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Efficacy and Pharmacology of the NLRP3 Inflammasome Inhibitor CP-456,773 (CRID3) in Murine Models of Dermal and Pulmonary Inflammation

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A critical component of innate immune response to infection and tissue damage is the NACHT, LRR, and PYD domains–containing protein 3 (NLRP3) inflammasome, and this pathway and its activation products have been implicated in the pathophysiology of a variety of diseases. NLRP3 inflammasome activation leads to the cleavage of pro–IL-1β and pro–IL-18, as well as the subsequent release of biologically active IL-1β, IL-18, and other soluble mediators of inflammation. In this study, we further define the pharmacology of the previously reported NLRP3 inflammasome–selective, IL-1β processing inhibitor CP-456,773 (also known as MCC950), and we demonstrate its efficacy in two in vivo models of inflammation. Specifically, we show that in human and mouse innate immune cells CP-456,773 is an inhibitor of the cellular release of IL-1β, IL-1α, and IL-18, that CP-456,773 prevents inflammasome activation induced by disease-relevant soluble and crystalline NLRP3 stimuli, and that CP-456,773 inhibits R848- and imiquimod-induced IL-1β release. In mice, CP-456,773 demonstrates potent inhibition of the release of proinflammatory cytokines following acute i.p. challenge with LPS plus ATP in a manner that is proportional to the free/unbound concentrations of the drug, thereby establishing an in vivo pharmacokinetic/pharmacodynamic model for CP-456,773. Furthermore, CP-456,773 reduces ear swelling in an imiquimod cream–induced mouse model of skin inflammation, and it reduces airway cytokines following acute i.p. challenge with house dust mite extract. These data implicate the NLRP3 inflammasome in the pathogenesis of dermal and airway inflammation, and they highlight the utility of CP-456,773 for interrogating the contribution of the NLRP3 inflammasome and its outputs in preclinical models of inflammation and disease. The Journal of Immunology, 2016, 197: 2421–2433.

The intracellular Nod-like receptor (NLR) family of proteins includes important immune sensors of microbial infection and cellular injury, and activation of a subset of NLR family members induces the formation of multimeric protein complexes known as inflammasomes (1). Upon activation, the NLR

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Abbreviations used in this article: ASC, apoptosis-associated speck-like protein containing a CARD; BAL, bronchoalveolar lavage; BMDM, bone marrow–derived macrophage; CAPS, cryopyrin-associated periodic syndrome; CRID, cytokine release inhibitory drug; DAMP, damage-associated molecular pattern; Dex, dexamethasone; HDM, house dust mite; Kir6.2, inwardly rectifying K+ channel 6.2; MSU, monosodium urate; NLR, Nod-like receptor; NLRP3, NACHT, LRR, and PYD domains–containing protein 3; qPCR, quantitative real-time PCR; siRNA, small interfering RNA; SUR, sulfonylurea receptor.

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Amelioration of disease sequelae via inhibition or deficiency of NLRP3 inflammasome components or products has been demonstrated in a wide range of preclinical models, including models of autoimmune inflammatory disease, pulmonary disease, renal disease, neurodegenerative disease, and metabolic disease (4, 8, 9, 12–15). The contribution of the NLRP3 inflammasome to human disease is evidenced by the pathology observed in patients with cryopyrin-associated periodic syndrome (CAPS). These individuals have gain-of-function mutations in NLRP3 resulting in hyperactivation of the NLRP3 inflammasome and a spectrum of autoinflammatory pathologies, including recurrent fevers, severe arthralgias, cutaneous lesions, and aseptic meningitis (16–18). Fortunately, IL-1β neutralizing agents have proven largely effective for these patients (17, 19).

Considering the evidence that excessive activation of the NLRP3 inflammasome and release of its outputs can contribute to disease, a means to pharmacologically inhibit the NLRP3 inflammasome might provide benefit in certain diseases. Putative inhibitors of the NLRP3 inflammasome pathway have been described, including glyburide (glibenclamide), Bay 11-7082, β-hydroxybutyrate, and parthenolide (3, 4, 20). In 2001, Perregaux et al. (21) described a class of diarylsulfonyleurea compounds with structural similarities to glyburide that are capable of inhibiting LPS plus ATP–induced processing and release of IL-1β. These authors named this series of compounds cytokine release inhibitory drugs (CRIDs). Subsequent publications provided further insight into the pharmacological activities of CRID compounds, and it was demonstrated that the molecule CP-456,773 (CAS 210826-40-7, sodium salt CAS 256373-96-3), which was originally termed CRID3 and later renamed MCC0950 by Coll et al. (22), prevents IL-1β processing and release by specifically inhibiting NLRP3 inflammasome activation in both innate immune cells and in vivo disease models (23, 24).

In this study, we describe CP-456,773 as a potent and selective inhibitor of NLRP3 inflammasome–dependent release of IL-1β, IL-1α, and IL-18 from innate immune cells. Additionally, using CP-456,773 we have interrogated the role of the NLRP3 inflammasome and its products in mediating inflammation in mouse models of peritonitis, imiquimod cream–induced skin inflammation, and human monocyte–derived macrophages (BMDMs) and human macrophages. Cells were incubated with various concentrations of test compound or vehicle (DMSO) for 30 min prior to addition of the NLRP3 activators. Mouse BMDMs were stimulated with 10 μg/ml nigericin or 5 mM ATP for 1 h. Human monocyte–derived macrophages were stimulated with 10 or 30 μg/ml nigericin, or 5 or 7.5 mM ATP for 1 h, or with 150 μg/ml MSU crystals or 350 μg/ml cholesterol crystals for 4 h. For measuring lactate dehydrogenase (LDH) release as an indicator of cell death, human monocyte–derived macrophages were stimulated with 10 μg/ml nigericin for 2 h and LDH was measured in cell supernatants using a cytotoxicity detection kit (LDH) from Roche (Indianapolis, IN).

### Materials and Methods

**Reagents**

*Escherichia coli* 0111:B4 LPS was purchased from Sigma-Aldrich (St. Louis, MO) and used for in vitro experiments. *E. coli* 055:B5 LPS (ultrapure; InvivoGen, San Diego, CA) was used for in vivo studies. Nigericin was obtained from Tocris Bioscience (Bio-Techne, Minneapolis, MN). ATP was from Sigma-Aldrich. Mouse GM-CSF was purchased from PeproTech (Rocky Hill, NJ). R848, imiquimod, and monosodium urate (MSU) crystals for in vitro studies were obtained from InvivoGen. Cholesterol crystals were prepared from a sterile 2 mg/ml cholesterol (Sigma-Aldrich) solution in 1-propanol. Crystallization was induced by addition of 1.5 vol of endotoxin-free water and crystals were allowed to stabilize for at least 30 min. Cholesterol crystals were dried at 75°C and resuspended by water bath sonication in sterile PBS containing 0.1% FCS.

