Activated prostaglandin D₂ receptors on macrophages enhance neutrophil recruitment into the lung

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Background: Prostaglandin (PG) D₂ is an early-phase mediator in inflammation, but its action and the roles of the 2 D-type prostanooid receptors (DPs) DP₁ and DP₂ (also called chemoattractant receptor–homologous molecule expressed on T₄₂ cells) in regulating macrophages have not been elucidated to date.

Objective: We investigated the role of PGD₂ receptors on 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, Ca²⁺ 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 DP₁, DP₂, or both receptors on human macrophages induced strong intracellular Ca²⁺ flux, cytokine release, and migration of macrophages. In a murine model of LPS-induced pulmonary inflammation, activation of each PGD₂ receptor resulted in aggravated airway neutrophilia, tissue myeloperoxidase activity, cytokine contents, and decreased lung compliance. Selective depletion of alveolar macrophages abolished the PGD₂-enhanced inflammatory response. Activation of PGD₂ receptors on human macrophages enhanced the migratory capacity and prolonged the survival of neutrophils in vitro. In human lung tissue specimens both DP₁ and DP₂ receptors were located on alveolar macrophages along with hematopoietic PGD synthase, the rate-limiting enzyme of PGD₂ synthesis.

Conclusion: For the first time, our results show that PGD₂ markedly augments disease activity through its ability to enhance the proinflammatory actions of macrophages and subsequent neutrophil activation. (J Allergy Clin Immunol 2016;137:833-43.)

Key words: D-type prostanooid receptor 1, D-type prostanooid receptor 2/chemoattractant receptor–homologous molecule expressed on T₄₂ cells, prostaglandin D₂, hematopoietic prostaglandin D synthase, macrophages, pulmonary inflammation, neutrophils

Prostaglandin (PG) D₂, 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. PGD₂ activates 2 G protein–coupled receptors, the D-type prostanooid receptors (DPs) DP₁ and DP₂, with the latter also known as chemoattractant receptor–homologous molecule expressed on T₄₂ cells. At higher concentrations, PGD₂ can also signal through the thromboxane A₂ receptor. Although PGD₂ exerts similar binding affinities toward the DP₁ and DP₂ receptors, its metabolites, formed rapidly by enzymatic and nonenzymatic pathways, can differentially induce DP₂-mediated effects.

In patients with allergic diseases, the role of PGD₂ has mostly been associated with its release from activated mast cells and induction of vasodilation. More recently, however, PGD₂ has been found to promote additional proinflammatory responses through activation of DP₂ receptors reflected by increased eosinophilic infiltration into the lungs and skin of mice. Consequently, DP₂ antagonists were shown to ameliorate eosinophilic pulmonary inflammation in murine ovalbumin-induced and house dust mite–induced models, a rat Alternaria species–induced model, and the setting of chronic allergic skin inflammation. Moreover, the DP₂ antagonist CAY10471 ameliorated weight
loss and intestinal inflammation in a dextran sodium sulfate–induced colitis model in mice. In asthmatic patients induction of hematopoietic prostaglandin D synthase (HPGDS), the rate-limiting enzyme of PGD2 synthesis, was observed in the epithelial compartment, and PGD2 levels in bronchoalveolar lavage (BAL) fluid correlated positively with the severity of the disease. DP receptors, homologous molecule expressed on TH2 cells, antagonists was found to have some effects in allergic rhinitis, allergic conjunctivitis, eosinophilic esophagitis, and bronchial asthma.

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. 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, subsequent edema formation, and dysfunction of the involved cell types, including epithelial cells, endothelial cells, and macrophages. Several studies have made it clear that macrophages orchestrate neutrophil infiltration and thus strongly modulate the outcome of ARDS.

