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# Resolvin D1 Attenuates Polyinosinic-Polycytidylic Acid–Induced Inflammatory Signaling in Human Airway Epithelial Cells via TAK1

**Hsi-Min Hsiao,* Thomas H. Thatcher,[†,‡] Elizabeth P. Levy,[†,‡] Robert A. Fulton,[†,‡] Kristina M. Owens,[‡] Richard P. Phipps,[†,§] and Patricia J. Sime[†,‡,§]**

**The respiratory epithelium consists of lung sentinel cells, which are the first to contact inhaled inflammatory insults, including air pollutants, smoke, and microorganisms. To avoid damaging exuberant or chronic inflammation, the inflammatory process must be tightly controlled and terminated once the insult is mitigated. Inflammation resolution is now known to be an active process involving a new genus of lipid mediators, called "specialized proresolving lipid mediators," that includes resolvin D1 (RvD1). We and others have reported that RvD1 counteracts proinflammatory signaling and promotes resolution. A knowledge gap is that the specific cellular targets and mechanisms of action for RvD1 remain largely unknown. In this article, we identified the mechanism whereby RvD1 disrupts inflammatory mediator production induced by the viral mimic polyinosinic-polycytidylic acid [poly(I:C)] in primary human lung epithelial cells. RvD1 strongly suppressed the viral mimic poly(I:C)-induced IL-6 and IL-8 production and proinflammatory signaling involving MAPKs and NF-κB. Most importantly, we found that RvD1 inhibited the phosphorylation of TAK1 (TGF-β–activated kinase 1), a key upstream regulatory kinase common to both the MAPK and NF-κB pathways, by inhibiting the formation of a poly(I:C)-induced signaling complex composed of TAK1, TAB1 (TAK1 binding protein), and TRAF6 (TNF receptor–associated factor 6). We confirmed that ALX/FPR2 and GPR32, two RvD1 receptors, were expressed on human small airway epithelial cells. Furthermore, blocking these receptors abrogated the inhibitory action of RvD1. In this article, we present the idea that RvD1 has the potential to be used as an anti-inflammatory and proresolving agent, possibly in the context of exuberant host responses to damaging respirable agents such as viruses.** ***The Journal of Immunology*****, 2014, 193: 4980–4987.**

The human respiratory tract acts as the front line of defense against inhaled hazards such as air pollution, viral and bacterial pathogens, and smoky toxicants. Inhalation of dangerous insults ideally results in transient inflammatory responses that help neutralize the threat. Inflammation is a beneficial host response as long as it is well controlled (1). However, failure to resolve inflammatory responses can lead to chronic inflammation characteristic of lung diseases, including chronic obstructive pulmonary disease (COPD), asthma, and certain microbial infections (2). Resolution of inflammation was once thought to be a passive consequence of the removal of the initiating stimulus and the winding down of inflammatory mediator production (2, 3). A new paradigm is that resolution of inflammation is an active process mediated by a new genus of lipid mediators called specialized proresolving lipid mediators (SPMs) that actively counterbalance the inflammatory response (2). SPMs are mainly derived from dietary ω-3- and ω-6–polyunsaturated fatty acids (PUFAs) and are categorized as lipoxins, resolvins, protectins, and maresins according to their specific chemical and structural assignments (2, 4). These SPMs are generated during inflammation and act as potent anti-inflammatory and proresolving agents by limiting neutrophil infiltration, enhancing macrophage uptake and clearance of apoptotic neutrophils and microbes (5), and stimulating mucosal antiviral and antibacterial responses (6–8).

Earlier studies provided evidence that diets enriched in ω-3–PUFAs are beneficial to patients with chronic lung disease, by both relieving symptoms and improving lung function (9–11). Recently, we demonstrated that resolvin D1 (RvD1), a ω-3–PUFA–derived lipid molecule, reduced acute cigarette smoke–induced lung inflammation and actively promoted resolution of inflammation after smoking cessation (5). RvD1 has also been shown to attenuate LPS-induced acute lung injury and OVA-initiated allergic airway inflammation in mice (12, 13).

Although a growing body of evidence indicates that SPMs regulate lung homeostasis and exert anti-inflammatory effects, the

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H.-M.H., R.A.F., T.H.T., R.P.P., and P.J.S. conceived the study and designed the experiments; H.-M.H., E.P.L., R.A.F., and K.M.O. performed experiments and collected data; and H.-M.H., T.H.T., E.P.L., R.A.F., R.P.P., and P.J.S. analyzed the data and wrote the manuscript.

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Abbreviations used in this article: COPD, chronic obstructive pulmonary disease; hSAEC, human small airway epithelial cell; poly(I:C), polyinosinic-polycytidylic acid; PUFA, polyunsaturated fatty acid; RvD1, resolvin D1; SPM, specialized proresolving lipid mediator; TAB1, TAK1 binding protein; TAK1, TGF-β–activated kinase 1; TRAF6, TNF receptor–associated factor 6; 5Z-OX, (5Z)-7-oxozeaenol.

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cellular targets for SPMs and their mechanism of action remain a major knowledge gap. In this study, we investigated the hypothesis that RvD1 dampens inflammatory signaling in primary human small airway epithelial cells (hSAECs). Little or nothing is known about resolvins and the effects on virally induced inflammation. To begin exploring this, we used polyinosinic-polycytidylic acid [poly(I:C)]—a double-stranded RNA analog of respiratory viruses such as respiratory syncytial virus, influenza A virus, and rhinovirus (14)—as a model stimulus, because its signaling pathways are well described and these viruses are important in inciting human lung disease. We report that RvD1 inhibits poly(I:C)-induced proinflammatory signaling in hSAECs, and we describe some of the key receptors and intracellular inflammatory signaling pathways involved.

## Materials and Methods

### *Reagents and primary Abs*

Resolvin D1 (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) was purchased from Cayman Chemical (Ann Arbor, MI). The TGF-β–activated kinase 1 (TAK1) inhibitor (5Z)-7-oxozeaenol (5Z-OX) was purchased from Tocris (Minneapolis, MN) and was dissolved in 100% ethanol according to the manufacturer's recommendation. As RvD1 and 5Z-OX were initially dissolved in 100% ethanol, control cultures were treated with the same final concentration of ethanol (<0.1%). ALX/FPR2-specific antagonist Boc-2 was purchased from Genscript (Piscataway, NJ). A GPR32-neutralizing Ab (GX71225) was purchased from GeneTex (Irvine, CA) and was used as described previously (15). Anti-FPR2 (ab26316), anti–β-actin (ab6046), and anti-GPR32 (ab79516) were purchased from Abcam (Cambridge, MA). Anti-p65 (sc-109), anti-pTAK (sc-130219), anti-TAK (sc-07162), and normal goat IgG (sc-2028) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-pERK (#9101), anti-total ERK (#4695), and normal rabbit IgG (#9300) were purchased from Cell Signaling (Boston, MA).

### *Cell culture and the treatments*

Two strains of primary hSAECs from different donors were purchased from Lonza (Allendale, NJ) and cultured in SAEC growth medium (Lonza) supplemented as recommended by the supplier, and used for experiments at early passage (16). For the preparation of SAECs containing an NF-κB–luciferase reporter, hSAECs were infected with commercially available ready-to-transduce lentiviral particles that express the firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the NF-κB transcriptional response element (SA Biosciences, Valencia, CA). Typically, hSAECs were preincubated for 24 h in basal medium (without supplements) to minimize the effect of medium components on inducing inflammatory signaling. Cells were then pretreated with RvD1 for 30 min, followed by 5 μg/ml high m.w. poly(I:C) (Invivogen, San Diego, CA) for the indicated time points.