**Animals**

All procedures using animals were performed under protocols approved by Pfizer’s Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act. Age-matched 6- to 10-wk-old female 129SvEsdTac mice and C57BL/6 mice were obtained from Taconic Farms (Germantown, NY). Age-matched 10- to 14-wk-old female BALB/c female mice were obtained from Charles River Laboratories (Wilmington, MA). Mice were acclimated in the vivarium for at least 5 d before use in a temperature-controlled room with a 12 h light/dark cycle and were allowed access to standard laboratory chow and water ad libitum.

**CP-456,773, glyburide, and KT53 compounds**

CP-456,773, sodium salt (CAS 256373-96-3), was synthesized at Pfizer as previously described (24). Glyburide was purchased from Sigma-Aldrich. KT53 was prepared as described (25).

**Preparation of mouse bone marrow–derived macrophages and human monocyte–derived macrophages**

To generate bone marrow–derived macrophages (BMDMs), bone marrow cells were harvested from femurs and tibias of C57BL/6 mice and collected into RPMI 1640 media containing 10% heat-inactivated FBS and supplemented with GlutaMAX, nonessential amino acids, sodium pyruvate, penicillin-streptomycin, and 2-ME (all reagents from Life Technologies, Grand Island, NY). RBCs were lysed using BD Pharm Lyse by BD Biosciences (San Jose, CA). Cells were cultured with 50 ng/ml GM-CSF in ultralow binding tissue culture plates (Corning) and harvested for use after 7–10 d in culture.

Human monocyte–derived macrophages were prepared by isolating monocytes from platelet apheresis buffy coats (Massachusetts General Hospital, Boston, MA) using the RosetteSep monocyte enrichment cocktail from Stemcell Technologies (Vancouver, BC, Canada) followed by separation over a Histopaque 1077 density gradient. Platelets were removed by low-speed centrifugation. Monocytes were resuspended in media containing 20 ng/ml human GM-CSF from R&D Systems (Minneapolis, MN) and cultured in Teflon-coated tissue culture bags (American Fluorooseal). Monocyte–derived GM-CSF–differentiated macrophages were harvested after 9–12 d of culture.

**Mouse and human macrophage activation**

NLRP3 inflammasome activation assays with mouse BMDMs (7.5 × 10^5 cells/well), human macrophages (1 × 10^5 cells/well), or mouse J774 cells (American Type Culture Collection, Manassas, VA) (1 × 10^5 cells/well) were performed in 96-well plates under serum-free conditions, unless otherwise stated, using RPMI 1640 without phenol red from Life Technologies. Cells were primed for 3–4 h with LPS (1 μg/ml LPS for J774 cells; 100 ng/ml LPS for BMDMs and human macrophages). Cells were incubated with various concentrations of test compound or vehicle (DMSO) for 30 min prior to addition of the NLRP3 activators. Mouse BMDMs were stimulated with 10 μg/ml nigericin or 5 mM ATP for 1 h. Human monocyte–derived macrophages were stimulated with 10 or 30 μg/ml nigericin, or 5 or 7.5 mM ATP for 1 h, or with 150 μg/ml MSU crystals or 350 μg/ml cholesterol crystals for 4 h. For measuring lactate dehydrogenase (LDH) release as an indicator of cell death, human monocyte–derived macrophages were stimulated with 10 μg/ml nigericin for 2 h and LDH was measured in cell supernatants using a cytotoxicity detection kit (LDH) from Roche (Indianapolis, IN).

**Human PBMC IL-1β and IL-6 release and cell viability**

Human PBMCs from Astarte Biologies (Bothell, WA) were removed from cryopreservation, washed, and resuspended in 1× Opti-MEM media (Life Technologies). PBMCs were then resuspended in serum-free media containing 100 ng/ml LPS and seeded at a density of 5 × 10^5 cells/well into 384-well cell culture plates. PBMCs were primed with LPS for 3 h and then treated with compound or vehicle for 30 min prior to addition of nigericin or media. PBMCs were stimulated with 3 μg/ml nigericin for 2 h and then supernatants were collected for analysis. IL-1β in the supernatant was determined using a human IL-1β HTRF assay kit (Cisbio assays, Bedford, MA). To assess compound effects on cell viability and cytokine stimulations of human PBMCs or purified human monocytes were performed in 96-well plates (1 × 10^5 cells/well) under serum-free conditions using RPMI 1640 (without phenol red) from Life Technologies. Cells were incubated with CP-456,773 or vehicle for 30 min prior to addition of the imidazoquinoline compounds. Purified monocytes were stimulated with...
1 μg/ml R848 for 4 h. PBMCs were stimulated with 1 μg/ml R848 or 1 μg/ml imiquimod for 20 h. Cell supernatants were analyzed for released cytokines.

Human macrophage NLRC4 activation

Human monocyte-derived GM-CSF–differentiated macrophages were treated as in Zhao et al. (26). Briefly, cells were primed with 1 ng/ml LPS for 2 h in RPMI 1640 containing 0.1% FBS and then incubated with 1 or 5 μM CP-456,773, or DMSO alone, for 30 min before treatment with LFn-Fpr1 fusion protein (4 μg/ml) (26) alone or in combination with anthrax protective Ag (1 μg/ml) or with nigericin (10 μM) for 4 or 2 h, respectively. Supernatants were harvested and IL-1β was measured using the IL-1β HTRF kit (Cisbio assays). Supernatant was precipitated as detailed in Horning et al. (27), resuspended, and separated using a 4–12% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane. The membrane was probed for IL-1β by immunoblot analysis (AF-201, used at 1:100 dilution; R&D Systems).

NLRP3 activation in mouse blood

Heparinized blood was collected from naive female 129S6/SvEvTac mice and pooled. Eighty microliters of whole blood was added per well of 96-well polypropylene plates and stimulated for 3 h with 1 μg/ml LPS. LPS–primed blood was treated with CP-456,773 or vehicle for 30 min prior to addition of 3 mM ATP for a final assay volume of 100 μl per well. After 1 h of stimulation with ATP the blood was centrifuged and the plasma collected for cytokine analyses.

Cytokine determinations

Mouse and human IL-1β, TNF-α, and IL-6 were measured with kits from Mesoscale Diagnostics (Rockville, MD), unless otherwise noted. Mouse IL-1α and IL-18 were measured with ELISA kits from R&D Systems and MBL International (Wobum, MA), respectively.

