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Activated prostaglandin D_2 receptors on macrophages enhance neutrophil recruitment into the lung

Katharina Jandl, MSc[a], **Elvira Stacher, MD**[b], **Zoltán Bálint, PhD**[c], **Eva Maria Sturm, PhD**[a], **Jovana Maric, MSc**[a], **Miriam Peinhaupt, MSc**[a], **Petra Luschnig, PhD**[a], **Ida Aringer, MD**[a,d], **Alexander Fauland, PhD**[e], **Viktoria Konya, PhD**[a,f], **Sven-Erik Dahlen, PhD**[g], **Craig E. Wheelock, PhD**[e], **Dagmar Kratky, PhD**[h], **Andrea Olschewski, MD**[c], **Gunther Marsche, PhD**[a], **Rufina Schuligoi, PhD**[a], and **Akos Heinemann, MD**[a]

[a]Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria

[b]Institute of Pathology, Medical University of Graz, Graz, Austria

[c]Ludwig Boltzmann Institute for Lung Vascular Research, Graz

[d]Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

[e]Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Stockholm, Sweden

[f]Center for Infectious Medicine, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden

[g]Institute of Environmental Medicine, Experimental Asthma and Allergy Research Unit, Karolinska Institutet, Stockholm, Sweden

[h]Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria

Abstract

Background—Prostaglandin (PG) D_2 is an early-phase mediator in inflammation, but its action and the roles of the 2 D-type prostanoid receptors (DPs) DP_1 and DP_2 (also called chemoattractant receptor–homologous molecule expressed on T_H2 cells) in regulating macrophages have not been elucidated to date.

Corresponding author: Akos Heinemann, MD, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Universitaetsplatz 4, 8010, Graz, Austria. akos.heinemann@medunigraz.at.

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Objective—We investigated the role of PGD_2 receptors on primary human macrophages, as well as primary murine lung macrophages, and their ability to influence neutrophil action *in vitro* and *in vivo*.

Methods—*In vitro* studies, including migration, Ca^{2+} flux, and cytokine secretion, were conducted with primary human monocyte-derived macrophages and neutrophils and freshly isolated murine alveolar and pulmonary interstitial macrophages. *In vivo* pulmonary inflammation was assessed in male BALB/c mice.

Results—Activation of DP_1, DP_2, or both receptors on human macrophages induced strong intracellular Ca^{2+} flux, cytokine release, and migration of macrophages. In a murine model of LPS-induced pulmonary inflammation, activation of each PGD_2 receptor resulted in aggravated airway neutrophilia, tissue myeloperoxidase activity, cytokine contents, and decreased lung compliance. Selective depletion of alveolar macrophages abolished the PGD_2-enhanced inflammatory response. Activation of PGD_2 receptors on human macrophages enhanced the migratory capacity and prolonged the survival of neutrophils *in vitro*. In human lung tissue specimens both DP_1 and DP_2 receptors were located on alveolar macrophages along with hematopoietic PGD synthase, the rate-limiting enzyme of PGD_2 synthesis.

Conclusion—For the first time, our results show that PGD_2 markedly augments disease activity through its ability to enhance the proinflammatory actions of macrophages and subsequent neutrophil activation.

Keywords

D-type prostanoid receptor 1; D-type prostanoid receptor 2/chemoattractant receptor–homologous molecule expressed on T_H2 cells; prostaglandin D_2; hematopoietic prostaglandin D synthase; macrophages; pulmonary inflammation; neutrophils

Prostaglandin (PG) D_2, a lipid mediator from the arachidonic acid/COX pathway, has been shown to play complex and often opposing roles in the development and resolution of inflammation, which can be attributed to differential activation of its receptors. PGD_2 activates 2 G protein–coupled receptors, the D-type prostanoid receptors (DPs) DP_1 and DP_2, with the latter also known as chemoattractant receptor–homologous molecule expressed on T_H2 cells.1 At higher concentrations, PGD_2 can also signal through the thromboxane A_2 receptor.2,3 Although PGD_2 exerts similar binding affinities toward the DP_1 and DP_2 receptors, its metabolites, formed rapidly by enzymatic and nonenzymatic pathways, can differentially induce DP_2-mediated effects.1

In patients with allergic diseases, the role of PGD_2 has mostly been associated with its release from activated mast cells and induction of vasodilation. More recently, however, PGD_2 has been found to promote additional proinflammatory responses through activation of DP_2 receptors reflected by increased eosinophilic infiltration into the lungs and skin of mice.4,5 Consequently, DP_2 antagonists were shown to ameliorate eosinophilic pulmonary inflammation in murine ovalbumin-induced6 and house dust mite–induced7 models, a rat *Alternaria* species–induced model,8 and the setting of chronic allergic skin inflammation.9 Moreover, the DP_2 antagonist CAY10471 ameliorated weight loss and intestinal inflammation in a dextran sodium sulfate–induced colitis model in mice.10 In asthmatic

patients induction of hematopoietic prostaglandin D synthase (HPGDS), the rate-limiting enzyme of PGD_2 synthesis, was observed in the epithelial compartment,11 and PGD_2 levels in bronchoalveolar lavage (BAL) fluid correlated positively with the severity of the disease.12 DP_2/chemoattractant receptor–homologous molecule expressed on T_H2 cell antagonists was found to have some effects in allergic rhinitis,13 allergic conjunctivitis,14 eosinophilic esophagitis,15 and bronchial asthma.16,17

Macrophages are essential in pulmonary inflammatory diseases by maintaining tissue homeostasis and mounting rapid responses to exogenous and endogenous stimuli. Because they are the main source of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, their role in inflammation is pivotal.18 Acute lung injury (ALI) or its more severe clinical manifestation, acute respiratory distress syndrome (ARDS), is a pulmonary inflammatory disease that can lead to respiratory failure. Pulmonary complications in this disease are mainly attributed to rapid neutrophil infiltration into the alveolar space,19 subsequent edema formation, and dysfunction of the involved cell types, including epithelial cells,20 endothelial cells,21 and macrophages.22–24 Several studies have made it clear that macrophages orchestrate neutrophilic infiltration and thus strongly modulate the outcome of ARDS.23–27

Stimulated by the dominant physiologic role of PGD_2 in the lung, we hypothesized that PGD_2 might govern disease activity and progression by acting on lung macrophages. Previous studies revealed anti-inflammatory effects of the PGD_2 metabolite 15d-PGJ_2 acting through peroxisome proliferator–activated receptor γ in RAW 264.7 macrophages28 and demonstrated the expression of PGD_2 receptors on human monocytes,29 whereas the role of PGD_2 in regulation of macrophage function has not been assessed yet.

Methods

Detailed description of ethical permits, materials, and procedures is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Isolation of peripheral blood polymorphonuclear neutrophils and PBMCs

Human peripheral blood polymorphonuclear cells and PBMCs were isolated from healthy donors independent of sex and age, as described previously.30

Differentiation from monocytes to macrophages

Human peripheral blood monocytes isolated from healthy donors were differentiated to human monocyte-derived macrophages (MDMs) for 7 to 10 days with 20 ng/mL human recombinant macrophage colony-stimulating factor.