### *ELISA*

Human IL-6 and IL-8 were measured in cell culture media by commercially available ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

### *Luciferase assay*

Cells were lysed with cell culture lysis reagent provided in the luciferase kit purchased from Promega (Madison, WI). The luciferase assay was carried out following the manufacturer's protocol.

### *Immunofluorescent staining*

SAECs were seeded onto cover slides in a 12-well culture plate. Cells were fixed and permeabilized with Cytofix/Cytoperm solution (BD Biosciences, San Jose, CA) for 15 min and subsequently washed with Cytoperm/Wash buffer following the manufacturer's protocol. Cells were then stained with anti-p65 Ab for 1 h followed by staining with Alexa 568–conjugated donkey anti-rabbit Ab (Invitrogen, Carlsbad, CA) for another 30 min on ice. Samples were counterstained and mounted with ProLong Gold antifade reagent that contains DAPI as a nuclear dye (Invitrogen).

### *RNA isolation and semiquantitative PCR*

Briefly, cells were lysed with lysis buffer provided by the manufacturer, and RNA was isolated using an RNeasy mini total RNA isolation kit according to the manufacturer's instructions (QIAGEN, Valencia, CA). RNA (1 μg) was reverse transcribed using iScript (Bio-Rad, Hercules, CA), and the cDNA was then subjected to a PCR reaction, which was performed under the following conditions: 1 cycle at 95°C for 5 min, then 40 cycles at 95°C for 10 s, 55°C for 30 s, and 72°C for 30 s, followed by inactivation at 95°C for 10 min. PCR products were then separated using the E-Gel system using a 2% precast agarose gel supplied by Invitrogen (Grand Island, NY). Primer sequences are listed below: 18sRNA: 5′-GGTCGCTCGCTCCTCTCCCA-3′; 18sRNA: 5′-AGGGGCTGACCGGGTTGGTT-3′; ALX/FPR2-F: 5′-AGTCTGCTGGCTACACTGTTC-3′; GPR2/ALX-R: 5′-AGCACCACCAATGGGAGGA-3′; GPR32-F: 5′-GTGATCGCTCTTGTTCCAGGA-3′; GPR32-R: 5′-GGACGCAGACAGGATAACCAC-3′.

### *Flow cytometric analysis*

Cells were lightly trypsinized, washed, and then stained with rabbit polyclonal Ab against GPR32 or ALX/FPR2 independent aliquots for 30 min on ice. Cells were washed and stained with Alexa Fluor 568 goat anti-rabbit for a further 30 min. Samples were run on a FACSCanto II (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR). Forward and side scatter corrections were used to exclude doublets.

### *Western blotting*

Cells were disrupted with lysis buffer with a supplement of protease (P-8340; Sigma-Aldrich, St. Louis, MO) and phosphatase inhibitors (P0044; Sigma-Aldrich). Total protein in the lysates was determined using a bicinchoninic acid detection assay (Pierce, Rockford, IL). Protein (10 μg) was separated via 12% SDS-PAGE, transferred onto an Immobilon-P membrane (Millipore), and blocked with 5% nonfat dry milk or BSA in 0.1% Tween 20 in TBS. Blots were then probed with the Abs, as indicated in the figure legends.

### *Immunoprecipitation*

Immunoprecipitation was performed as described (17). Briefly, hSAECs were washed once with ice-cold PBS and lysed in 300 μl RIPA lysis buffer (Cell Signaling) containing a final concentration of 1 mM PMSF. Cell extracts were incubated with TNF receptor–associated factor 6 (TRAF6) or TAK1 binding protein (TAB1) Abs or with a matching IgG control overnight, followed by a 2-h incubation with 50 μl washed protein A/G-Sepharose beads (Thermo Scientific, Rockford, IL). The immune complex was washed with lysis buffer four times, then boiled in SDS loading buffer and analyzed by Western blotting, as described earlier. An HRP-conjugated anti-rabbit secondary Ab was purchased from Rockland (Gilbertsville, PA) and was used to avoid the detection of pre-existing heavy and light chain IgG.

### *Statistical analysis*

All results are reported as the mean ± SD. One-way ANOVA with Bonferroni multiple comparisons was performed using GraphPad Prism version 5.0d for Mac (GraphPad Software, La Jolla CA; www.graphpad.com). A $p$ value $< 0.05$ was considered significant.

## Results

### *RvD1 attenuates poly(I:C)-induced proinflammatory signaling in hSAECs*

To assess whether RvD1 modulates poly(I:C)-induced inflammatory mediator production, we used two strains of primary hSAECs derived from different donors. These cells were pretreated with RvD1 for 30 min prior to the addition of poly(I:C). Supernatants were collected, and the levels of IL-6 and IL-8 were determined. Poly(I:C) is a powerful inflammatory agonist, as proven by its ability to induce a high level of IL-6 and IL-8 production in hSAECs (Fig. 1A–D). Pretreatment with RvD1 significantly reduced production of both proinflammatory mediators in both cell strains. (Fig. 1A–D). Previously, we and others (5, 13) showed that RvD1 promoted the resolution of inflammation in vivo when given after the insult. In this study, we next determined whether RvD1 is capable of limiting the inflammatory responses after activation by poly(I:C). RvD1 added 15 min after poly(I:C) significantly attenuated the production of IL-8 (Fig. 1F), whereas there was a nonsignificant trend toward reduced IL-6 production (Fig. 1E).

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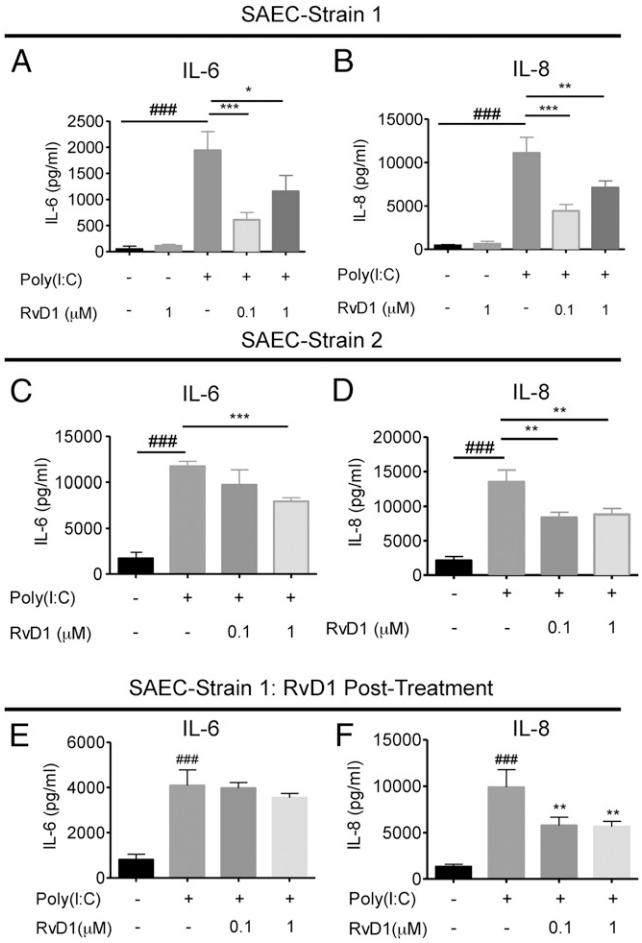


**FIGURE 1.** RvD1 attenuates poly(I:C)-induced IL-6 and IL-8 production in primary hSAECs. Two strains of human primary SAECs were pretreated with RvD1 for 30 min prior to the addition of poly(I:C) (5 μg/ml) for 24 h. Supernatants from the cultures were collected and subjected to ELISA to determine the levels of (**A** and **C**) IL-6 and (**B** and **D**) IL-8. (**E** and **F**) hSAECs were treated with poly(I:C) (5 μg/ml) for 15 min prior to treatment with RvD1 (100 nM) for an additional 24 h. Supernatants were collected, and the levels of IL-6 and IL-8 were determined as described earlier. Data shown are mean ± SD of triplicate cultures, from one representative experiment of three performed. $^{###}p < 0.001$ compared with the nontreated control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with poly(I:C)-stimulated culture, using one-way ANOVA with Bonferroni posttests.