ASC speck assay

Mouse 19.5 reporter cells (28), grown in DMEM plus 10% FBS, were detached with trypsin-EDTA and washed once with PBS. Cells were resuspended in 1× Opti-MEM media (Life Technologies) with 250 ng/ml LPS, or with or without 10% FBS, to a density of 5×10^6 cells/ml in 96-well plates. After 2.5 h the titration of CP-456,773 was added, and 1 h later 10 μM nigericin was added. After a subsequent 1.5 h, the plates were briefly centrifuged, 60 μl of supernatant was removed, and 6 μl of 16% formaldehyde containing 1:300 Draq5 was added to each well. Plates were stored at 4°C for subsequent specking analysis with four images taken per field using a Zeiss Axio Observer Z1 fluorescence microscope.

GST Omega 1-1 gene expression knockdown

Immortalized murine BALB/c macrophages prepared as described (27) were transfected with small interfering RNAs (siRNAs) in six-well plates. The siRNAs were Silencer selected siRNAs from Thermofisher Scientific (GST-O-1-1 no. 1, s07151; GST-O-1-1 no. 2, s07154; GST-O-1-1 no. 3, s071431; NLRP3, s03711; negative control no. 1, 4390847; negative control no. 2, 4390844). After 2 d the cells were harvested, pooled, and plated at 8×10^5 cells/well in 96-well plates. After 1.5 h the media were replaced with 100 μl of media containing 200 ng/ml LPS. After a subsequent 2 h, 50 μl of supernatant was removed for TNF-α measurement by ELISA (R&D Systems), and 100 μl of CP-456,773 diluted in culture media was added for a final concentration of 2.5 μM (or 100 μl of media alone was added to control wells). After a subsequent 1 h, 50 μl of nigericin or ATP was added for final concentrations of 10 μM and 2 mM, respectively. After 1.5 h, 100 μl was collected for IL-1β measurement by ELISA (R&D Systems). Each condition was performed in triplicate. Five wells of LPS-treated cells were pooled for RNA extraction and further quantitative real-time PCR (qPCR) analysis.

qPCR analysis of gene expression knockdown

RNA was isolated according to the manufacturer’s protocol (Qiagen) and synthesized into cDNA using SuperScript III (Thermo Fisher Scientific). qPCR was performed on cDNA using the Maxima SYBR Green/ROX qPCR master mix (Thermo Fisher Scientific) in a QuantStudio 6 flex system (Thermo Fisher Scientific). The mouse targeted qPCR primer pairs used were: hypoxanthine phosphoribosyltransferase forward, 5′-TGAAGTACCTCATTAGAATCAGGGCA-3′; reverse, 5′-CTGCTGGAATACGCTTCTTCG-3′; reverse, 5′-AGGCTGATGCTTCGTGTTCCA-3′; GST Omega 1-1 forward, 5′-GGCTGAGGAACGATTTCAAA-3′; reverse, 5′-GAGCTCCCATGCGTCCT-3′; reverse, 5′-AGGCTGATGCTTCGTGTTCCA-3′; GST Omega 1-1 forward, 5′-GGCTGAGGAACGATTTCAAA-3′; reverse, 5′-GAGCTCCCATGCGTCCT-3′; reverse, 5′-GAGCTCCCATGCGTCCT-3′. Selectivity assays

Selectivity assays, summarized in Supplemental Table I, were carried out at Eurofins Cerep (Celle L’Evescault, France), DiscoverX ( Freemont, CA), Reaction Biology (Malvern, PA), Carma Biosciences (Natick, MA), and WuXi AppTec (Shanghai, China).

Compound preparation and administration

In studies where vehicle or compound was administered, animals were dosed via oral gavage with a stainless steel animal feeding needle 1 h prior to LPS plus ATP challenge. Compound was formulated for oral delivery in 2% Tween 80/0.5% methylcellulose.

Plasma exposure of CP-456,773 and inhibition of induction of cytokine production in vivo

Female mice (129S6/SvEvTac) were distributed into eight CP-456,773 treatment groups that received a single dose of 0.4, 1.2, 4, 7, 12, 20, 70, or 200 mg/kg CP-456,773 or vehicle by oral gavage. At 3.5 h after oral dose, blood was collected from each animal via cardiac puncture and was allowed to clot for 2 h. Serum was analyzed for CP-456,773 using liquid chromatography–tandem mass spectrometry to separate CP-456,773 from endogenous matrices and mass spectrometry to detect CP-456,773. For cytokine determinations, mice were given 1 μg of E. coli 055:B5 ultrapure LPS in PBS by i.p. injection. Two hours later, an i.p. injection of 5 mM ATP disodium salt (Sigma-Aldrich) in 0.5 ml of PBS was administered. The pH of the ATP solution was adjusted to 7.2 prior to injection. Thirty minutes after ATP administration blood was collected via cardiac puncture, and peritoneal cavities were lavaged with ice-cold PBS containing 25 U/ml heparin (sodium salt; Sigma-Aldrich) and 10% heat-inactivated FBS (Life Technologies). A protease inhibitor mixture was added to the lavage buffer just prior to use at one tablet per 50 ml of buffer (cOmplete tablets; Roche). Blood was kept at room temperature and allowed to clot for 2 h before separation by centrifugation. Serum and centrifugation-clarified lavage fluid were stored at −80°C until analysis. Plasma protein binding of CP-456,773 was measured by equilibrium dialysis of the in vitro fraction unbound of CP-456,773 at a concentration of 2 μM in plasma from pooled male and female CD-1 mice. Plasma was centrifuged and supernatant was analyzed by liquid chromatography–tandem mass spectrometry to detect CP-456,773 by York Bioanalytical Solutions (York, U.K.).