Stimulated by the dominant physiologic role of PGD2 in the lung, we hypothesized that PGD2 might govern disease activity and progression by acting on lung macrophages. Previous studies revealed anti-inflammatory effects of the PGD2 metabolite 15d-PGJ2 acting through peroxisome proliferator–activated receptor γ in RAW 264.7 macrophages and demonstrated the expression of PGD2 receptors on human monocytes, whereas the role of PGD2 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.

### Differentiation from monocytes to macrophages

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

### Live cell fluorescent Ca2+ 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.

### Monocyte Ca2+ flux

Ca2+ flux was measured by means of flow cytometry, as previously described.

### Neutrophil apoptosis

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

### 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. Migration of human MDMs was assessed by using Transwell inserts, as described previously.

### Flow cytometric staining

The following antibodies and concentrations were used: DP2 (20 μg/mL), DP1 (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.

### 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.

### Abbreviations used

- ALI: Acute lung injury
- ARDS: Acute respiratory distress syndrome
- BAL: Bronchoalveolar lavage
- DK-PGD2: 13,14-Dihydro-15-keto prostaglandin D2
- DP: D-type prostanooid receptor
- HPGDS: Hematopoietic prostaglandin D synthase
- KC: Keratinocyte-derived chemokine
- MDM: Monocyte-derived macrophages
- MPO: Myeloperoxidase
- PG: Prostaglandin
- PX: Thromboxane
- RANTES: Regulatory T cell–attracting chemokine
- TH2: T helper 2 cell
- TLR: Toll-like receptor
- TNF: Tumor necrosis factor
- PGD2: Prostaglandin D2
- TGF-β: Transforming growth factor-β
- TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand
- TX: Thromboxane
- VEGF: Vascular endothelial growth factor
- VEGFR-2: Vascular endothelial growth factor receptor 2
- VLA-4: Very late antigen 4
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-DP2 (1:200), anti-DP1 (1:100), or anti-HPGDS (1:200) antibodies.

Lipid mediator analysis

PGD₂, PGE₂, TXB₂, 12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid, and 6-keto-PGF₁α 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. Therefore mouse lung function was determined by using the flexiVent system (SCIREQ, Montreal, Quebec, Canada), as described previously. 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

DP1 and DP2 are expressed on human macrophages and induce Ca2+ signaling

We first set out to elucidate whether PGD2 receptors are expressed on human monocytes and macrophages and found that...
both cell types express DP1 and DP2 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 DP2 expression being more abundant than DP1 expression in both monocytes and macrophages (see Fig E1). Next, we probed for DP1 and DP2 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 DP1 and DP2 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 PGD2-DP1-DP2 axis in the modulation of macrophage biology in patients with pulmonary diseases.

Then we sought to determine the biological activities of DP1 and DP2 in vitro. Ca2+ imaging revealed that superfusion of human MDMs with 1 μmol/L PGD2 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 Ca2+ response to PGD2 stimulation (see Fig E5 in this article’s Online Repository at www.jacionline.org). Both DP2- and DP1-selective agonists (13,14-dihydro-15-keto PGD2, and BW245C, respectively; 1 μmol/L) were likewise able to elicit Ca2+ flux, although to a lesser extent than PGD2 (Fig 1, C, and see Fig E4, A). In agreement with this observation, blockade of either DP2 (CAY10471, 10 μmol/L) or DP1 (MK0524, 10 μmol/L) slightly decreased, whereas simultaneous antagonism of both receptors abolished the PGD2-induced Ca2+ signal (see Fig E4, B and C). Ca2+ release induced by both PGD2 and 13,14-dihydro-15-keto prostaglandin D2 (DK-PGD2) was prevented by overnight incubation with pertussis toxin (100 ng/mL), indicating involvement of Goi heterotrimers in this process (see Fig E4, D and E).