Taken together, these results demonstrate that RvD1 attenuates production of proinflammatory mediators in hSAECs following stimulation with poly(I:C).

### *RvD1 attenuates poly(I:C)-induced MAPK and NF-κB activation*

Poly(I:C) is a potent activator of MAPK and NF-κB pathways, which are essential in upregulating the production of inflammatory mediators (16, 18). Given that RvD1 suppresses the production of poly(I:C)-induced IL-6 and IL-8, we investigated the effect of RvD1 on the NF-κB and MAPK pathways. Cells were pretreated with RvD1 for 30 min prior to the addition of poly(I:C) and harvested at key time points. As a potent MAPK activator, poly(I:C) activated ERK phosphorylation at 15 and 30 min (Fig. 2A; panel p-ERK). RvD1 inhibited ERK activation in a dose-dependent manner (Fig. 2A). We also examined MEK, the immediate upstream ERK kinase, and found that RvD1 also inhibited MEK phosphorylation in a dose-dependent manner. (Fig. 2A).

To evaluate NF-κB activity, we created a hSAEC strain that constitutively expresses a luciferase reporter under control of an NF-κB–responsive element (hSAEC/NF-κB–Luc). As a positive control, cells treated with poly(I:C) showed a significant increase in NF-κB transactivation. This activation was strongly inhibited by RvD1 (Fig. 2B). We further probed NF-κB activation by assessing p65 nuclear translocation using immunocytochemistry. Poly(I:C) elicited the translocation of p65 into the nucleus at the 1 h time point. Of interest, RvD1 inhibited poly(I:C)-induced nuclear translocation of p65 (Fig. 2C).

### *hSAECs express the RvD1 receptors GPR32 and ALX/FPR2*

Two RvD1 receptors have been identified in human cells, ALX/FPR2 and GPR32 (15, 19). However, it is unknown whether these two receptors are expressed in SAECs and, if so, whether they play roles in regulating inflammatory responses. To determine whether hSAECs express ALX/FPR2 and GPR32, we harvested RNA and analyzed the expression of these two receptors by semiquantitative PCR. Flow cytometry was performed to evaluate cell surface expression. The hSAECs contain mRNA for both receptors (Fig. 3A) and express both receptors on their cell surface (Fig. 3B).

### *The actions of RvD1 are mediated through the receptor ALX/FPR2 and GPR32*

Pharmacological inhibition of ALX/FPR2 can be achieved using a receptor antagonist (20). To determine whether RvD1 acts on SAECs via this receptor, hSAECs were pretreated with Boc-2, an ALX/FPR2-specific antagonist, for 30 min (20). Cells were then treated with or without RvD1 for another 30 min prior to the addition of poly(I:C). Poly(I:C) induced strong production of IL-6 and IL-8 that was potently attenuated by RvD1. Of interest, Boc-2 alone neither triggered an inflammatory response (Fig. 4) nor induced cell death (data not shown), whereas Boc-2 pretreatment partially neutralized the anti-inflammatory effect of RvD1 in hSAECs, implicating ALX/FPR2 as an essential component for RvD1 activation in airway epithelial cells (Fig. 4A, 4B). Moreover, Boc-2 also partially reversed the inhibitory effect of RvD1 on NF-κB activation (Fig. 4C). To further interrogate whether RvD1 also acts through GPR32, hSAECs were preincubated with a GPR32-neutralizing Ab (10 μg/ml), in the presence or absence of Boc-2 for 30 min, prior to treatment with RvD1. These cells were then stimulated with poly(I:C) for a further 24 h to elicit the production of inflammatory mediators. Consistent with the above results, RvD1 inhibited the production of IL-6 and IL-8, and this inhibition was partially blunted by the GPR32-neutralizing Ab (Fig. 4D, 4E). In the presence of both the GPR32 Ab and Boc-2, the inhibitory effect of RvD1 on production of IL-6 and IL-8 was fully reversed, indicating that the anti-inflammatory signaling by RvD1 is receptor mediated.

### *TAK1 inhibition is sufficient to ablate poly(I:C)-induced inflammation in hSAECs*

Given that RvD1 suppresses both ERK and NF-κB, we postulate that RvD1 might act on a molecule that controls both ERK and NF-κB. on the basis of literature suggesting that TAK1 is responsible for phosphorylation of both MEK and NF-κB (p65/RelA) (21), we reasoned that TAK1 could be a target of RvD1 that accounts for RvD1's dual activity. We first evaluated the role of TAK1 in poly(I:C)-induced inflammatory signaling. The hSAECs were pretreated with the TAK1-specific inhibitor 5Z-OX or RvD1 for 30 min, prior to the addition of poly(I:C). Inhibition of TAK1 was sufficient to terminate poly(I:C)-induced

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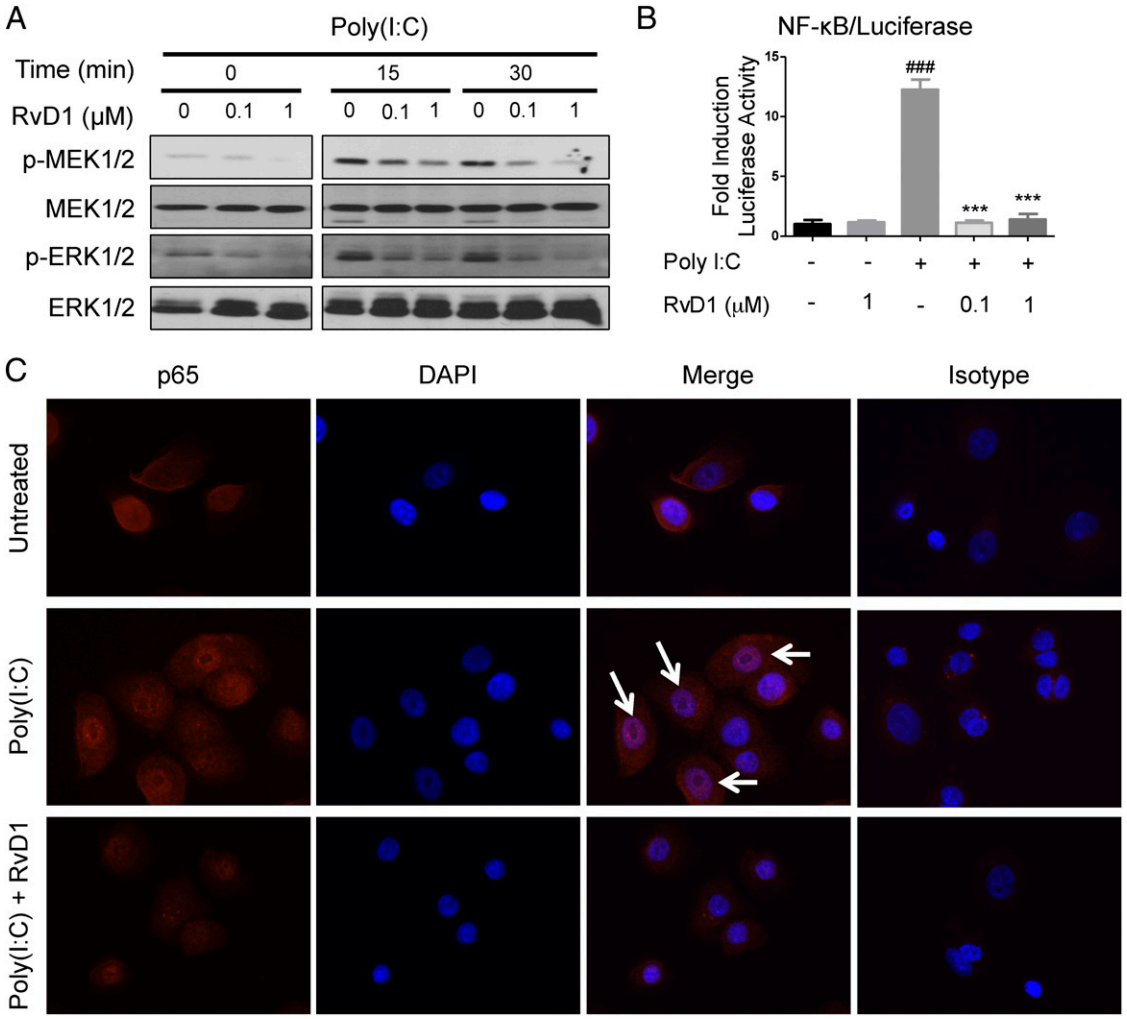