Live cell fluorescent Ca^{2+} imaging

Macrophages were loaded with 2 μmol/L Fura-2/AM. Fluorescence images were obtained with alternate excitation at 340 and 380 nm, and emitted light was collected at 510 nm. Intracellular calcium levels were calculated, as previously described.31

Monocyte Ca^{2+} flux

Ca^{2+} flux was measured by means of flow cytometry, as previously described.30

Neutrophil apoptosis

Neutrophil survival was assessed by using Annexin V/propidium iodide staining, as described previously.32

Neutrophil and macrophage chemotaxis

Neutrophils were placed in the upper compartment of a Transwell chamber (Corning, Inc, New York, NY) in the absence or presence of MDMs. After 1 hour, neutrophils that migrated to the bottom well were collected, suspended in 150 μL of fixative solution, and enumerated by means of flow cytometric analysis.33 Migration of human MDMs was assessed by using Transwell inserts, as described previously.30

Flow cytometric staining

The following antibodies and concentrations were used: DP_2 (20 μg/mL), DP_1 (20 μg/mL), anti-mouse MHC class II (2.5 μg/mL), anti-mouse Siglec-F (5 μg/mL), anti-mouse CD3ε (5 μg/mL), anti-mouse B220 (2 μg/mL), and anti-mouse CD11c (2 μg/mL) antibodies.

LPS-induced lung injury

Pulmonary inflammation was induced in 8- to 10-week-old BALB/c mice by means of intranasal application of 1 mg/kg LPS. Agonists or antagonists were applied 24 hours before LPS application subcutaneously every 12 hours. Unless stated otherwise, mice were killed 4 hours after LPS administration.

Myeloperoxidase assay

Myeloperoxidase (MPO) activity was determined, as described previously.34

BAL protein content

BAL protein concentrations were measured by using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Rockford, Ill), according to the manufacturer's protocol.

Vascular permeability in lung tissue was assessed by using Evans blue dye extravasation after 60 minutes of circulation. Evans blue protein leak was determined, as described in the Methods section in this article's Online Repository.

Murine lung histology

Paraffin-embedded murine lungs were cut (5-μm sections), deparaffinized, and immunostained with anti-Ly6G and hematoxylin.

Isolation of murine alveolar and interstitial macrophages

Alveolar and interstitial macrophages were isolated from BAL fluid, as described in the Methods section in this article's Online Repository.

Depletion of murine lung macrophages

Three hundred micrograms (in 60 μL volume) of clodronate or control liposomes were intranasally applied to mice 24 hours before LPS challenge.35

Cytokine measurements

Cytokine levels were determined by using either a multianalyte immunoassay (Bender Medsystems, Vienna, Austria), ELISA (PeproTech, Rocky Hill, NJ), or the ProcartaPlex Mouse Cytokine Kit (eBioscience, San Diego, Calif).

Immunohistochemistry of human lung tissue

Human paraffin-embedded lung samples were stained with anti-DP_2 (1:200), anti-DP_1 (1:100), or anti-HPGDS (1:200) antibodies.

Lipid mediator analysis

PGD_2, PGE_2, TXB_2, 12S-hydroxy-5Z,8E,10E-heptoadectrienoic acid, and 6-*keto*-$PGF_{1\alpha}$ were analyzed by using liquid chromatography–tandem mass spectrometry, as previously published36 and as described in the Methods section in this article's Online Repository.

Measurement of murine lung function

Decreased lung compliance caused by pulmonary edema and atelectasis is a hallmark of human ALI/ARDS and a preferred readout in mouse models.37 Therefore mouse lung function was determined by using the flexiVent system (SCIREQ, Montreal, Quebec, Canada), as described previously.38

Statistical analysis

Data were analyzed by using either the Student *t* test, 1-way ANOVA, or 2-way ANOVA, followed by the Bonferroni or Dunnett posttest, with GraphPad Prism software (version 5.04; GraphPad Software, La Jolla, Calif). *In vitro* experiments were performed in duplicates, with *n* numbers indicating biological replicates with cells from different donors. *P* values of less than .05 were considered significant.

Results

DP_1 and DP_2 are expressed on human macrophages and induce Ca^{2+} signaling

We first set out to elucidate whether PGD_2 receptors are expressed on human monocytes and macrophages and found that both cell types express DP_1 and DP_2 on their cell surface (Fig 1, *A*, and see Fig E1 in this article's Online Repository at www.jacionline.org). Interestingly, expression of both receptors increased during differentiation of MDMs, with DP_2 expression being more abundant than DP_1 expression in both monocytes and macrophages (see Fig E1). Next, we probed for DP_1 and DP_2 expression in human lung tissue with different pathologies (see Table E1 in this article's Online Repository at www.jacionline.org). Both were highly and consistently expressed on human alveolar macrophages (Fig 1, *B*) because 60% to 75% of alveolar macrophages were positively stained for both DP_1 and DP_2 independent of the underlying disease (see Figs E2 and E3 in this article's Online

Repository at www.jacionline.org). This expression pattern suggested a possible role for the PGD_2-DP_1-DP_2 axis in the modulation of macrophage biology in patients with pulmonary diseases.

Then we sought to determine the biological activities of DP_1 and DP_2 *in vitro*. Ca^{2+} imaging revealed that superfusion of human MDMs with 1 μmol/L PGD_2 led to a strong release of intracellular calcium (Fig 1, *C*, and see Fig E4, *A*, in this article's Online Repository at www.jacionline.org), whereas human monocytes showed no Ca^{2+} response to PGD_2 stimulation (see Fig E5 in this article's Online Repository at www.jacionline.org). Both DP_2- and DP_1-selective agonists (13,14-dihydro-15-keto PGD_2, and BW245C, respectively; 1 μmol/L) were likewise able to elicit Ca^{2+} flux, although to a lesser extent than PGD_2 (Fig 1, *C*, and see Fig E4, *A*). In agreement with this observation, blockade of either DP_2 (CAY10471, 10 μmol/L) or DP_1 (MK0524, 10 μmol/L) slightly decreased, whereas simultaneous antagonism of both receptors abolished the PGD_2-induced Ca^{2+} signal (see Fig E4, *B* and *C*). Ca^{2+} release induced by both PGD_2 and 13,14-dihydro-15-keto prostaglandin D_2 ($DK\text{-}PGD_2$) was prevented by overnight incubation with pertussis toxin (100 ng/mL), indicating involvement of Gαi heterotrimers in this process (see Fig E4, *D* and *E*).