Imiquimod cream–induced skin inflammation in mice

Female 129S6/SvEvTac mice received a daily topical dose of 62.5 mg of cream containing 5% imiquimod and 25% isoocteric acid (Global Pharmaceuticals, Philadelphia, PA) on the shaved back and left ear for 3 consecutive days. This is equivalent to a daily dose of 3.12 mg of active imiquimod compound. Mice were dosed twice a day by oral gavage with either CP-456,773 at 200 mg/kg or vehicle. The final dose of the study was given on day 5, 1 h prior to euthanasia and blood collection via cardiac puncture. On days 1 (baseline), 4, and 5 ear thicknesses were measured in quadruplicate using a microcaliper (Mitutoyo, Elk Grove Village, IL). Percentage change from baseline ear measurement was calculated for each animal. Blood serum was separated and stored at −80°C. Each left ear was harvested, snap frozen in liquid nitrogen, and stored at −80°C. Frozen mouse ear tissue was processed using a Covaris CP02 cryoPREP machine. Tissue powder was resuspended in T-Pot tissue extraction buffer from Thermofisher Scientific (Waltham, MA) supplemented with 1% protease inhibitor mixture (Thermo Scientific) and 1% phosphatase inhibitors (phosphatase inhibitor cocktails 2 and 3; Sigma-Aldrich). Tissue lysates were centrifuged for 10 min at 10,000 rpm at 4°C. Supernatants were collected and cytokines were measured with a mouse Th17 magnetic bead panel assay kit from EMD Millipore (Billerica, MA) using the Lumexin platform.

Murine model of acute house dust mite challenge

Age-matched 10- to 14-wk-old female BALB/c mice received a single intratracheal instillation of saline or 100 μg of house dust mite (HDM) extract from Dermatophagoides pteronyssinus (Greer Laboratories, Lenoir, NC) resuspended in saline using a 100-μl Hamilton glass syringe, as previously described (29). Animals were dosed by oral gavage with saline (vehicle), 2 mg/kg dexamethasone (Dex) USP (Sigma-Aldrich), or 200 mg/kg CP-456,773, twice daily, on the day before and the day of HDM challenge. All mice were terminally anesthetized 24 h after HDM challenge, and bronchoalveolar lavage (BAL) samples were collected for analyses. Total BAL cellularity was assessed with an IDEXX ProCyte analyzer (Sysmex, Westbrook, ME), and cell differentials were determined from cytospin slides stained with the Wright–Giemsa method using Protocol Hema 3 from York Bioanalytical Solutions (York, U.K.).
Results

**CP-456,773 is a potent inhibitor of NLRP3 inflammasome activation in human and mouse cells**

In 2001, Perregaux et al. (21) described a class of sulfonylurea-containing compounds capable of inhibiting LPS plus ATP–induced release of mature IL-1β, which they termed CRIDs. Since then, the sulfonylurea-containing compound glyburide has been shown to prevent IL-1β release by inhibiting activation of the NLRP3 inflammasome (30). We compared the potency of glyburide and the CRID compound CP-456,773 (Fig. 1A) for inhibition of NLRP3 inflammasome–induced IL-1β release in human PBMCs that were first primed with LPS and then stimulated with the antibiotic ionophore nigericin, which is a well-established selective activator of the NLRP3 inflammasome (2, 30). CP-456,773 was a much more potent inhibitor of IL-1β release from PBMCs than was glyburide, with IC50 values of 35 and 9813 nM, respectively (Fig. 1B). CP-456,773 did not affect PBMC viability (Supplemental Fig. 1A), and it was specific for IL-1β inasmuch as LPS-induced IL-6 release was unaffected (Supplemental Fig. 1B). We extended these observations by demonstrating CP-456,773–dependent inhibition of IL-1β and IL-18 release from human monocyte-derived macrophages stimulated with LPS plus nigericin, with IC50 values of 7.2 and 10.3 nM, respectively (Fig. 1C, 1D). Moreover, CP-456,773 potently prevented LPS plus nigericin–induced LDH release (IC50 = 17.4 nM), indicating that CP-456,773 inhibits NLRP3–dependent pyroptosis in human monocyte-derived macrophages (Fig. 1E).

Similar to the results in human cells, CP-456,773 potently inhibited IL-1β release from mouse BMDMs when primed with LPS and then stimulated with nigericin or ATP, with IC50 values of 22 and 9.7 nM, respectively (Fig. 1F). As the NLRP3 inflammasome–dependent release of IL-1α had been reported previously (7), we investigated the potential for CP-456,773 to reduce NLRP3–driven IL-1α release from mouse immune cells. CP-456,773 potently inhibited IL-1α release from LPS–primed BMDMs stimulated with nigericin or ATP, with IC50 values of 18 and 12 nM, respectively (Fig. 1G). We conclude, therefore, that CP-456,773 is capable of inhibiting the NLRP3–dependent release of IL-1α.

**CP-456,773 inhibits release of IL-1β induced with both soluble and crystalline NLRP3 inflammasome activators**

We investigated the ability of CP-456,773 to inhibit NLRP3 activation induced by crystalline activators that have been associated with inflammatory diseases (2, 4, 9, 27). In the results shown in Fig. 1H, CP-456,773 markedly reduced the amount of IL-1β released from human monocyte-derived macrophages primed with LPS and then stimulated with MSU or cholesterol crystals. Moreover, CP-456,773 is selective for NLRP3 relative to NLRC4 inflammasome activation, because it did not inhibit NLRC4 inflammasome–dependent IL-1β release induced by LFn–PrgI fusion protein treatment in human monocyte–derived macrophages (Fig. 1I) (26). The LFn–PrgI fusion protein consists of the amino terminal domain of anthrax lethal toxin (LFn) in frame with the nNAP1 domain for the TIIHSS S. syphirum. This is delivered into the cytosol of the cell via the anthrax protective Ag delivery system, where it activates NLRC4 in an nNAP-dependent manner. Immunoblot detection of IL-1β confirmed that stimulation of LPS–primed human macrophages with LFn–PrgI fusion protein or with nigericin resulted in generation and release of cleaved IL-1β into cell supernatants (Fig. 1J). CP-456,773 did not inhibit LFn–PrgI fusion protein–induced IL-1β cleavage and release, but CP-456,773 did prevent generation and release of cleaved IL-1β following nigericin stimulation. Because CP-456,773 prevents NLRP3–dependent pyroptosis and the resulting extracellularization of pro–IL-1β, it is not surprising that pro–IL-1β was not detected in the CP-456,773–treated nigericin–stimulated cell supernatants.

**CP-456,773 potency and activity in mouse blood and impact of serum proteins**

To profile the potency of CP-456,773 in a whole-blood matrix, for the purpose of tracking pharmacodynamic exposures in vivo using an ex vivo assay, we established an in vitro mouse blood assay system that measures inflammasome–dependent release of IL-1β. The ex vivo stimulation of naive mouse blood with LPS followed by ATP resulted in a significant release of IL-1β in the plasma, whereas minimal amounts of IL-1β were detected in mouse blood treated with either LPS alone or ATP alone (Fig. 2A). In this mouse blood system, CP-456,773 demonstrated a concentration–dependent inhibition of IL-1β release, with an IC50 of 913 nM (Fig. 2B). Correcting this whole blood IC50 for plasma protein binding (Supplemental Table I) and blood/plasma partition (∼1:1) gives an estimated free (i.e., unbound) IC50 for CP-456,773 of 6.8 nM, a similar value to that observed with our in vitro assays under serum–free conditions (Fig. 1F). As a measure of selectivity of the compound for the NLRP3 inflammasome pathway, we observed that CP-456,773 at 10 μM had no effect on the release of TNF-α from LPS plus ATP–stimulated mouse blood (Fig. 2C).