**FIGURE 2.** RvD1 attenuates poly(I:C)-induced MEK, ERK, and NF-κB activation and translocation. (**A**) SAECs were pretreated with the indicated concentration of RvD1, 30 min prior to the addition of poly(I:C) (5 μg/ml) for the indicated times. Cell lysates were collected and subjected to Western blotting to visualize the phosphorylation of MEK (p-MEK) and ERK (p-ERK). Data shown are from the same blot from one representative experiment of three. (**B**) SAECs stably expressing a luciferase reporter were pretreated with the indicated concentration of RvD1prior to the addition of poly(I:C). Cell lysates were collected, and NF-κB activation was determined by a luciferase assay. Data shown are mean ± SD of triplicate cultures, from one representative experiment of three performed. $^{###}p < 0.001$ compared with a nontreated control culture. $^{***}p < 0.001$ compared with poly(I:C)-treated culture using one-way ANOVA with Bonferroni posttests. (**C**) SAECs were pretreated with RvD1 (100 nM) for 30 min prior to stimulation with poly(I:C) (5 μg/ml) for an additional 1 h. Samples were then stained with Ab against the NF-κB subunit p65. Cells were mounted and counterstained with DAPI. Arrows indicate colocalization of p65 and the DAPI nuclear stain. The experiment was performed twice with duplicate wells in each experiment, and representative images are shown.

IL-6 and IL-8 production (Fig. 5A, 5B). Moreover, 5Z-OX treatments also inhibited poly(I:C)-induced ERK phosphorylation and NF-κB activation (Fig. 5C, 5D, respectively), suggesting that TAK1 is a main regulator of poly(I:C)-elicited inflammation in hSAECs.

*Resolvin D1 attenuates poly(I:C)-induced TAK1 activation*

To assess whether RvD1 modulates TAK1 activation, hSAECs were treated with RvD1 for 30 min prior to stimulation for 10 min with poly(I:C). We found that poly(I:C) was able to activate TAK1 phosphorylation and that this activation was suppressed by RvD1 (100 nM) (Fig. 6A). To determine if the inhibition of TAK1 phosphorylation by RvD1 was a receptor-mediated process, we pretreated the cells with or without Boc-2, and in the presence or absence of a GPR32-neutralizing Ab for 30 min, prior to addition of RvD1. Cells were then stimulated with poly(I:C) to activate inflammatory signaling. Again, in the presence of Boc-2, the inhibition of TAK1 phosphorylation by RvD1 was partially reversed (Fig. 6A, *lane 5*; Fig. 6B, *lane 4*). Pretreatment with GPR32-neutralizing Ab also partially reversed the effect of RvD1 (Fig. 6B, *lane 7*). Finally, the combination of Boc-2 and GPR32-neutralizing Ab completely reverses the inhibitory effect of RvD1 (Fig. 6B, *lane 8*).

*RvD1 interferes with the association of the poly(I:C)-induced TRAF6/TAK1/TAB1 signaling complex*

Upon stimulation with poly(I:C), TAK1 becomes activated through the formation of a signaling complex with TRAF6 and TAB1. The formation of this signaling complex has proven to be essential for TAK1 activation, which in turn activates downstream signaling, including MAPKs and NF-κB (21, 22). Given that the specific inhibition of TAK1 blocks proinflammatory signaling and that RvD1 attenuates TAK1 activation, we hypothesized that RvD1 inhibits TAK1 activation by interfering with the formation of poly(I:C)-mediated signaling components. To evaluate this hypothesis, cells were pretreated with RvD1

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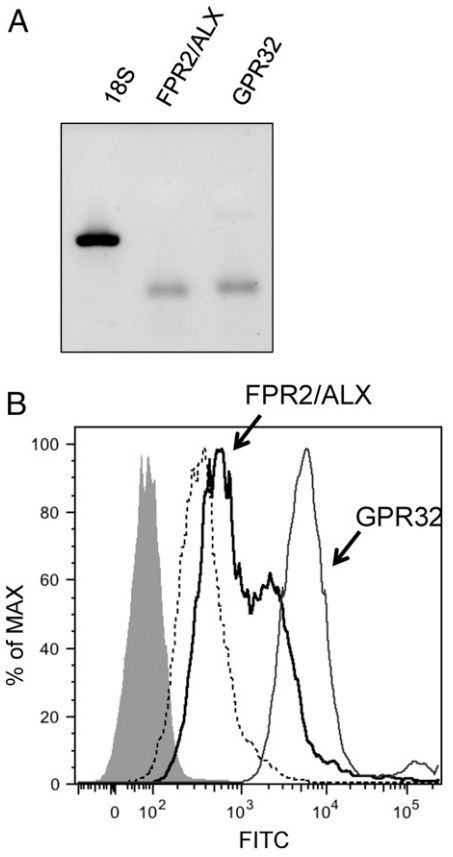


**FIGURE 3.** SAECs express the RvD1 receptors ALX/FPR2 and GPR32. (**A**) Total RNA from the SAECs was isolated and subjected to a semiquantitative PCR. PCR samples were analyzed on a 2% agarose gel. (**B**) Nonpermeabilized SAECs were stained with Abs against ALX/FPR2 (black line) or GPR32 (gray line) and analyzed by flow cytometry. Dotted line, a secondary Ab control; solid shaded area, a nonstained control.

(100 nM) for 30 min prior to stimulation by poly(I:C) for an additional 10 min. Cell lysates were collected and immunoprecipitated with TRAF6 or TAB1 Abs, followed by Western blot analyses using TAK1 Ab. In unstimulated cells, there is some association of TRAF6 and TAK1, consistent with previous reports in other cell types (Fig. 6C). Poly(I:C) strongly increased the association of TAK1 with TRAF6 and TAB1, the first time this complex has been demonstrated in hSAECs. In cell cultures pretreated with RvD1, the interaction of TAK1 with either TRAF6 or TAB1 was decreased, which suggests that RvD1 interferes with the formation of poly(I:C)-induced signaling complex (Figs. 6C, 7).