PGD_2 induces migration of macrophages through DP_1 and DP_2 and enhances TNF-α secretion

We next evaluated the chemotactic potential of PGD_2 toward MDMs by using Transwell inserts, followed by enumeration of fluorescently labeled cell nuclei of the migrated cells on the lower surface of the filter (see Fig E6, *B*). We found that PGD_2 exerted a concentration-dependent chemotactic activity toward MDMs, with the highest responses observed at 100 nmol/L (Fig 1, *D*, and see Fig E6, *A*, in this article's Online Repository at www.jacionline.org). This could be partially blocked by either the DP_2- or DP_1-specific antagonist (CAY10471 or MK0524, 1 μmol/L, respectively; Fig 1, *D*). Inhibition of both receptors completely abolished the migratory activity of MDMs toward PGD_2 (Fig 1, *D*). Because PGD_2 was shown to be critical in mediating macrophage migration toward LPS in a murine model,39 we hypothesized that PGD_2 could alter the LPS-induced cytokine secretion from MDMs. Although almost no TNF-α was detectable in the supernatants of vehicle-treated cells, LPS stimulation (100 ng/mL) induced a strong release of the proinflammatory cytokine. Indeed, this response was markedly enhanced in cells pretreated with PGD_2 or $DK\text{-}PGD_2$ (see Fig E6, *C*). Although the DP_1-specific agonist BW245C was not able to induce changes in TNF-α secretion, involvement of both DP_1 and DP_2 receptors in the PGD_2 response is still likely because only antagonism of both receptors was able to completely block the PGD_2-mediated increase in TNF-α secretion.

Endotoxin-induced lung injury is aggravated by PGD_2

Prompted by these observations, we investigated the effect of PGD_2 in an *in vivo* model of pulmonary inflammation. Here neutrophil infiltration is a main pathogenic feature that essentially depends on macrophage function.23 LPS significantly increased neutrophil influx into the bronchoalveolar space after 4 hours of treatment. Of particular interest, this effect was almost doubled when PGD_2 (5 mg/kg) was administered subcutaneously before LPS challenge (Fig 2, *A*). Selective DP_1 and DP_2 agonists induced an even more pronounced

response; in detail, the selective DP_2 agonist DK-PGD_2 (5 mg/kg) enhanced neutrophil influx by 2.5-fold and the DP_1 agonist BW245C (5 mg/kg) enhanced neutrophil influx by 2.3-fold (Fig 2, *B*). Histologically, the inflammatory state of the lungs was indicated by dense Ly6G-positive neutrophil infiltrates in the bronchiolar region, with disturbed alveolar morphology in LPS-treated animals. When PGD_2 was administered before LPS treatment, even more Ly6G-positive neutrophils were found around the bronchioli accompanied by neutrophil infiltrates in the alveolar space, which was largely absent in vehicle/LPS-treated mice (Fig 2, *C*, and see Fig E7, *A*, in this article's Online Repository at www.jacionline.org). MPO activity, a marker for lung inflammation, was likewise increased depending on the applied dose of PGD_2 (Fig 2, *D*). These observations were reflected by increased protein content in the BAL fluid in PGD_2-treated animals (see Fig E7, *B*), as well as enhanced Evans blue extravasation in the lung tissue that depended on both DP_1 and DP_2 (Fig 2, *E*). It is noteworthy that the capacity of PGD_2 to increase neutrophil recruitment was sustained after 24 hours (see Fig E7, *C*). Only minor alterations were found in the number of other cell types, such as monocytes, macrophages, lymphocytes, and eosinophils (see Fig E8 in this article's Online Repository at www.jacionline.org). The observation that PGD_2 aggravated pulmonary inflammation was also reflected by a decrease in lung function. In these experiments we found that LPS alone caused hyperresponsiveness to methacholine with respect to airway resistance and compliance (Fig 2, *F*), which was further accentuated after combined treatment of mice with PGD_2 and LPS (Fig 2, *F*). In all experimental readouts, including cell infiltration into the alveolar space, MPO activity, and lung function, PGD_2 treatment in the absence of LPS had no effect (data not shown).

Blocking of endogenous PGD_2 reduces neutrophilic infiltration into the lungs

Mice were pretreated with the PGD_2 receptor antagonists MK0524 or CAY10471 (5 mg/kg) followed by LPS challenge to delineate the pathogenic role of endogenous PGD_2. Analysis of BAL cells revealed that the DP_1 antagonist MK0524 reduced endotoxin-induced neutrophilia (Fig 3, *A*), whereas the DP_2 antagonist CAY10471 reduced MPO activity (Fig 3, *B*). Next, we compared lipid mediator levels in the BAL fluid of vehicle- and LPS-treated animals and found that there was a marked increase in PGD_2 levels in the latter (Fig 3, *C*). In agreement with previous findings, PGE_2 levels also increased, as did TXB_2 and 12S-hydroxy-5Z,8E,10E-heptoadectrienoic acid levels. We could not detect any significant changes in PGI2 levels (estimated through its metabolite 6-keto-$PGF_{1\alpha}$).

PGD_2 treatment enhances proinflammatory cytokine release *in vivo* and *in vitro*

In addition to prostanoids, increased keratinocyte-derived chemokine (KC) and monocyte chemotactic protein 1 levels were observed in the cell-free BAL fluid of LPS-treated mice. Importantly, the increase in levels of these chemokines was significantly stronger in PGD_2-treated animals (see Fig E9, *A*, in this article's Online Repository at www.jacionline.org). To determine whether macrophages were responsible for the increased release of these chemokines, we isolated alveolar and interstitial macrophages and mimicked the *in vivo* model. Both alveolar and interstitial pulmonary macrophages increased secretion of the neutrophil chemoattractant KC when pretreated with PGD_2 (see Fig E9, *B* and *C*), implicating macrophages as the cell type responsible for the enhanced neutrophil recruitment *in vivo*. Interestingly, monocyte chemotactic protein 1 secretion by alveolar and

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interstitial macrophages was influenced by neither LPS nor PGD_2 treatment (see Fig E9, *B* and *C*).

Macrophage depletion prevents the increased inflammatory response mediated by PGD_2

To highlight the role of macrophages in the PGD_2-induced enhancement of pulmonary inflammation, we next selectively reduced alveolar macrophage counts in the pulmonary LPS model. Intranasal application of 300 μg of clodronate led to a marked reduction of macrophage counts by approximately 70% throughout the groups (see Fig E10 in this article's Online Repository at www.jacionline.org). This dose was chosen to avoid proinflammatory responses caused by clodronate and/or liposomes themselves, which were observed at higher doses (data not shown). In animals that had received control liposomes, LPS treatment led to neutrophil recruitment into the bronchoalveolar space, which was again further enhanced when the animals were pretreated with PGD_2 or any of the specific agonists for DP_1 and DP_2. After reduction of alveolar macrophage counts, LPS still enhanced neutrophil counts in the lungs, suggesting that 30% residual macrophages were sufficient to induce lung neutrophilia, but no further increase in neutrophil counts was seen in PGD_2-, DK-PGD_2–, or BW245C-treated animals (Fig 4, *A*, and see Fig E11 in this article's Online Repository at www.jacionline.org). Concomitant to decreased alveolar neutrophil counts, the PGD_2-induced increase in MPO activity, as well as compromised lung function, were reversed by alveolar macrophage reduction (Fig 4, *B* and *C*).