**PGD2 induces migration of macrophages through DP1 and DP2 and enhances TNF-α secretion**

We next evaluated the chemotactic potential of PGD2 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 PGD2 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 DP2- or DP1-specific antagonist (CAY10471 or MK0524, 1 μmol/L, respectively; Fig 1, D). Inhibition of both receptors completely abolished the migratory
activity of MDMs toward PGD₂ (Fig 1, D). Because PGD₂ was shown to be critical in mediating macrophage migration toward LPS in a murine model,⁴⁹ we hypothesized that PGD₂ 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 PGD₂ or DK-PGD₂ (see Fig E6, C). Although the DP₁-specific agonist BW245C was not able to induce changes in TNF-α secretion, involvement of both DP₁ and DP₂ receptors in the PGD₂ response is still likely because only antagonism of both

**FIG 4.** Macrophage depletion prevents the proinflammatory effect of PGD₂ 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 PGD₂ (n = 6–8). *P < .05, **P < .01, and ***P < .001 versus vehicle/LPS.
receptors was able to completely block the PGD₂-mediated increase in TNF-α secretion.

Endotoxin-induced lung injury is aggravated by PGD₂

Prompted by these observations, we investigated the effect of PGD₂ in an in vivo model of pulmonary inflammation. Here neutrophil infiltration is a main pathogenic feature that essentially depends on macrophage function. LPS significantly increased neutrophil influx into the bronchoalveolar space after 4 hours of treatment. Of particular interest, this effect was almost doubled when PGD₂ (5 mg/kg) was administered subcutaneously before LPS challenge (Fig 2, A). Selective DP₁ and DP₂ agonists induced an even more pronounced response; in detail, the selective DP₂ agonist DK-PGD₂ (5 mg/kg) enhanced neutrophil influx by 2.5-fold and the DP₁ 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 PGD₂ 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). The observation that PGD₂ 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 PGD₂ and LPS (Fig 2, F). In all experimental readouts, including cell infiltration into the alveolar space, MPO activity, and lung function, PGD₂ treatment in the absence of LPS had no effect (data not shown).

Blocking of endogenous PGD₂ reduces neutrophilic infiltration into the lungs

Mice were pretreated with the PGD₂ receptor antagonists MK0524 or CAY10471 (5 mg/kg) followed by LPS challenge to delineate the pathogenic role of endogenous PGD₂. Analysis of BAL cells revealed that the DP₁ antagonist MK0524 reduced endotoxin-induced neutrophilia (Fig 3, A), whereas the DP₂ antagonist CAY10471 reduced MPO activity (Fig 3, B). Next,
through its metabolite 6-keto-PGF1α. PGD2 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 PGD2-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 PGD2 (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 interstitial macrophages was influenced by neither LPS nor PGD2 treatment (see Fig E9, B and C).

**Macrophage depletion prevents the increased inflammatory response mediated by PGD2**

To highlight the role of macrophages in the PGD2-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 PGD2 or any of the specific agonists for DP1 and DP2. 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 PGD2-, DK-PGD2−, 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 PGD2-induced increase in MPO activity, as well as compromised lung function, were reversed by alveolar macrophage reduction (Fig 4, B and C).

**PGD2 receptor activation on macrophages modulates neutrophil function**

*In vitro* neutrophil migration assays were performed in the presence of human MDMs treated with vehicle or PGD2 to further clarify the mechanisms through which PGD2 receptor activation on macrophages augmented the LPS-induced neutrophil recruitment. In this coculture experiment, PGD2-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 PGD2 treatment of MDMs enhanced the basal migratory capacity and IL-8-induced migration of neutrophils alike. Importantly, neutrophil migration was unaffected by PGD2 when macrophages were absent (Fig 5, B). Therefore PGD2 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 PGD2 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 PGD2 (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 PGD₂ and showed the same survival rate as in the presence of vehicle (Fig 5, D). These data show that PGD₂ receptor activation on macrophages profoundly influences neutrophil function by enhancing their migratory capacity and survival.