## Discussion

The airway epithelium is the first line of contact for inhaled allergens, hazardous particles, and infectious agents such as viruses. Despite continuous exposure to these noxious agents, the overall homeostasis of the airway epithelium is remarkably stable owing to tight coupling of proinflammatory and proresolving processes. Previously, resolution of inflammation was thought to be a passive process involving the deactivation of proinflammatory mediators and removal of the stimuli. Previous findings from our group and others support the concept that, in the lung, resolution is an active process controlled by a family of novel lipid-derived mediators termed SPMs (5, 12, 23, 24). Poly(I:C) is a potent stimulus that causes acute inflammatory responses in vitro and in vitro (16, 18, 25, 26) resembling acute disease in human lungs (26). In this study, we report the new findings that RvD1 suppresses the inflammatory response to poly(I:C) in airway epithelial cells through the inhibition of TAK1, leading to a subsequent reduction of both ERK and NF-κB activation. We further illustrate that RvD1 inhibits TAK1 activation, at least partly, via perturbing the association of a poly(I:C)-mediated complex involving TAK1, TAB1, and TRAF6. Overall, we provide compelling evidence to show that the anti-inflammatory actions of RvD1 are mediated by receptors ALX/FPR2 and GPR32.

Activation of epithelial cells by viral infection results in an immediate host defense response causing the production of proinflammatory mediators such as IL-6 and IL-8 (14, 27). In this study, we chose to use undifferentiated hSAECs as our in vitro model based on previous studies showing that hSAECs respond to various inflammatory stimuli and produce corresponding proinflammatory mediators similar to those in human airway pathophysiology (16, 28, 29). The production of these proinflammatory mediators can further amplify the inflammatory response by activating nearby cells, including fibroblasts, macrophages, and neutrophils (14). IL-6 and IL-8 are elevated during viral infection and during acute COPD exacerbations, and this increase is highly associated with persistent neutrophilic disease in the human airway (30). As a potent activator and chemoattractant of neutrophils, IL-8 may be a potential therapeutic target to reduce neutrophilic inflammation.

In the current study, we demonstrate that both ALX/FPR2 and GPR32 are expressed by hSAECs. We also demonstrate that by blocking these two receptors, RvD1 is no longer able to inhibit poly(I:C)-induced inflammatory responses. This finding supports the concept that both ALX/FPR2 and GPR32 are responsible for the inhibitory signaling mediated by RvD1 (15). ALX/FPR2 is known to be a receptor for several SPMs, including RvD1 and lipoxin $A_4$ (31, 32). RvD1 attenuates allergic airway inflammation via ALX/FPR2 in a mouse model (24). Of note, ALX/FPR2 can deliver either proinflammatory or proresolving signals based on its conformational status (31). ALX/FPR2 is also elevated in the lungs of patients with COPD, although whether this indicates chronic proinflammatory signaling or an unsuccessful attempt to respond to proresolving signaling is unknown (33, 34). GPR32 was recently identified as a receptor for RvD1, RvD3, and RvD5 in human cell culture models (15, 19, 35), but its role in human disease states has not yet been determined. Given that both GPR32 and ALX/FPR2 signaling is active in airway epithelial cells, it will be of therapeutic interest to use SPMs and their derived analogs to treat inflammatory airway disease caused by inhaled stimuli such as microorganisms and cigarette smoke (1, 5, 36).

In the current study, we identified TAK1 as a master regulator of multiple proinflammatory pathways and as a physiological target of RvD1 in hSAECs (Fig. 7). TAK1 was once thought to be activated by TGF-β to promote cell growth and differentiation. However, more recent data show that TAK1 is a key regulator of the immune response and inflammatory signaling that promotes tumorigenesis, fibrosis, and multiple inflammatory disorders (37). Given that TAK1 is a common regulator of many important physiologically relevant pathways, the pharmacological targeting of TAK1 may have therapeutic benefits. In our current study, we show that RvD1 treatment largely blocked the TRAF6/TAK1/TAB1 association and TAK1 phosphorylation. It is worthwhile to note that docosahexaenoic acid, a precursor of RvD1, was shown to inhibit TAK1 activation via a GPR120-dependent signaling pathway. This inhibition involves the ac-

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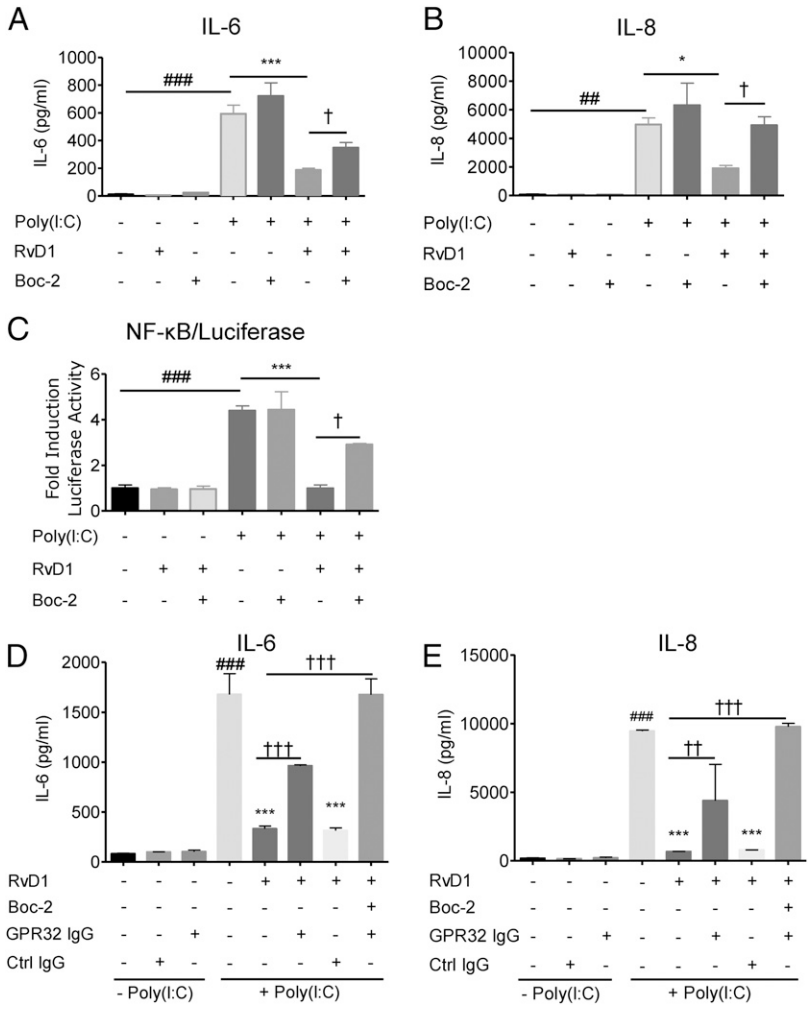


**FIGURE 4.** The actions of RvD1 are receptor mediated. SAECs were first treated with the receptor ALX/FPR2 antagonist Boc-2 (1 μM) for 30 min prior to the addition of RvD1 (100 nM) or vehicle for another 30 min. Cells were then treated with poly(I:C) (5 μg/ml) for 24 h to activate inflammatory signaling. Supernatants were collected and analyzed to determine their levels of (**A**) IL-6 and (**B**) IL-8. (**C**) SAEC/NF-κB–Luc cells were treated with Boc-2 for 30 min, followed by a treatment of RvD1 (100 nM) for another 30 min prior to the addition of poly(I:C). Cell lysates were collected and analyzed using a luciferase assay. (**D** and **E**) SAECs were treated with Boc-2 and/or a GPR32-neutralizing Ab for a total of 30 min prior to treatment with100 nM RvD1. Cells were then treated with poly(I:C) (5 μg/ml) for 24 h, and (D) IL-6 and (E) IL-8 were determined as described previously. Data shown are the mean ± SD of triplicate cultures, from one representative experiment of two performed. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with untreated control. $^{*}p < 0.05$, $^{***}p < 0.001$ compared with poly(I:C)-treated culture. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, as compared with RvD1-treated, poly(I:C)-exposed culture, by one-way ANOVA with Bonferroni posttests.