PGD_2 receptor activation on macrophages modulates neutrophil function

In vitro neutrophil migration assays were performed in the presence of human MDMs treated with vehicle or PGD_2 to further clarify the mechanisms through which PGD_2 receptor activation on macrophages augmented the LPS-induced neutrophil recruitment. In this coculture experiment PGD_2-treated macrophages enhanced the migration of neutrophils when compared with basal neutrophil migration in the presence of untreated MDMs (Fig 5, *A*). This effect did not depend on IL-8 concentrations because PGD_2 treatment of MDMs enhanced the basal migratory capacity and IL-8–induced migration of neutrophils alike. Importantly, neutrophil migration was unaffected by PGD_2 when macrophages were absent (Fig 5, *B*). Therefore PGD_2 does not exert its effect through direct stimulation of neutrophils but rather through a macrophage-dependent pathway. In addition to migration, we monitored neutrophil survival in further coculture experiments. MDMs grown on 48-well plates were treated with vehicle or PGD_2 24 hours before neutrophils were added. Neutrophil apoptosis was monitored over 24 hours. The portion of viable neutrophils (Annexin V/propidium iodide negative) was higher when macrophages were treated with PGD_2 (Fig 5, *C*). Importantly, this effect was not due to enhanced macrophage phagocytosis of apoptotic/necrotic neutrophils because neutrophil numbers did not change throughout the experiment (see Fig E12 in this article's Online Repository at www.jacionline.org). Furthermore, neutrophils cultivated without macrophages did not react to PGD_2 and showed the same survival rate as in the presence of vehicle (Fig 5, *D*). These data show that PGD_2 receptor activation on macrophages profoundly influences neutrophil function by enhancing their migratory capacity and survival.

Cells expressing hematopoietic PGD_2 synthase are abundant in lungs of patients with ARDS

We finally examined whether levels of HPGDS, the rate-limiting enzyme responsible for the production of PGD_2, differed in patients with ARDS and control subjects. Indeed, immunohistochemistry revealed that, compared with control lung samples (Fig 6, *A*), there was a clear increase in the number of cells highly expressing HPGDS in patients with ARDS (Fig 6, *B*). Moreover, although 50% to 60% of macrophages, as identified by means of morphology, were positive for HPGDS, this value increased to 85% to 95% in lung sections from patients with ARDS.

Discussion

In this study we propose a novel role for PGD_2 and its corresponding receptors in pulmonary inflammation. Here, for the first time, we show that human macrophages express active DP_1 and DP_2, which are associated with alterations in cytokine profiles and migratory responses, factors that contribute greatly to inflammation in pulmonary diseases. First, we confirmed basal expression of PGD_2 receptors on monocytes,29 but we also showed an upregulation that was more pronounced in the expression of DP_2 than DP_1 during differentiation to macrophages. Furthermore, we were able to demonstrate that both PGD_2 receptors are functional on human macrophages: not only did macrophages themselves migrate toward PGD_2, but the PG also stimulated the interaction of macrophages with neutrophils, thereby supporting neutrophil migration and survival. This finding might be explained by the increased production of cytokines after PGD_2 stimulation of macrophages prompted by DP_1/DP_2-mediated Ca^{2+} flux.

Previous reports suggested that DP_1- and DP_2-mediated actions oppose each other, being anti-inflammatory and proinflammatory, respectively.10,40 Such divergent actions of PGD_2 have not been observed here because we could show that on human MDMs, selective activation of both receptors increases intracellular free Ca^{2+} levels and induces migration. Furthermore, these responses were only partially inhibited by either selective antagonist, and only blockade of DP_1 along with DP_2 was sufficient to abolish the PGD_2-induced activation of macrophages. In this respect PGD_2 receptors on macrophages differed from other cell types, such as eosinophils, where these receptors engage in a crosstalk and both are needed for the complete functional response,41 and bronchial epithelial cells, which express DP_2 but not DP_1.11

We also observed DP_1 and DP_2 expression on human macrophages in the lung, both in the healthy state and in several pathologic conditions, from organizing pneumonia to diffuse alveolar damage. Moreover, it was mainly the macrophages themselves that were positive for the PGD_2-synthesizing enzyme HPGDS in the lungs of patients with ARDS. Although upregulation of HPGDS has up to now mostly been described as a consequence of increased numbers of epithelial and submucosal mast cells,42 our finding proposes a new important role for PGD_2 in the regulation of macrophages in the lung.

Next, we investigated the biological role of PGD_2 in lung pathophysiology *in vivo* and used a murine, LPS-induced lung injury model that relies largely on functional macrophages.

22,23 As predicted by our *in vitro* data, systemic application of PGD_2, acting through both DP_1 and DP_2, aggravated LPS-induced lung pathology in several ways by (1) increasing neutrophil influx into the airways, (2) promoting MPO activity in lung tissue, (3) increasing cytokine levels, and (4) impairing lung function. Thus PGD_2 enhances neutrophilic inflammatory responses in the presence of disease-activating triggers. In parallel with increased levels of PGD_2 in the BAL fluid of LPS-treated animals, TXB_2 levels also increased. This upregulation of the TX pathway can further substantiate our finding of DP_2 activation in pulmonary inflammation because we previously identified the TX metabolite 11-dehydro-TXB_2 as a full agonist for DP_2.43 Finally, we provided evidence that these PGD_2 responses essentially depended on macrophages because murine alveolar macrophages were a rich source of the neutrophil chemoattractant KC and macrophage depletion reversed the ability of PGD_2 to enhance lung inflammation. Two types of macrophages can be found in the lungs: alveolar and interstitial macrophages. Both are long-lived resident cells that orchestrate tissue homeostasis and can react rapidly to endogenous and exogenous stimuli, thus forming a first line of defense,22 and both seemed to respond to PGD_2 when KC secretion was analyzed. Human cell studies, which we conducted in parallel, identified 2 novel mechanisms of how PGD_2 receptor activation on macrophages can regulate neutrophil function, namely enhanced migratory capacity and survival of the latter. A recent study showed that activated T_H2 cells in response to PGD_2 treatment react with secretion of IL-6 and GM-CSF at levels that are able to modulate neutrophil functions. 44 Our data strongly suggest that this mechanism is not restricted to T_H2 cells but also applies to macrophages. One could speculate that type 2 activated macrophages would be even more susceptible in this respect.

Finally, using the same mouse model, we could also demonstrate a clear proinflammatory role of endogenous PGD_2. Blocking of endogenous PGD_2 either by DP_1- or DP_2-specific antagonists ameliorated the inflammatory response, although in a differential manner. On the one hand, the DP_1-specific antagonist MK0524 decreased neutrophilia in the bronchoalveolar space. On the other hand, the DP_2-specific antagonist CAY10471 markedly reduced MPO activity measured in lung tissue. One reason for that discrepancy might be that here we are looking at 2 different lung compartments. Neutrophils migrate from the blood through the interstitium into the alveolar space, and the 2 receptors might play different roles in this process, such as DP_2 regulating neutrophil recruitment from the blood to the tissue and DP_1 promoting alveolar evasion of neutrophils. Along with the increase in PGD_2 levels in the BAL fluid, these observations clearly highlight endogenous PGD_2 in the development of experimental pulmonary inflammation.