The impact of plasma protein binding on in vitro (cell-based) potency of CP-456,773 was demonstrated by the addition of 10% FBS to the culture media. In LPS plus nigericin–stimulated mouse J774 cells, this caused a right shift in the IC50 compared with serum–free conditions, with IC50 values of 116 and 7.5 nM, respectively (Supplemental Fig. 1C). In immortalized mouse macrophages the IC50 for CP-456,773–mediated inhibition of nigericin–induced ASC speck formation shifted from 130 nM in serum–free conditions to 1190 nM in the presence of 10% FBS (Supplemental Fig. 1D).

**In vivo inhibition of acute LPS plus ATP–induced IL-1α, IL-1β, and IL-18 cytokine release by CP-456,773**

To correlate in vivo exposures of CP-456,773, following oral dosing, with the pharmacodynamic effects of CP-456,773 on the release of NLRP3–dependent cytokines, we employed an acute model in which 129Sv/ScEvTac mice were challenged with LPS followed by ATP (31). Specifically, mice were dosed with varying amounts of CP-456,773 or vehicle at t = 0 and then challenged by i.p. injection of LPS at t = 1 h, followed by i.p. injection of ATP at t = 3 h. Serum and peritoneal lavage were collected at t = 3.5 h and total CP-456,773 levels in serum were determined. Free/unbound concentrations of CP-456,773 in serum 3.5 h after dosing demonstrated a linear relationship with dose (Fig. 2D). In a single oral (100 mg/kg) dose pharmacokinetic study in male CD-1 mice, CP-456,773 demonstrated an apparent plasma clearance of 0.5 l/h and an apparent volume of distribution of 5.7 l, resulting in a t1/2 of 8 h. Moreover, the systemic exposure of CP-456,773 orally administered once daily during a period of 5 d increased in a linear manner between 100 and 200 mg/kg (area under the curve [0–2] and maximum concentration) (Supplemental Table I). No accumulation in CP-456,773 was observed in mice from repeated dosing up to 5 consecutive days.

It has been demonstrated that IL-1β production in mice challenged with LPS plus ATP is NLRP3–dependent, and that IL-6 production under these conditions is not (32). As evidence of CP-456,773 in vivo...
LPS plus ATP–challenged mice were reduced then stimulated with nigericin or LFn-PrgI fusion protein for 1.5 h. Data are representative of two independent experiments. (1 h. Graph of mean IL-1

pharmacology, levels of IL-1β in serum and peritoneal lavage from LPS plus ATP–challenged mice were reduced >90%, relative to vehicle-treated animals, following single oral doses of CP-456,773 between 4 and 200 mg/kg (Fig. 2E, 2F). At doses of 0.4 and 1.2 mg/kg, IL-1β release was inhibited by 50 and 90%, respectively, relative to vehicle-treated animals. Release of both IL-1α and IL-18 was also significantly inhibited with oral dosing of CP-456,773 relative to vehicle-treated animals (Fig. 2G, 2H). In contrast, the elevated level of IL-6 observed in serum and peritoneal lavage from mice challenged with LPS followed by ATP was not inhibited at the high dose of 200 mg/kg CP-456,773 (Fig. 2I, 2J), thereby demonstrating the in vivo selectivity of CP-456,773 for inhibition of the NLRP3-dependent cytokines IL-1β, IL-1α, and IL-18. Average calculated free concentrations of CP-456,773 in mice at 3.5 h after oral administration with 0.4 and 1.2 mg/kg were 3.5 ± 1.1 and 13.6 ± 2.1 nM, respectively, and at these doses the LPS plus ATP–induced peritoneal IL-1β levels were inhibited by 40 and 74%, respectively. Thus, in vivo free concentrations of CP-456,773 capable of inhibiting LPS plus ATP–induced IL-1β were highly similar to those observed for CP-456,773–mediated inhibition of LPS plus ATP–induced IL-1β release in mouse BMDMs under serum-free conditions (IC50 = 9.7 nM). These results establish a 1:1 relationship between the intrinsic potency of CP-456,773 in vitro and the free/unbound concentrations required to show efficacy in vivo.

CP-456,773 inhibits IL-1β release induced by imidazquinoline compounds in cells

The imidazquinoline compounds imiquimod and R848 have been shown to activate IL-1β release from immune cells in a manner that requires NLRP3, ASC, and caspase-1 (33). Therefore, we evaluated the potential for CP-456,773 to inhibit imidazquinoline-induced IL-1β release in cultured immune cells. In purified human monocytes, 1 μM CP-456,773 selectively reduced R848-induced IL-1β, but it did not inhibit R848-induced IL-6 or TNF-α release from these same cells (Fig. 3A, 3B). In human PBMCs, CP-456,773 reduced both R848- and imiquimod-induced IL-1β release with IC50 values of 26 and 10 nM, respectively (Fig. 3C, 3D). Thus, in human PBMCs the potency of CP-456,773 for inhibiting imidazquinoline-induced IL-1β is similar to the potency observed for inhibition of IL-1β release induced with the canonical NLRP3 trigger nigericin (Fig. 1B). We conclude that CP-456,773
FIGURE 2. CP-456,773 activity in mouse blood and pharmacodynamics effects on local and systemic cytokine release in mice. (A) IL-1β in plasma from mouse blood primed with 1 μg/ml LPS for 3 h and then stimulated with 3 mM ATP for 1 h. (B) Effect of CP-456,773 on IL-1β release from mouse blood primed with 1 μg/ml LPS for 3 h and then stimulated with 3 mM ATP for 1 h. (C) TNF-α in plasma from mouse blood primed with 1 μg/ml LPS for 3 h and then stimulated with 3 mM ATP for 1 h treated with 10 μM CP-456,773. (A–C) CP-456,773 preincubated with blood for 30 min prior to addition of ATP. Graphs of mean ± SEM (n = 3 experiments). (D) Concentrations of CP-456,773 measured in blood collected from mice 3.5 h following oral gavage dosing of CP-456,773 and free concentrations of CP-456,773 were calculated. (E–J) Cytokines in serum or peritoneal lavage from mice dosed with CP-456,773 by oral gavage at time = 0 h, then challenged i.p. with LPS at time = 1 h, then challenged with ATP at time = 3 h, followed by blood and peritoneal lavage collection at time = 3.5 h. (E) IL-1β levels in serum. (F) IL-1β levels in peritoneal lavage. (G) IL-18 levels in peritoneal lavage. (H) IL-1α levels in peritoneal lavage. (D–H) Combined results from two independent studies with intercalated doses. Study 1 contained 7, 20, 70, and 200 mg/kg treatment groups (gray boxes, five mice per group). Study 2 contained 0.4, 1.2, 4, and 12 mg/kg treatment groups (unfilled boxes, seven mice per group). (I) IL-6 measured in serum (five mice per group). (J) Figure legend continues.
potently inhibits imidazoquinoline compound–mediated activation of the NLRP3 inflammasome and the concomitant IL-1β release. The similarity of IC50 values suggests that CP-456,773–mediated inhibition of imidazoquinoline- and nigericin-induced NLRP3 inflammasome activation is likely accomplished through a common mechanism.