tivation of β-arrestin, which sequesters TAB1, leading to a reduction in TAK1 activation (38). Given that RvD1 inhibits TAK1 activation and activates β-arrestin, it is very likely that RvD1 also interferes with the complex formation via a similar mechanism (19, 38). It is also known that TAK1 can be negatively regulated by certain phosphatases (39, 40). As such, it

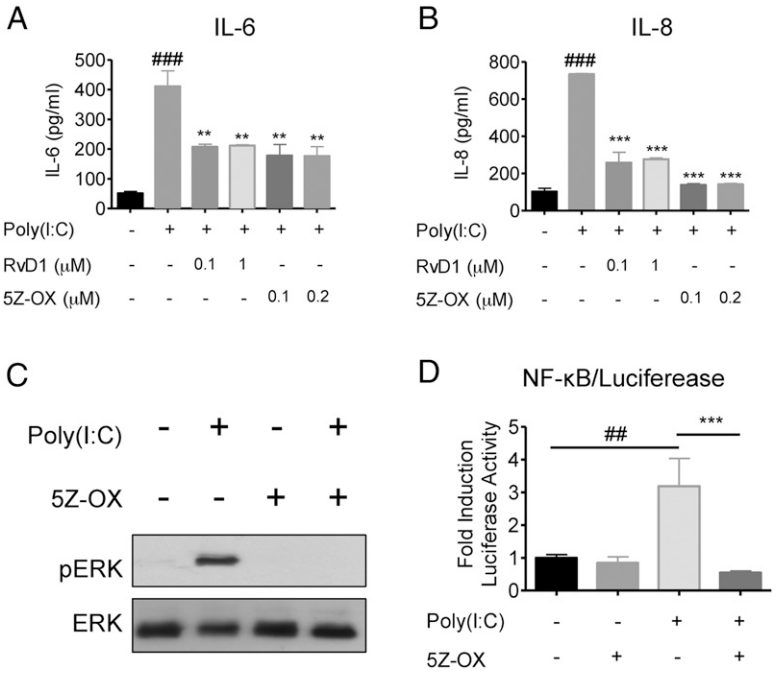


**FIGURE 5.** Blockade of TAK1 is sufficient to dampen poly(I:C)-induced inflammation in SAECs. SAECs were pretreated with either the TAK1 inhibitor 5Z-OX (100 or 200 nM) or RvD1 for 30 min prior to the addition of poly(I:C) (5 μg/ml) for 24 h. Supernatant from the culture was collected, and the levels of (**A**) IL-6 and (**B**) IL-8 were analyzed by ELISA following the manufacturer's protocol. (**C**) SAECs were pretreated with 200 nM of 5Z-OX or 100 nM of RvD1 for 30 min prior to the addition of poly(I:C) for another 15 min. Cell lysates were collected and analyzed by Western blotting to visualize ERK phosphorylation. The membrane was stripped and reprobed, for total ERK as a loading control. Data shown are from the same blot from one representative experiment of three. (**D**) SAEC/NF-κB–Luc cells were treated as previously stated. Cell lysates were collected, and the level of luciferase was determined using a luciferase assay. Data shown are mean ± SD of triplicate cultures, from one representative experiment of three performed. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with untreated culture. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with poly(I:C)-treated culture, using one-way ANOVA with Bonferroni posttests.

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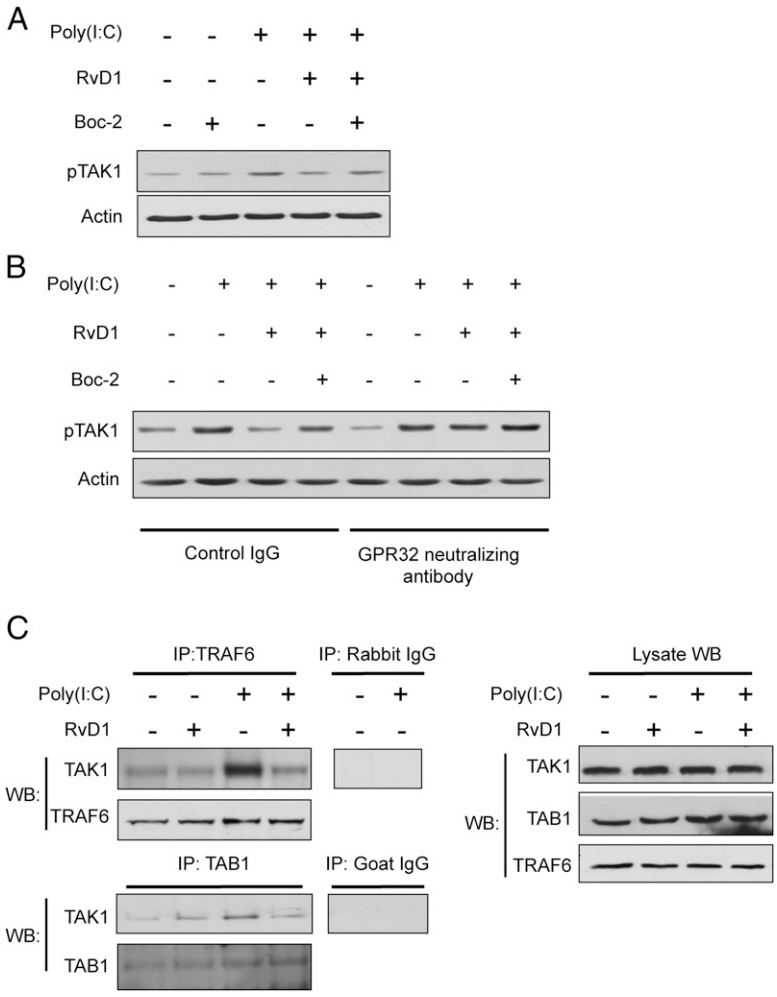


**FIGURE 6.** Blockade of FPR2/ALX and GPR32 reverses the inhibitory effect of RvD1 on poly(I:C)-induced TAK1 activation. (**A**) SAECs were pretreated with Boc-2 (1 μM) for 30 min, followed by RvD1(100nM) for an additional 30 min. Cells were then exposed to poly(I:C) (5 μg/ml) for 10 min to activate TAK1. Cell lysates were collected and TAK1 phosphorylation (p-TAK1) was analyzed by Western blotting. The same membrane was stripped and reprobed using total actin as a loading control. Data shown are from the same blot from one representative experiment of three. (**B**) SAECs were pretreated with GPR32-neutralizing Ab, with or without Boc-2, for 30 min. Cells were then treated with RvD1 (100 nM) for another 30 min, followed by poly(I:C) for 10 min. Cell lysates were collected and analyzed as described previously. Data shown are from the same blot from one representative experiment of two. Total actin was used as a loading control. (**C**) Cells were pretreated with RvD1 (100 nM) prior to the addition of poly(I:C) for an additional 10 min. The poly(I:C)-induced signaling complex was immunoprecipitated with Abs against TRAF6 or TAB1 followed by Western blotting analyses with anti-TAK1 Ab. IgG control lanes were precipitated with nonspecific rabbit or goat control Ab. Lysate indicates 1/10 input in each experiment. Levels of total TAK, TAB1, and TRAF6 in each sample were determined by Western blotting.

would be interesting to examine whether RvD1 has a direct impact on phosphatase activity.