Recently, it was shown that mice lacking the DP_1 receptor display aggravated neutrophil influx and increased mortality in experimental ALI.45 Although this study seemingly contradicts our results, several experimental details make a direct comparison difficult. First, the authors used almost 4 times higher doses of LPS and observed the ensuing effects for 3 days. Second, DP_1 was knocked out unconditionally, so that mice might have experienced compensational mechanisms, such as an upregulation of DP_2 receptors, thereby driving inflammation. A significant advantage of our study is the use of pharmacologic approaches, which lends our findings clear translational potential toward clinical application.

Collectively, we propose that the PGD_2-DP_1-DP_2 axis on macrophages is of pivotal importance in regulating inflammatory responses, and thus also in tissue damage, by triggering and maintaining a proinflammatory environment. Given the involvement of both PGD_2 receptors in macrophage regulation, recently developed dual DP_1/DP_2 antagonists46 appear to be a promising approach to treating distinct inflammatory diseases that involve macrophage activation and neutrophil accumulation in the lung.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
BAL	Bronchoalveolar lavage
DK-PGD_2	13,14-Dihydro-15-keto prostaglandin D_2
DP	D-type prostanoid receptor
HPGDS	Hematopoietic prostaglandin D synthase
KC	Keratinocyte-derived chemokine
MDM	Monocyte-derived macrophages
MPO	Myeloperoxidase
PG	Prostaglandin
TX	Thromboxane

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Key messages

- DP_1 and DP_2 are expressed and functionally active on isolated MDMs and pulmonary macrophages.
- PGD_2 receptor activation of macrophages promotes their interaction with neutrophils and enhances neutrophil function.
- The presence of hematopoietic PGD synthase, DP_1, and DP_2 in macrophages highlights a novel role for PGD_2 in pulmonary inflammation.

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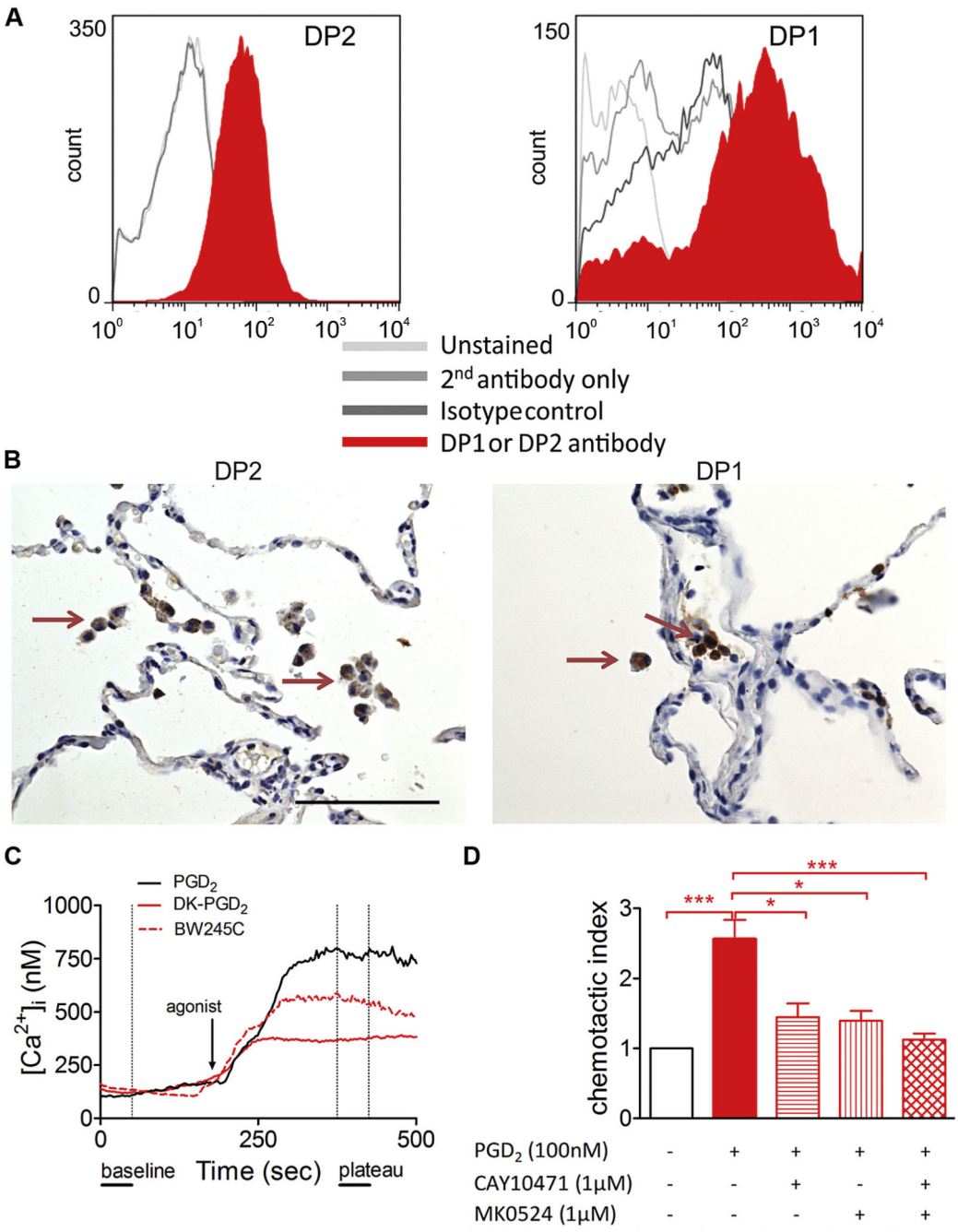

Fig 1.

PGD_2 receptors DP_1 and DP_2 are expressed on macrophages and induce Ca^{2+} flux and migration. **A,** Flow cytometric histograms of DP_2 and DP_1 staining *(filled histograms)* on MDMs, respectively. **B,** Immunohistochemistry of healthy human lung tissue showing DP_2- and DP_1-positive alveolar macrophages *(arrows)*. **C,** Representative Ca^{2+} responses of MDMs over time. **D,** MDM migration toward PGD_2 is blocked by DP_1- and DP_2-specific antagonists (n = 4-5). **P* < .05 and ****P* < .001.