The pharmacological target of CP-456,773 remains unknown

CP-456,773 was inactive or only very weakly potent (>10 μM) when screened against a large panel of molecules, including enzymes, receptors, and channels (Supplemental Table II). In these assays CP-456,773 did not inhibit targets such as caspase-1, SYK, JNK, GPR40, and GPR120, which have been shown to regulate NLRP3 inflammasome activity (34, 35). Consistent with this high degree of selectivity of CP-456,773, we note that in previous reports CP-456,773 (described as CAS 210826-40-7) was evaluated as part of the ToxCast initiative and was found to be inactive or only weakly active when assessed in >400 assays/endpoints (36, 37).

In one publication GST Omega 1-1, an atypical member of the GST superfamily, was reported to be a potential target of the CRID molecules (23). We tested KT53, a potent and selective inhibitor of GST Omega 1-1, in our NLRP3 inflammasome–dependent IL-1β release assay (25). KT53 weakly inhibited LPS plus nigericin–induced IL-1β release from PBMCs with an IC50 of 3505 nM, but KT53 was approximately equipotent for inhibiting LPS-induced IL-6 with an IC50 of 4181 nM (data not shown). This weak KT53 potency in IL-1β release assays strongly suggests that GST Omega 1-1 is not the pharmacological target of CP-456,773. As an alternative approach in these investigations, we knocked down GST Omega 1-1 mRNA expression in mouse macrophages. The release of IL-1β induced by stimulating LPS-primed mouse macrophages with nigericin or ATP was not abrogated when GST Omega 1-1 mRNA expression was knocked down (Fig. 4A, 4B). Moreover, CP-456,773 was capable of inhibiting NLRP3 inflammasome–dependent release of IL-1β in cells with >90% knockdown of GST Omega 1-1 mRNA expression (Fig. 4A, 4B). As a positive control for these experiments, the knockdown of NLRP3 mRNA greatly reduced IL-1β release from LPS-primed mouse macrophages stimulated with nigericin or ATP (Fig. 4A, 4B, 4D). Taken together, these results indicate that GST Omega 1-1 is highly unlikely to be the target of CP-456,773, and that inhibition of GST Omega 1-1 is not the mechanism by which CP-456,773 blocks NLRP3 inflammasome activation.

![Graph Image](https://example.com/graph.png)

**FIGURE 3.** R848- or imiquimod-induced IL-1β release from human immune cells is inhibited by CP-456,773. (A) IL-1β measured in cell supernatants from purified human monocytes stimulated with R848 for 4 h with or without 1 μM CP-456,773. Graph of mean ± SD (n = 2 donors). (B) TNF-α and IL-6 measured in cell supernatants from purified human monocytes stimulated with R848 for 4 h with or without 1 μM CP-456,773. Graph of mean ± SD (n = 2 donors). (C and D) Effect of CP-456,773 on IL-1β released from human PBMCs stimulated with R848 (C) or imiquimod (D) for 20 h. (C) Graph of mean ± SEM (n = 4 donors). (D) Graph of mean ± SD (n = 2 donors). (A–D) CP-456,773 preincubated with cells for 30 min prior to R848 or imiquimod stimulations.

IL-6 measured in peritoneal lavage in a third study (five mice per group). (D–J) Box–and-whisker graphs of median line in box, box extending from 25th to 75th percentiles, and whiskers from minimum to maximum values. (E–H) *p ≤ 0.05, **p ≤ 0.001, ***p ≤ 0.0001 compared with appropriate vehicle group by unpaired t test. NS, not significant.
As mentioned above, CP-456,773 is structurally similar to the sulfonylurea compound glyburide, which has been described to inhibit NLRP3 inflammasome activation and IL-1β release (21, 30). Glyburide is used in the treatment of type 2 diabetes and regulates insulin secretion by binding to sulfonylurea receptors (SURs) on pancreatic β cells and agonizing SUR-mediated antagonism of the

**FIGURE 4.** GST Omega 1-1 mRNA knockdown does not affect NLRP3 inflammasome-dependent IL-1β release or the ability of CP-456,773 to inhibit IL-1β release. (A and B) Effect of NLRP3 or GST Omega 1-1 siRNA-mediated knockdown on IL-1β in supernatants from mouse macrophages stimulated with 200 ng/ml LPS for 2 h followed by 1 h treatment with or without 2.5 μM CP-456,773 and then stimulated for 1.5 h with 10 μM nigericin (A) or 2 mM ATP (B). (C and D) Effect of NLRP3 or GST Omega 1-1 siRNA-mediated knockdown on TNF-α in supernatants from mouse macrophages stimulated with 200 ng/ml LPS for 2 h and collected prior to treatment with or without 2.5 μM CP-456,773 and 10 μM nigericin (C) or 2 mM ATP (D). (E and F) Effect of GST Omega 1-1 or NLRP3 siRNA-mediated knockdown on GST Omega 1-1 mRNA (E) or NLRP3 mRNA (F) determined by quantitative PCR and relative to CTRL-1 normalized to 1. (A and B) Graphs of mean ± SEM (n = 3–4 experiments). *p < 0.05, **p < 0.01, ***p < 0.0001 compared with CTRL-1.
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inwardly rectifying K+ channel 6.2 (Kir6.2) (30). Lamkanfi et al. (30) demonstrated that SUR2 and Kir6.2 proteins are not required for NLRP3 inflammasome activation, and thus the target of CP-456,773–mediated inhibition of NLRP3 inflammasome activation is likely distinct from the targets that are known to be responsible for glyburide’s ability to promote insulin secretion. Consistent with this notion, we have observed that CP-456,773 does not inhibit SUR1, SUR2a, or SUR2b-mediated regulation of Kir6.2 currents in recellularin cell lines. As a positive control, glyburide demonstrated potent inhibition of these SUR1-, SUR2a-, or SUR2b-mediated currents under these conditions (IC50 values of 4, 104, and 7 nM, respectively) (data not shown).