One disease in which chronic inflammation is of critical clinical importance is COPD (emphysema and chronic bronchitis). COPD patients have increased levels of proinflammatory cytokines and cells in their lungs, exhaled breath condensate, and serum, long after smoking cessation (41). This chronic proinflammatory environment leaves them susceptible to viral and bacterial infections, resulting in a rapid decline in lung function during acute exacerbations (42). Because patient mortality increases significantly with multiple exacerbations, finding therapies that can reverse the chronic inflammation and restore immune homeostasis is a critical clinical need. The currently recommended therapy for exacerbations of COPD includes treatments with corticosteroids, which have serious side effects. It should also be noted that current therapies for lung inflammation, including lipoxygenase inhibitors and cyclooxygenase inhibitors, block the production not only of proinflammatory leukotrienes and PGs but also of anti-inflammatory and proresolving lipoxins and resolvins (43–45). This fact highlights the significant therapeutic potential of endogenous proresolving mediators.

In this article, we show that RvD1 attenuates inflammatory responses to poly(I:C), a double-stranded RNA analog of important respiratory viruses implicated in chronic inflammatory conditions, including COPD exacerbations. We propose that RvD1 will be effective in reducing excess inflammation in viral infections. Furthermore, RvD1 and other SPMs are anti-inflammatory and proresolving, without being immunosuppressive, which supports the idea that they have significant clinical potential in COPD and other diseases involving chronic inflammation and viral infection (5, 24, 46).

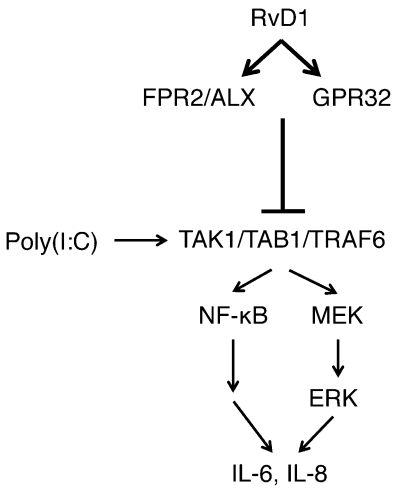


**FIGURE 7.** A diagram illustrating how RvD1 modulates poly(I:C)-induced inflammation in SAECs. In hSAECs, RvD1 potently inhibits proinflammatory signaling elicited by a viral mimetic ligand poly(I:C). RvD1 inhibits activation of TAK1, a common upstream regulatory protein of both MAPK and NF-κB pathways, via perturbing the formation of a poly(I:C)-induced signaling complex composed of TAK1, TAB1, and TRAF6. By blocking RvD1's receptors ALX/FPR2 and GPR32, the anti-inflammatory effect of RvD1 was abolished, suggesting that the actions of RvD1 are receptor dependent.

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## Disclosures

The authors have no financial conflicts of interest.