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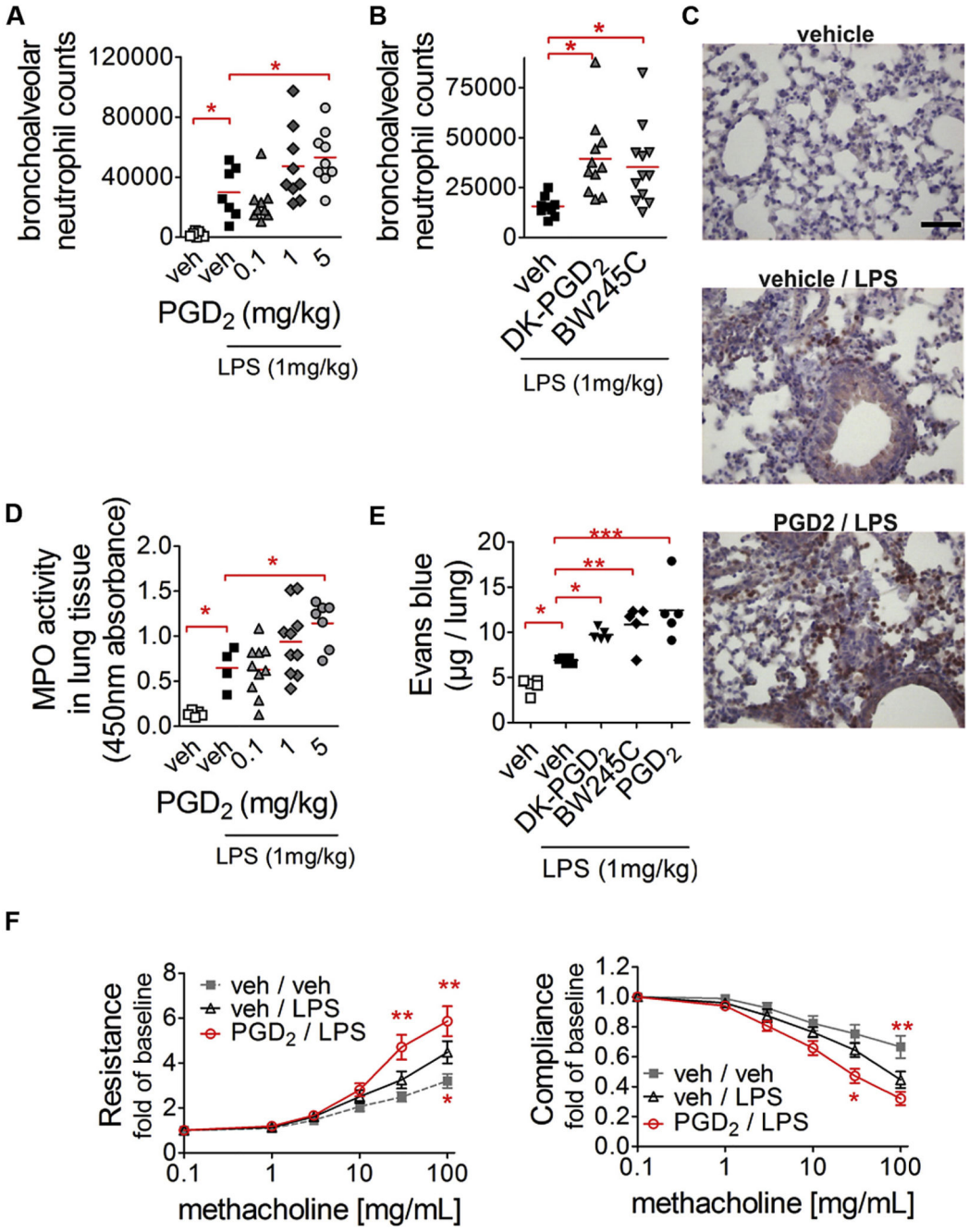

Fig 2.

PGD_2 acting through DP_1 and DP_2 promotes neutrophil influx into lungs and aggravates airway hyperreactivity. **A** and **B**, Neutrophil infiltration is increased in animals pretreated with PGD_2 (Fig 2, *A*) or DP_1- and DP_2-selective agonists (Fig 2, *B*). **C**-**F**, PGD_2 pretreatment increases Ly6G-positive neutrophil infiltration in the peribronchial and alveolar space (representative pictures, *scale bar* = 50µm; Fig 2, *C*), MPO activity (Fig 2, *D*), Evans blue dye extravasation (Fig 2, *E*), and airway hyperreactivity (vs vehicle/LPS; Fig 2, *F*). $*P < .05$, $**P < .01$, and $***P < .001$.

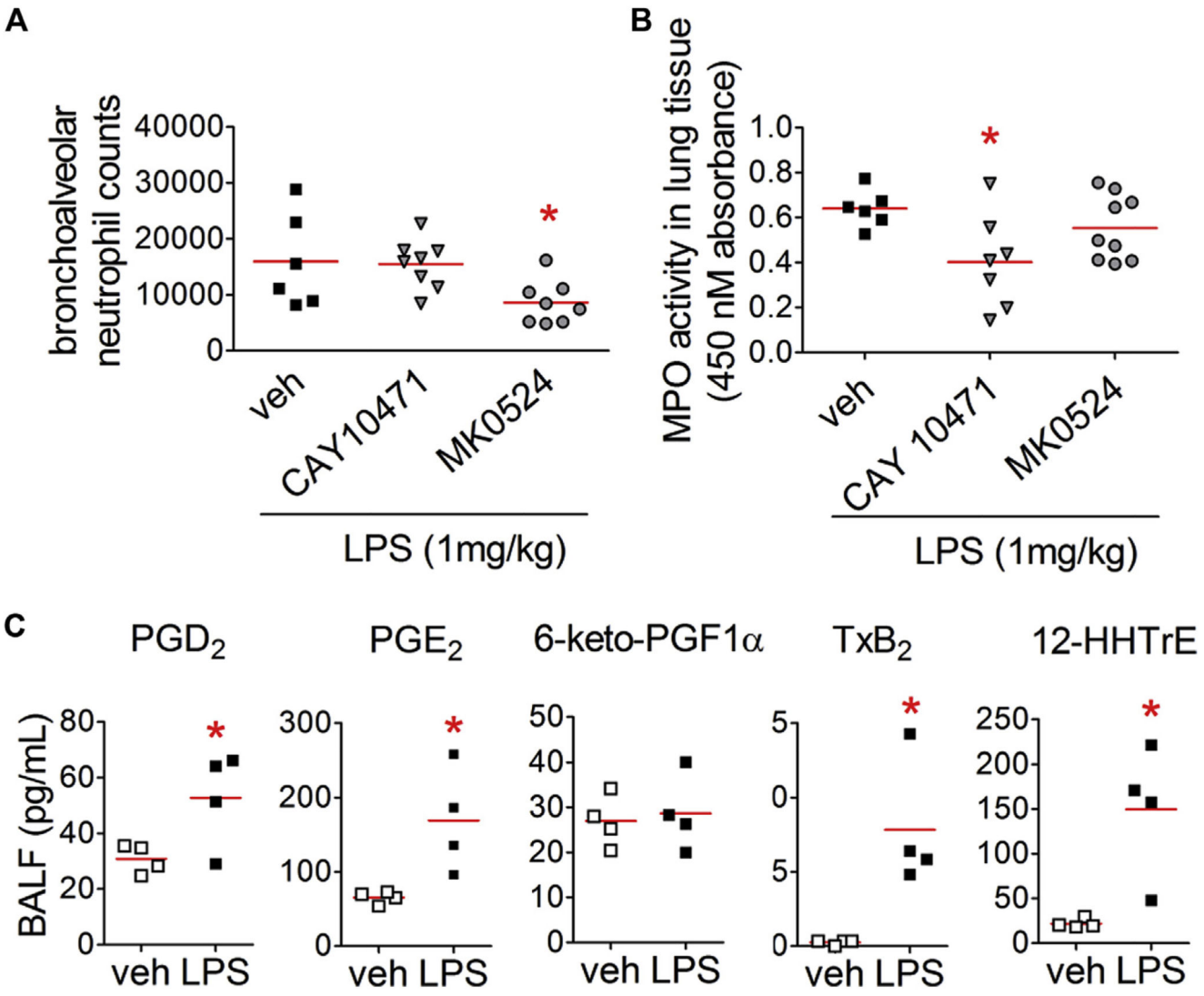

Fig 3.