Collectively, our results build on previous work but dismiss GST Omega 1-1, SUR1, SUR2a, and SUR2b as the pharmacological targets of CP-456,773. Moreover, our selectivity panel screening indicates that CP-456,773 does not target several cellular proteins that have been reported to modulate NLRP3 inflammasome activity (e.g., caspase-1, SYK, JNK, GPR40, and GPR120) (34, 35). To our knowledge, the pharmacological target of CP-456,773 remains unknown.

**CP-456,773 efficacy in a mouse model of skin inflammation**

IL-1β expression is increased in psoriatic lesions, and a genetic association exists between polymorphisms in NLRP3 and susceptibility to psoriasis (38–40). Furthermore, a role for IL-1β in promoting Th17 responses has been implicated in the pathogenesis of skin inflammation and psoriasis (38, 41, 42). Together with our observation that R848 and imiquimod induce IL-1β in human immune cells and that these responses are sensitive to CP-456,773 (Fig. 3), we were prompted to evaluate CP-456,773 efficacy in a mouse model of imiquimod cream–induced skin inflammation. Application of imiquimod cream to the back skin and ear of mice for 3 consecutive days resulted in increased ear thickness on days 4 and 5. Oral twice-daily dosing of mice with 200 mg/kg CP-456,773 significantly reduced ear thickness measured on days 4 and 5 by 46.1 and 41.4%, respectively, relative to imiquimod cream–treated mice administered vehicle alone (Fig. 5A).

Protein concentrations of several cytokines that have been associated with inflammation both in this model and in human psoriatic skin samples were elevated in the inflamed imiquimod cream–challenged ears (38, 42). Notably, CP-456,773 treatment resulted in a significant reduction in levels of IL-22, IL-17A, and IL-17F in the imiquimod cream–challenged ears (Fig. 5B–D). These data suggest the involvement of the NLRP3 inflammasome and its outputs in the pathogenesis of imiquimod cream–induced skin inflammation and in the induction of cytokines associated with psoriasis.

**CP-456,773 modulates HDM-induced acute airway inflammation**

Pulmonary exposure to certain microbial and environmental agents can activate the NLRP3 inflammasome in cells of the airways, thereby contributing to and augmenting airway inflammation (13). The HDM *D. pteronyssinus* is a common airborne allergen capable of stimulating innate and adaptive immune pathways in the airways, and HDM extract has been shown to activate the NLRP3 inflammasome (43). We used CP-456,773 to investigate a role for the NLRP3 inflammasome in a mouse model of HDM-induced acute lung inflammation. As shown in Fig. 6A–C, mice exposed to a single challenge with HDM developed airway inflammation dominated by neutrophilia. Treatment with Dex or CP-456,773 slightly decreased total BAL cellularity that did not achieve statistical significance (p = 0.082 for CP-456,773 treatment) (Fig. 6A). However, both Dex and CP-456,773 significantly reduced BAL neutrophil numbers in HDM-challenged mice (p < 0.0001 and p < 0.001, respectively) (Fig. 6B). This single HDM challenge stimulates an acute inflammatory response that does not recapitulate a full asthma-like phenotype. Expectedly, BAL eosinophil numbers remained negligible in all groups 24 h after a single HDM challenge, with no significant change between HDM-challenged mice and saline controls (data not shown). Also, BAL macrophage numbers remained similar in all groups following HDM challenge (data not shown). HDM challenge evoked a modest but significant increase in lymphocytes detected in the BAL that was reduced by Dex or CP-456,773 treatment (p < 0.05 and p < 0.01 respectively) (Fig. 6C). Thus, CP-456,773 treatment significantly reduced acute neutrophil and lymphocyte infiltration in the airways of mice exposed to a single HDM challenge.

We also evaluated the presence of several inflammatory mediators in the mouse airways following HDM challenge. When BAL fluid samples were assayed for the presence of 36 different cytokines and chemokines, the following nine analytes were detected at significantly increased levels 24 h after challenge, compared with controls: CXCL1, CXCL2, CXCL5, CXCL10, CCL3, CCL4, CCL7, IL-5, and TNF-α. Fig. 6D–G show that treatment with Dex or CP-456,773 was able to significantly reduce levels of CXCL1, CXCL5, CXCL10, and CCL7 in the mouse airways. This is particularly interesting in the case of CXCL1 and CXCL5, as these are major chemoattractants for neutrophils. Increased levels of CCL3 (MIP-1α), CCL4 (MIP-1β), CXCL2, and TNF-α were also found in BAL from HDM-challenged mice, and although these were reduced to baseline levels by Dex treatment, CP-456,773 treatment had no significant effect on these four inflammatory mediators (despite a trend toward reduced TNF-α levels that remained below statistical significance; p = 0.0595) (Fig. 6H, 6I and data not shown). Taken together, these results indicate that activation of the NLRP3 inflammasome contributes to pulmonary inflammation in response to a single acute challenge with HDM allergen, and that treatment with CP-456,773 can modulate the levels of key chemoattractants and reduce the influx of neutrophils and lymphocytes in the mouse airways.

**Discussion**

Increasing evidence implicates the NLRP3 inflammasome and its activation products IL-1β, IL-18, IL-1α, and high-mobility group box 1 in the pathogenesis of tissue injury and disease (4, 8, 9, 12–15, 44). Therefore, a potent, selective, and orally available pharmacological inhibitor of NLRP3 inflammasome should be useful for elucidating the pathogenic contributions of NLRP3 inflammasome activation and its multiple products in preclinical disease models. Such a molecule may also be beneficial for treating CAPS and other NLRP3-dependent maladies in the clinic. Recently, Coll et al. (22) demonstrated that CP-456,773 (renamed MCC950 in their study) prevented IL-1β processing and release via inhibition of NLRP3 inflammasome activation, and it reduced disease in an experimental autoimmune encephalomyelitis model and rescued CAPS-like disease in transgenic mice expressing mutant NLRP3 protein. In the present study, we provide further evidence that CP-456,773 is a potent and selective inhibitor of NLRP3-dependent release of IL-1β, IL-18, and IL-1α from cultured immune cells. We also establish an in vivo concentration/effect relationship for CP-456,773 in a mouse model of acute peritoneal challenge with LPS followed by ATP, and we demonstrate that treatment with CP-456,773 significantly reduces skin inflammation in mice induced by imiquimod cream application and pulmonary inflammation in mice induced by acute HDM allergen challenge. These latter findings implicate the NLRP3 inflammasome and its products in the pathogenesis of dermal and airway inflammation. Our results, together with those provided by Coll et al. (22), firmly...
establish CP-456,773 as a potent and selective tool compound for studying the NLRP3 inflammasome in cell-based and mouse model systems.