## References

1. Serhan, C. N., and N. Chiang. 2013. Resolution phase lipid mediators of inflammation: agonists of resolution. *Curr. Opin. Pharmacol.* 13: 632–640.
2. Dalli, J., R. A. Colas, and C. N. Serhan. 2013. Novel n-3 immunoresolvents: structures and actions. *Sci. Rep.* 3: 1940.
3. Henson, P. M. 1991. Resolution of inflammation. A perspective. *Chest* 99(3, Suppl)2S–6S.
4. Serhan, C. N. 2010. Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? *Am. J. Pathol.* 177: 1576–1591.
5. Hsiao, H. M., R. E. Sapinoro, T. H. Thatcher, A. Croasdell, E. P. Levy, R. A. Fulton, K. C. Olsen, S. J. Pollock, C. N. Serhan, R. P. Phipps, and P. J. Sime. 2013. A novel anti-inflammatory and pro-resolving role for resolvin D1 in acute cigarette smoke-induced lung inflammation. *PLoS ONE* 8: e58258.
6. Campbell, E. L., N. A. Louis, S. E. Tomassetti, G. O. Canny, M. Arita, C. N. Serhan, and S. P. Colgan. 2007. Resolvin E1 promotes mucosal surface clearance of neutrophils: a new paradigm for inflammatory resolution. FASEB J. 21: 3162–3170.
7. Godson, C., S. Mitchell, K. Harvey, N. A. Petasis, N. Hogg, and H. R. Brady. 2000. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J. Immunol.* 164: 1663–1667.
8. Canny, G., O. Levy, G. T. Furuta, S. Narravula-Alipati, R. B. Sisson, C. N. Serhan, and S. P. Colgan. 2002. Lipid mediator-induced expression of bactericidal/ permeability-increasing protein (BPI) in human mucosal epithelia. *Proc. Natl. Acad. Sci. USA* 99: 3902–3907.
9. Mickleborough, T. D. 2005. Dietary omega-3 polyunsaturated fatty acid supplementation and airway hyperresponsiveness in asthma. *J. Asthma* 42: 305–314.
10. Arm, J. P., C. E. Horton, B. W. Spur, J. M. Mencia-Huerta, and T. H. Lee. 1989. The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. *Am. Rev. Respir. Dis.* 139: 1395–1400.
11. Shahar, E., A. R. Folsom, S. L. Melnick, M. S. Tockman, G. W. Comstock, V. Gennaro, M. W. Higgins, P. D. Sorlie, W. J. Ko, and M. Szklo, Atherosclerosis Risk in Communities Study Investigators. 1994. Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. *N. Engl. J. Med.* 331: 228–233.
12. Wang, B., X. Gong, J. Y. Wan, L. Zhang, Z. Zhang, H. Z. Li, and S. Min. 2011. Resolvin D1 protects mice from LPS-induced acute lung injury. *Pulm. Pharmacol. Ther.* 24: 434–441.
13. Rogerio, A. P., O. Haworth, R. Croze, S. F. Oh, M. Uddin, T. Carlo, M. A. Pfeffer, R. Priluck, C. N. Serhan, and B. D. Levy. 2012. Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. *J. Immunol.* 189: 1983–1991.
14. Vareille, M., E. Kieninger, M. R. Edwards, and N. Regamey. 2011. The airway epithelium: soldier in the fight against respiratory viruses. *Clin. Microbiol. Rev.* 24: 210–229.
15. Norling, L. V., J. Dalli, R. J. Flower, C. N. Serhan, and M. Perretti. 2012. Resolvin D1 limits polymorphonuclear leukocyte recruitment to inflammatory loci: receptor-dependent actions. *Arterioscler. Thromb. Vasc. Biol.* 32: 1970–1978.
16. Ritter, M., D. Mennerich, A. Weith, and P. Seither. 2005. Characterization of Toll-like receptors in primary lung epithelial cells: strong impact of the TLR3 ligand poly(I:C) on the regulation of Toll-like receptors, adaptor proteins and inflammatory response. *J. Inflamm. (Lond.)* 2: 16.
17. Takaesu, G., J. Ninomiya-Tsuji, S. Kishida, X. Li, G. R. Stark, and K. Matsumoto. 2001. Interleukin-1 (IL-1) receptor-associated kinase leads to activation of TAK1 by inducing TAB2 translocation in the IL-1 signaling pathway. *Mol. Cell. Biol.* 21: 2475–2484.
18. Rezaee, F., N. Meednu, J. A. Emo, B. Saatian, T. J. Chapman, N. G. Naydenov, A. De Benedetto, L. A. Beck, A. I. Ivanov, and S. N. Georas. 2011. Polyinosinic: polycytidylic acid induces protein kinase D-dependent disassembly of apical junctions and barrier dysfunction in airway epithelial cells. J. Allergy Clin. Immunol. 128: 1216–1224 e1211.
19. Krishnamoorthy, S., A. Recchiuti, N. Chiang, S. Yacoubian, C. H. Lee, R. Yang, N. A. Petasis, and C. N. Serhan. 2010. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. *Proc. Natl. Acad. Sci. USA* 107: 1660–1665.
20. Stenfeldt, A. L., J. Karlsson, C. Wennerås, J. Bylund, H. Fu, and C. Dahlgren. 2007. Cyclosporin H, Boc-MLF and Boc-FLFLF are antagonists that preferentially inhibit activity triggered through the formyl peptide receptor. *Inflammation* 30: 224–229.
21. Jiang, Z., M. Zamanian-Daryoush, H. Nie, A. M. Silva, B. R. Williams, and X. Li. 2003. Poly(I-C)-induced Toll-like receptor 3 (TLR3)-mediated activation of NFkappa B and MAP kinase is through an interleukin-1 receptor-associated kinase (IRAK)-independent pathway employing the signaling components TLR3-TRAF6-TAK1-TAB2-PKR. *J. Biol. Chem.* 278: 16713–16719.
22. Ninomiya-Tsuji, J., K. Kishimoto, A. Hiyama, J. Inoue, Z. Cao, and K. Matsumoto. 1999. The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* 398: 252–256.
23. Xu, M. X., B. C. Tan, W. Zhou, T. Wei, W. H. Lai, J. W. Tan, and J. H. Dong. 2013. Resolvin D1, an endogenous lipid mediator for inactivation of inflammation-related signaling pathways in microglial cells, prevents lipopolysaccharide-induced inflammatory responses. *CNS Neurosci. Ther.* 19: 235–243.
24. Levy, B. D. 2012. Resolvin D1 and Resolvin E1 promote the resolution of allergic airway inflammation via shared and distinct molecular counter-regulatory pathways. *Front. Immunol.* 3: 390.
25. Webster Marketon, J. I., and J. Corry. 2013. Poly I:C and respiratory syncytial virus (RSV) inhibit glucocorticoid receptor (GR)-mediated transactivation in lung epithelial, but not monocytic, cell lines. *Virus Res.* 176: 303–306.
26. Lindh, H. F., H. L. Lindsay, B. R. Mayberry, and M. Forbes. 1969. Polyinosinic-cytidylic acid complex (poly I:C) and viral infections in mice. *Proc. Soc. Exp. Biol. Med.* 132: 83–87.
27. Numata, M., H. W. Chu, A. Dakhama, and D. R. Voelker. 2010. Pulmonary surfactant phosphatidylglycerol inhibits respiratory syncytial virus-induced inflammation and infection. *Proc. Natl. Acad. Sci. USA* 107: 320–325.
28. Dakhama, A., M. Kraft, R. J. Martin, and E. W. Gelfand. 2003. Induction of regulated upon activation, normal T cells expressed and secreted (RANTES) and transforming growth factor-beta 1 in airway epithelial cells by *Mycoplasma* pneumoniae. *Am. J. Respir. Cell Mol. Biol.* 29: 344–351.
29. Yadav, U. C., K. V. Ramana, L. Aguilera-Aguirre, I. Boldogh, H. A. Boulares, and S. K. Srivastava. 2009. Inhibition of aldose reductase prevents experimental allergic airway inflammation in mice. *PLoS ONE* 4: e6535.
30. Wedzicha, J. A. 2004. Role of viruses in exacerbations of chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* 1: 115–120.
31. Cooray, S. N., T. Gobbetti, T. Montero-Melendez, S. McArthur, D. Thompson, A. J. Clark, R. J. Flower, and M. Perretti. 2013. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. *Proc. Natl. Acad. Sci. USA* 110: 18232–18237.
32. Filep, J. G. 2013. Biasing the lipoxin A4/formyl peptide receptor 2 pushes inflammatory resolution. *Proc. Natl. Acad. Sci. USA* 110: 18033–18034.
33. Bozinovski, S., M. Uddin, R. Vlahos, M. Thompson, J. L. McQualter, A. S. Merritt, P. A. Wark, A. Hutchinson, L. B. Irving, B. D. Levy, and G. P. Anderson. 2012. Serum amyloid A opposes lipoxin $A_4$ to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease. *Proc. Natl. Acad. Sci. USA* 109: 935–940.
34. Bozinovski, S., D. Anthony, G. P. Anderson, L. B. Irving, B. D. Levy, and R. Vlahos. 2013. Treating neutrophilic inflammation in COPD by targeting ALX/FPR2 resolution pathways. *Pharmacol. Ther.* 140: 280–289.
35. Chiang, N., G. Fredman, F. Bäckhed, S. F. Oh, T. Vickery, B. A. Schmidt, and C. N. Serhan. 2012. Infection regulates pro-resolving mediators that lower antibiotic requirements. *Nature* 484: 524–528.
36. Morita, M., K. Kuba, A. Ichikawa, M. Nakayama, J. Katahira, R. Iwamoto, T. Watanebe, S. Sakabe, T. Daidoji, S. Nakamura, et al. 2013. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. *Cell* 153: 112–125.
37. Sakurai, H. 2012. Targeting of TAK1 in inflammatory disorders and cancer. *Trends Pharmacol. Sci.* 33: 522–530.
38. Oh, D. Y., S. Talukdar, E. J. Bae, T. Imamura, H. Morinaga, W. Fan, P. Li, W. J. Lu, S. M. Watkins, and J. M. Olefsky. 2010. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 142: 687–698.
39. Kajino, T., H. Ren, S. Iemura, T. Natsume, B. Stefansson, D. L. Brautigan, K. Matsumoto, and J. Ninomiya-Tsuji. 2006. Protein phosphatase 6 down-regulates TAK1 kinase activation in the IL-1 signaling pathway. *J. Biol. Chem.* 281: 39891–39896.
40. Zheng, H., Q. Li, R. Chen, J. Zhang, Y. Ran, X. He, S. Li, and H. B. Shu. 2013. The dual-specificity phosphatase DUSP14 negatively regulates tumor necrosis factor- and interleukin-1-induced nuclear factor-κB activation by dephosphorylating the protein kinase TAK1. *J. Biol. Chem.* 288: 819–825.
41. Barnes, P. J. 2009. The cytokine network in chronic obstructive pulmonary disease. *Am. J. Respir. Cell Mol. Biol.* 41: 631–638.
42. Wedzicha, J. A., and G. C. Donaldson. 2003. Exacerbations of chronic obstructive pulmonary disease. *Respir. Care* 48: 1204–1213; discussion 1213–1205.
43. Schwab, J. M., N. Chiang, M. Arita, and C. N. Serhan. 2007. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* 447: 869–874.
44. Levy, B. D., C. B. Clish, B. Schmidt, K. Gronert, and C. N. Serhan. 2001. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* 2: 612–619.
45. Serhan, C. N., and N. Chiang. 2008. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br. J. Pharmacol.* 153(Suppl 1): S200–S215.
46. Lee, H. N., J. K. Kundu, Y. N. Cha, and Y. J. Surh. 2013. Resolvin D1 stimulates efferocytosis through p50/p50-mediated suppression of tumor necrosis factor-α expression. *J. Cell Sci.* 126: 4037–4047.

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