Blocking of endogenous PGD_2 ameliorates LPS-induced neutrophil influx into the alveolar space and pulmonary tissue. **A** and **B,** DP_1 antagonist reduced neutrophil counts in the bronchoalveolar space (Fig 3, *A*), whereas DP_2 antagonist reduced MPO activity (Fig 3, *B*; n = 6-9). **P* < .05 versus vehicle. **C,** Lipid mediators in BAL fluid 4 hours after vehicle or LPS treatment were quantified by using liquid chromatography– tandem mass spectrometry (n = 4). *HHTrE*, 12S-hydroxy-5Z,8E,10E-heptoadectrienoic acid. **P* < .05 versus vehicle.

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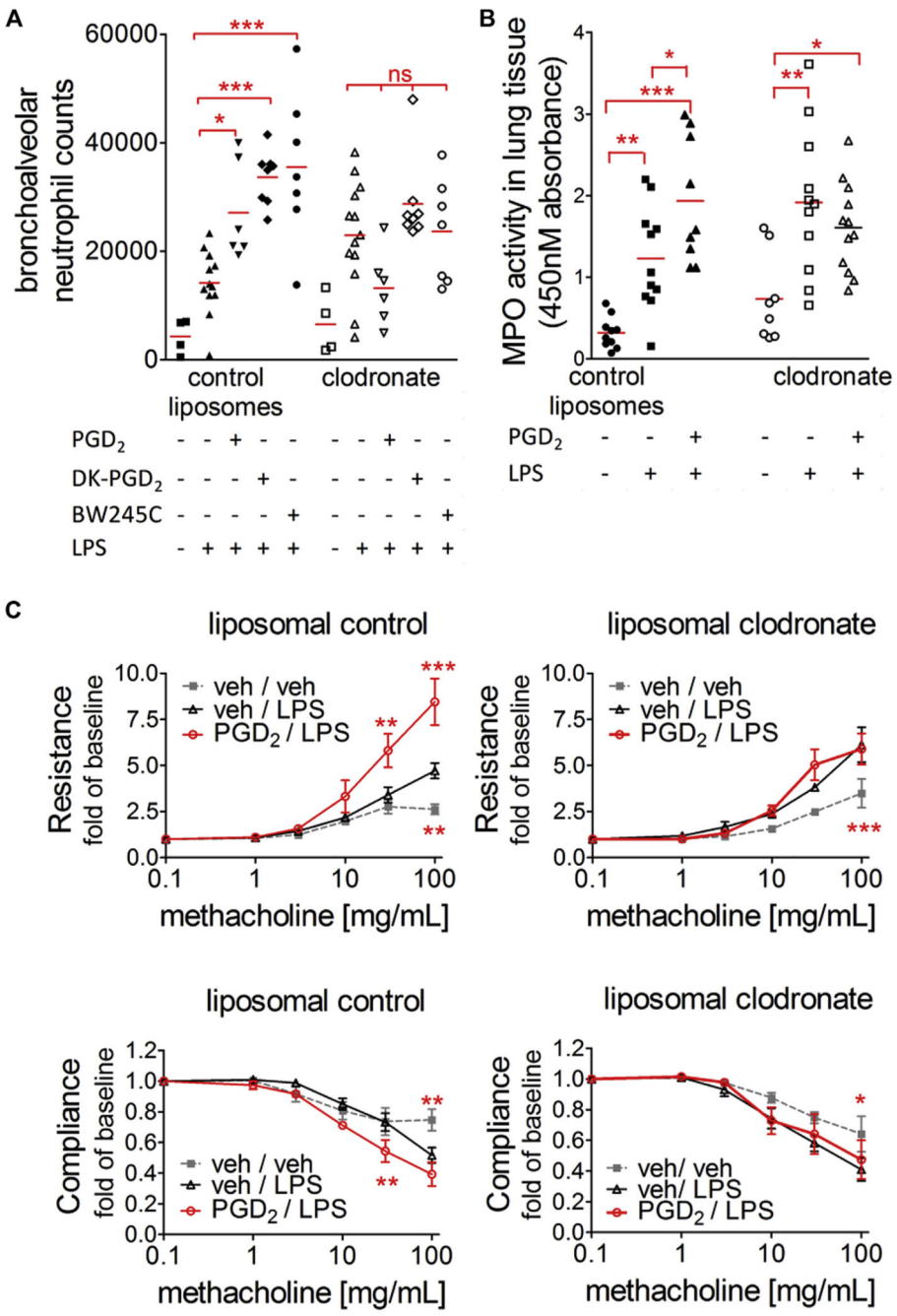

Fig 4.

Macrophage depletion prevents the proinflammatory effect of PGD_2 on neutrophil recruitment. **A,** Neutrophil numbers in BAL fluid. **B,** MPO activity in lung tissue. $*P < .05$, $**P < .01$, and $***P < .001$. **C,** Macrophage depletion prevents reduction in lung function induced by PGD_2 (n = 6-8). $*P < .05$, $**P < .01$, and $***P < .001$ versus vehicle/LPS.