The results presented in this study and elsewhere demonstrate that CP-456,773 can inhibit NLRP3 inflammasome activation induced by a variety of stimuli capable of activating the NLRP3 inflammasome via seemingly disparate cellular pathways, including K⁺ efflux, Ca²⁺ mobilization, reactive oxygen species generation, “frustrated” phagocytosis of crystals leading to lysosome rupture, and NLRP3 gain-of-function mutations (1, 3, 22). Thus, CP-456,773 regulates a cellular target that is downstream of, but common to, these various NLRP3 activation stimuli. CP-456,773 does not prevent K⁺ efflux, Ca²⁺ mobilization, or NLRP3 association with the adaptor protein ASC (22). The identification of the molecular target of CP-456,773 is a compelling area of research, and one that may lead to elucidation of a novel signaling molecule that is required for NLRP3 inflammasome activation in response to a wide variety of stimuli.

Our demonstration that CP-456,773 treatment of mice significantly reduces imiquimod cream–induced skin inflammation further supports a role for the NLRP3 inflammasome in the sequelae of skin inflammation and disease. CAPS patients often develop cutaneous lesions, and transgenic mice expressing NLRP3 protein harboring the orthologous NLRP3 mutations develop cutaneous inflammation mediated by enhanced IL-1β release from skin-resident mast cells (17, 45). CP-456,773 efficacy in the imiquimod cream–induced skin inflammation model might result from direct prevention of imiquimod cream–induced NLRP3 inflammasome activation and IL-1β release, because imiquimod stimulation (with LPS priming) is capable of activating the NLRP3 inflammasome and IL-1β release from mast cells (45, 46). Alternatively, as IL-17 and IL-22 are present and contribute to skin disease in this model, CP-456,773 may act by preventing IL-17– and IL-22–mediated activation of the NLRP3 inflammasome in keratinocytes (42, 47). Reduced skin inflammation and dermal thickness induced by imiquimod cream has been observed in caspase-1–deficient mice (47). However, in a recent publication from Rabeony et al. (48), imiquimod cream–induced skin inflammation was not reduced in NLRP3-, caspase-1–, and ASC-deficient mice. It is unclear why the results from Rabeony et al. disagree with previously published results demonstrating reduced imiquimod cream–induced skin inflammation in caspase-1–deficient mice, or with our results with CP-456,773 in the present study (47). However, Rabeony et al. (48) do demonstrate that IL-1α and IL-1β together contribute to dermatitis and that caspase-1 activity is upregulated in response to imiquimod cream in their model. Furthermore, it has been reported that the isostearic acid component of the imiquimod cream can activate NLRP1 inflammasome–dependent cleavage and release of IL-1β from human keratinocytes and that isostearic acid might contribute to inflammation (49). Although Coll et al. (22) have demonstrated that CP-456,773 does not inhibit NLRP1 inflammasome activation in cultured mouse macrophages, we cannot rule out the possibility that CP-456,773 might inhibit inflammasomes other than NLRP3 in vivo. On balance, our results together with previously published data indicate that the NLRP3/caspase-1/IL-1 axis likely plays a key role.
in the development of skin inflammation driven by imiquimod cream, and that the NLRP3 inflammasome pathway may represent a useful therapeutic target for skin disorders such as psoriasis and dermatitis.

The pathogenic contributions of NLRP3 inflammasome components or products have been described for a range of pulmonary inflammation disease models (13). It is thought that in preclinical asthma models an initial challenge with HDM recapitulates the sensitization phase of the disease, where a TLR4/IL-1α/IL-1R1 axis likely plays a critical role (50). Allergen challenge is capable of inducing release of the NLRP3 stimuli ATP and uric acid in the airways of sensitized mice and allergic patients, and P2X7R-deficient mice develop reduced pulmonary inflammation following HDM challenge, thus supporting a role for ATP in driving airway inflammation (51–53). However, there are conflicting data regarding the role of NLRP3 and IL-1 in various murine asthma models (52, 54–56). These discrepancies may be a result of the nature of the allergen used in combination (or not) with adjuvant, or they may possibly be due to differences in animal microbiome composition, as Allen et al. (56) have hypothesized. Our observation that CP-456,773 significantly reduces airway neutrophilia and lung chemokine levels following a single HDM challenge points to a role for activation of the NLRP3 inflammasome in the initial release of inflammatory mediators and the early recruitment of lymphocytes and granulocytes into the airways, and our results suggest that the NLRP3 inflammasome may contribute to the sensitization phase of allergic asthma.

In summary, our results confirm and extend our understanding of the in vitro and in vivo pharmacologic properties of CP-456,773, and they underscore the utility of CP-456,773 as a potent and highly selective inhibitor of NLRP3 inflammasome activation in preclinical disease models. Additionally, our mouse model data implicate NLRP3 inflammasome activation in mediating inflammation of both skin and airways, and, by extension, in the progression of certain dermal and pulmonary diseases.

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FIGURE 6. CP-456,773 treatment reduces mouse airway inflammation following acute HDM challenge. (A–I) BAL cellularity and BAL cytokines/chemokines in mice challenged with intratracheal saline or HDM, and treated with saline, Dex (2 mg/kg), or CP-456,773 (200 mg/kg twice daily). Total cellularity (A) and differential numbers of neutrophils (B) or lymphocytes (C) in BAL samples 24 h after HDM challenge. (D–I) Concentrations of cytokine/chemokine proteins CXCL1/KC (D), CXCL5/GCP-2 (E), CXCL10/IP-10 (F), CCL7/MARC (G), CCL3/MIP-1α (H), and TNF-α (I) in BAL samples 24 h after HDM challenge. All data are representative of two to three independent experiments with 5–10 mice per group. Statistical significance was determined using a one-way ANOVA followed by a Dunnett test for multiple comparisons where all groups were compared with HDM/vehicle-treated animals. For the determination of individual p values, a Mann–Whitney U test was used to compare a specific group to HDM/vehicle-treated animals. For all groups, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with HDM/vehicle animals.
Disclosures
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