and cytokine analysis

Eicosanoids were quantified by LC-MS/MS similar to a previously published method (*47*). Cytokines were quantified using commercially available multiplex assays or enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. More detailed procedures can be found in the Supplementary Materials.

Chemotaxis assays

PMNs were resuspended to a concentration of 1×10^6 cells/ml in the presence of human GM-CSF (100 ng/ml; Miltenyi Biotec) and stimulated overnight with *HpbE* (10 μg/ml). When mentioned, PMNs were pretreated with 1 μM FP (Sigma-Aldrich), 10 μM MK (Cayman Chemical), or conditioned medium from MDM stimulated overnight with *Hpb* extract (10 μg/ml) ± 100 μM indomethacin for 1 hour. Nasal polyp secretions or a chemokine cocktail [RANTES (2 ng/ml), IL-8 (20 ng/ml; Miltenyi Biotec), and LTB_4 (2 ng/ml; Cayman Chemical)] was placed in the lower wells of a chemotaxis plate (3 μm pore size; Corning). After mounting the transwell unit, 2×10^5 PMNs were added to the top of each well and migration was allowed for 3 hours at 37°C, 5% CO_2. The number of cells migrating to the lower well was counted microscopically. In some experiments, manual counting was validated by flow cytometry.

Statistical analysis

All statistical analyses for biological data were performed using GraphPad Prism (GraphPad Software Inc.). Where two groups were compared, statistical significance was determined by Wilcoxon-Mann-Whitney test. Where more than two groups were compared, Kruskal-Wallis followed by Dunn's multiple comparisons test, Friedman test (paired samples), or two-way analysis of variance (ANOVA) (unpaired samples) was used. Heat maps were generated using MetaboAnalyst version 3.0 (McGill University), a free online tool for metabolomics data analysis.

SUPPLEMENTARY MATERIALS

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Supplementary Materials and Methods

Fig. S1. PUFA metabolites produced by human MDM in response to treatment with *HpbE* and impact of *HpbE*-treated *Ptgs2*$^{-/-}$ BMDM on eosinophilic airway inflammation.

Fig. S2. *HpbE* has stronger eicosanoid-modulatory effects than glucocorticosteroids.

Fig. S3. *HpbE* has a distinct potential to induce type-2–suppressive mediators compared to other helminth products or helminth-associated bacteria.

Fig. S4. Viability and LTA4H expression in human eosinophils and neutrophils and PUFA metabolites produced by human PMNs in response to treatment with *HpbE*.

Fig. S5. *HpbE* induces a regulatory eicosanoid and cytokine profile in mixed and isolated CD14$^+$ human PBMCs.

Fig. S6. Effect of COX-2, NF-κB, PI3K, PTEN, or PKA inhibition on *HpbE*-driven modulation of cytokines and eicosanoid pathways.

Fig. S7. Effect of neutralizing antibodies against PRRs (TLR2 and dectin-1/2) or IL-1β on *HpbE*-driven modulation of eicosanoids and IL-10 in human MDM.

Fig. S8. Bithionol does not affect cell viability and L3 stage *HpbE* shows a higher GDH activity as compared to L4 or L5 extracts.

Fig. S9. Newly generated monoclonal antibodies recognize *Hpb* GDH, clone 4F8 reduces *HpbE*-induced PGE_2 and IL-10 production, and bithionol partially inhibits activity of recombinant *Hpb* GDH.

Fig. S10. Sequence of *Hpb* GDH is distinct from human GDH and contains potential predicted glycosylation sites.

Table S1. LC-MS/MS panel of PUFAs and PUFA metabolites.

Table S2. Proteins present in active fractions of *HpbE* identified by MS.

Table S3. Primer sequences for qPCR.

Table S4. Reagents and resources.

Data file S1. Primary data.

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View/request a protocol for this paper from *Bio-protocol*.

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An anti-inflammatory eicosanoid switch mediates the suppression of type-2 inflammation by helminth larval products

Marta de los Reyes Jiménez, Antonie Lechner, Francesca Alessandrini, Sina Bohnacker, Sonja Schindela, Aurélien Trompette, Pascal Haimerl, Dominique Thomas, Fiona Henkel, André Mourão, Arie Geerlof, Clarissa Prazeres da Costa, Adam M. Chaker, Bernhard Brüne, Rolf Nüsing, Per-Johan Jakobsson, Wolfgang A. Nockher, Matthias J. Feige, Martin Haslbeck, Caspar Ohnmacht, Benjamin J. Marsland, David Voehringer, Nicola L. Harris, Carsten B. Schmidt-Weber and Julia Esser-von Bieren

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Tamping down type-2 inflammation

Type-2 immunity in allergy and asthma is driven by nonredundant pathways, sometimes making therapeutic targeting difficult. De los Reyes Jiménez *et al.* investigated whether factors from a known mediator of type-2 immunity, the helminth *Heligmosomoides polygyrus bakeri*, could have added beneficial anti-inflammatory effects. They found that helminth larvae modulated eicosanoids in human macrophages and granulocytes. Both the larvae and the helminth protein glutamate dehydrogenase alleviated inflammation in a house dust mite model of asthma. These results suggest that helminth products have the potential to broadly modulate pathways in type-2 immune diseases.

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