Cells expressing hematopoietic PGD₂ 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 PGD₂, 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 PGD₂ and its corresponding receptors in pulmonary inflammation. Here, for the first time, we show that human macrophages express active DP₁ and DP₂, 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 PGD₂ receptors on monocytes, but we also showed an upregulation that was more pronounced in the expression of DP₂ than DP₁ during differentiation to macrophages. Furthermore, we were able to demonstrate that both PGD₂ receptors are functional on human macrophages: not only did macrophages themselves migrate toward PGD₂, 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 PGD₂ stimulation of macrophages prompted by DP₁/DP₂-mediated Ca²⁺ flux.

Previous reports suggested that DP₁- and DP₂-mediated actions oppose each other, being anti-inflammatory and proinflammatory, respectively. Such divergent actions of PGD₂ have not been observed here because we could show that on human MDMs, selective activation of both receptors increases intracellular free Ca²⁺ levels and induces migration. Furthermore, these responses were only partially inhibited by either selective antagonist, and only blockade of DP₁ along with DP₂ was sufficient to abolish the PGD₂-induced activation of macrophages. In this respect PGD₂ 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, and bronchial epithelial cells, which express DP₂ but not DP₁.

We also observed DP₁ and DP₂ 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 PGD₂-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, our finding proposes a new important role for PGD₂ in the regulation of macrophages in the lung.

Next, we investigated the biological role of PGD₂ in lung pathophysiology in vivo and used a murine, LPS-induced lung injury model that relies largely on functional macrophages. As predicted by our in vitro data, systemic application of PGD₂, acting through both DP₁ and DP₂, 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 PGD₂ enhances neutrophilic inflammatory responses in the presence of disease-activating triggers. In parallel with increased levels of PGD₂ in the BAL fluid of LPS-treated animals, TXB₂ levels also increased. This upregulation of the TX pathway can further substantiate our finding of DP₂ activation in pulmonary inflammation because we previously identified the TX metabolite 11-dehydro-TXB₂ as a full agonist for DP₂. Finally, we provided evidence that these PGD₂ 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 PGD₂ 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, and both seemed to respond to PGD₂ when KC secretion was analyzed. Human cell studies, which we conducted in parallel, identified 2 novel mechanisms of how PGD₂ receptor activation on macrophages can regulate neutrophil function, namely enhanced migratory capacity and survival of the latter. A recent study showed that activated Th2 cells in response to PGD₂ treatment react with secretion of IL-6 and GM-CSF at levels that are able to modulate neutrophil functions. Our data strongly suggest that this mechanism is not restricted to Th2 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 PGD₂. Blocking of endogenous PGD₂ either by DP₁- or DP₂-specific antagonists ameliorated the inflammatory response, although in a different manner. On the one hand, the DP₁-specific antagonist MK0524 decreased neutrophilia in the bronchoalveolar space. On the other hand, the DP₂-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 DP₂ regulating neutrophil recruitment from the blood to the tissue and DP₁ promoting alveolar evasion of neutrophils. Along with the increase in PGD₂ levels in the BAL fluid, these observations clearly highlight endogenous PGD₂ in the development of experimental pulmonary inflammation.

Recently, it was shown that mice lacking the DP₁ receptor display aggravated neutrophil influx and increased mortality in experimental ALI. 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, DP₁ was knocked out unconditionally, so that mice might have experienced compensational mechanisms, such as an upregulation of
DP2 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 PGD2-DP1-DP2 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 PGD2 receptors in macrophage regulation, recently developed dual DP1/DP2 antagonists appear to be a promising approach to treating distinct inflammatory diseases that involve macrophage activation and neutrophil accumulation in the lung.

Key messages
- DP1 and DP2 are expressed and functionally active on isolated MDMs and pulmonary macrophages.
- PGD2 receptor activation of macrophages promotes their interaction with neutrophils and enhances neutrophil function.
- The presence of hematopoietic PGD synthase, DP1, and DP2 in macrophages highlights a novel role for PGD2 in pulmonary inflammation.

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