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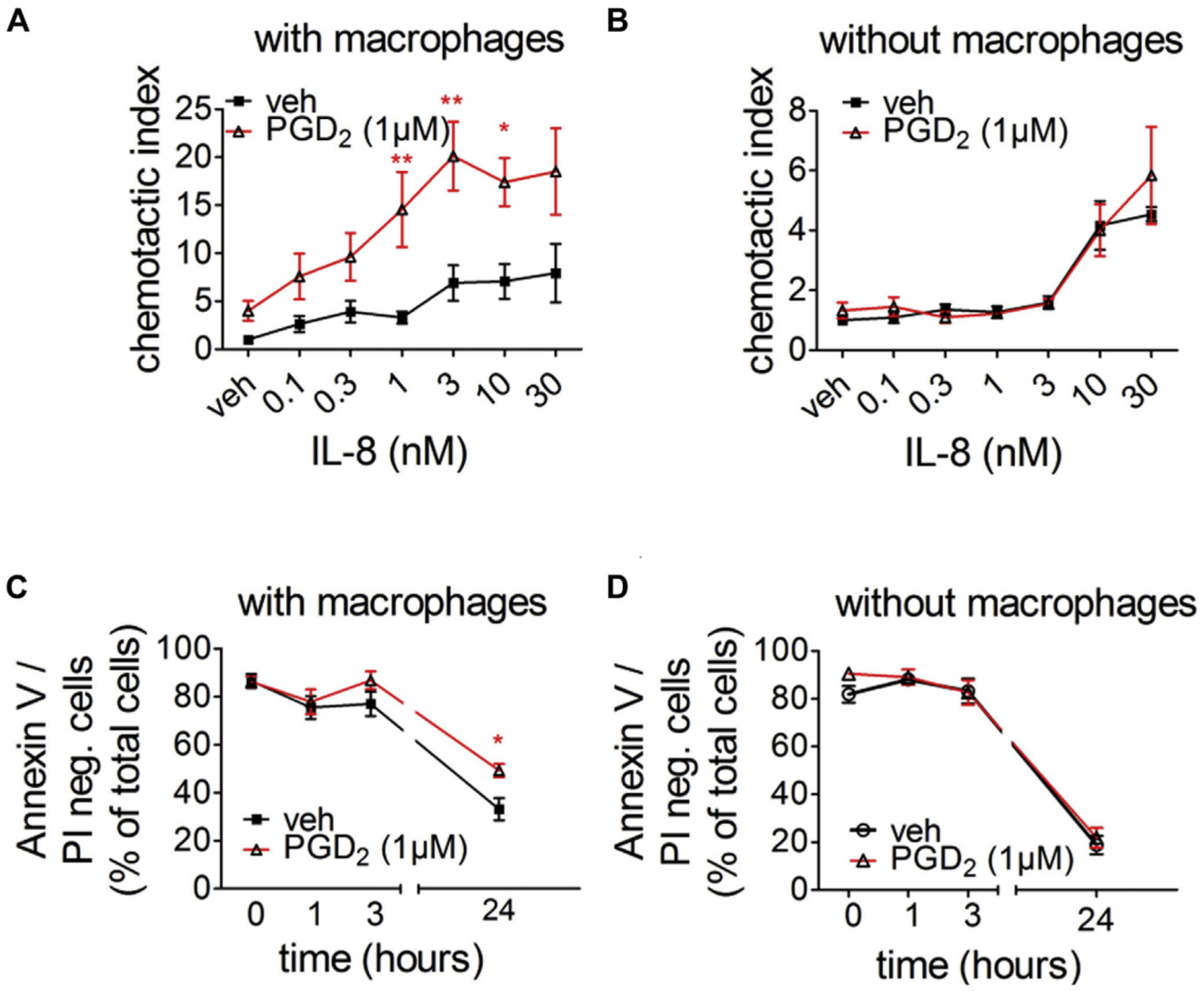

Fig 5.

PGD_2 receptor activation on MDMs increases neutrophil migration toward IL-8 (**A** and **B**) and prolongs neutrophil survival (**C** and **D**) *in vitro*. Fig 5, *A*, *C*, and *D*: n = 5-10; Fig 5, *B*: n = 3-5. **P* < .05 and ***P* < .01 versus vehicle. *PI*, Propidium iodide.

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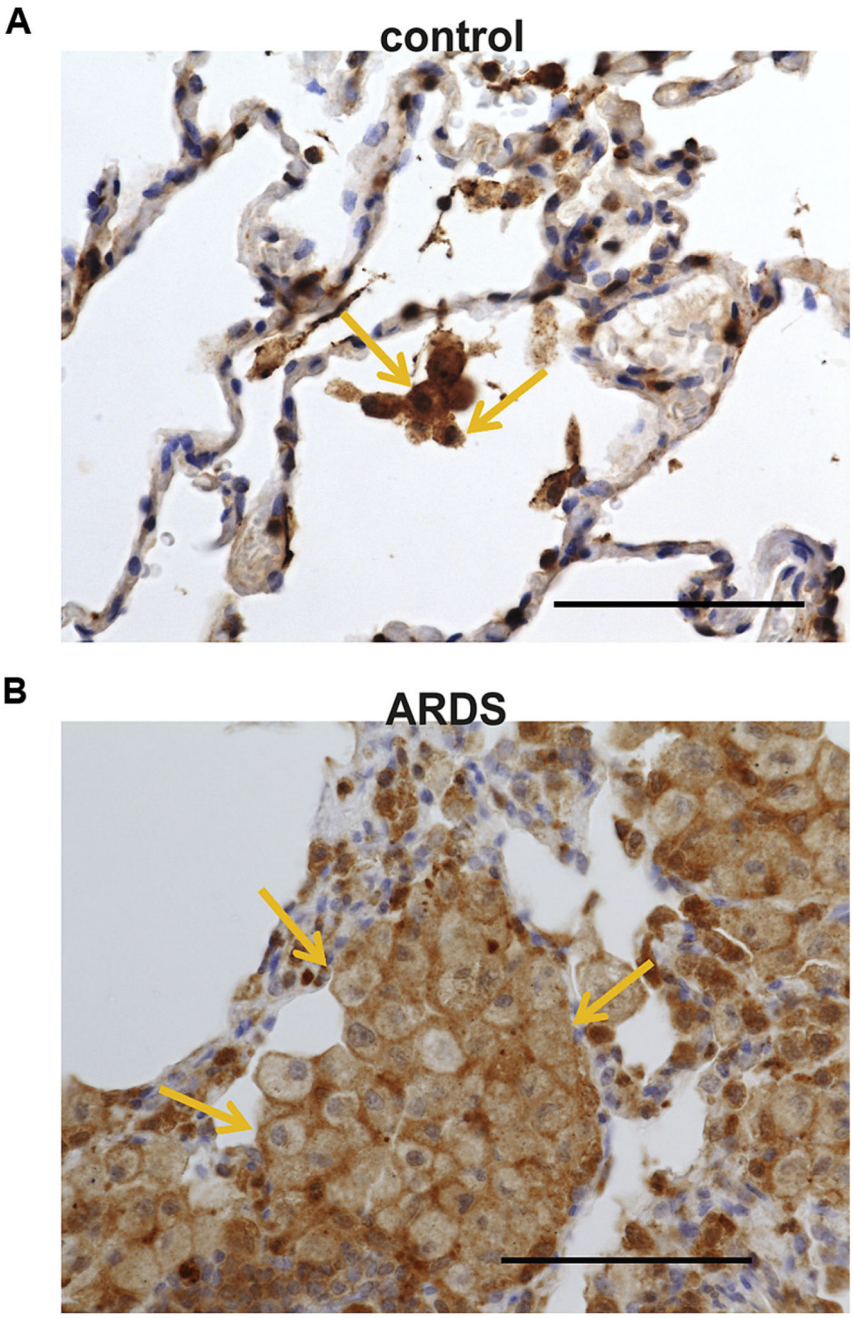

Fig 6.

Increased numbers of HPGDS-expressing cells in lungs of patients with ARDS. Representative immunohistochemical staining of human lung samples showing positive cells for HPGDS (brown) in a control subject **(A)** and a patient with ARDS **(B)**. Stainings are representative pictures of 5 patients and control subjects. Note the high amount of HPGDS found in alveolar macrophages. *Bar* = 100 μm.