Resolvin D1 and Resolvin E1 Promote the Resolution of Allergic Airway Inflammation via Shared and Distinct Molecular Counter-Regulatory Pathways

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Accessibility
Resolvins D1 and resolvin E1 promote the resolution of allergic airway inflammation via shared and distinct molecular counter-regulatory pathways

Bruce D. Levy*

Pulmonary and Critical Care Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA

RESOLVIN SIGNALING PATHWAYS IN ASTHMA

INTRODUCTION

The recent identification of specialized mediators that promote tissue resolution from acute inflammation and injury has opened a new window for discovery of cellular and molecular mechanisms governing chronic inflammation and adaptive immunity. Chronic “unresolved” inflammation is a pathologic response that is associated with several common human diseases for which there is no cure. Asthma is an exemplary illness with chronic inflammation that has a lifetime prevalence of nearly 1 in 10 in Western countries (Fanta, 2009). The chronic inflammation in asthma consists of airway infiltration of eosinophils and T-lymphocytes with increased levels of pro-phlogistic cytokines and lipid mediators (Busse and Lemanske, 2001). Of interest, many patients with uncontrolled lung inflammation, including clinically severe asthma, display a defect in the generation of specialized pro-resolving mediators (Levy et al., 2005, 2007; Planaguma et al., 2008; Table 1), consistent with a failure to establish sufficient protective counter-regulatory pathways in the asthmatic lung.

Catabasis is a healthy host tissue response to noxious stimuli. The literal definition of catabasis refers to a military retreat and the term has been adopted for use to describe the resolution process of returning an inflamed or injured tissue to homeostasis after the “battle” of inflammation. Catabasis requires well orchestrated cellular responses in which soluble mediators appear to play critical roles (Serhan, 2007). For resolution (Majno, 1996), restitution of endothelial and epithelial cell barrier integrity is necessary to prevent continued edema formation. Additional granulocyte recruitment is blocked and those granulocytes that have infiltrated the tissue undergo programmed cell death. The apoptotic cells are then cleared, principally by macrophages. The phagocytes also clear tissue microbes and debris, and structural cells re-establish organ function. One family of mediators for these pro-resolving cellular actions is the resolvins, which are enzymatically derived from the omega-3 fatty acids eicosapentaenoic acid (i.e., E-series resolvins) and docosahexaenoic acid (i.e., D-series resolvins; Serhan et al., 2000, 2002). Together with the lipoxins, protectins, and maresins, the resolvins comprise a new genus of endogenous, specialized pro-resolving mediators (Serhan, 2007). These mediators serve as agonists at select receptors to transduce their cell type specific pro-resolving actions (Serhan, 2007).

Defects in resolvin signaling pathways can be resolution “toxic” in model systems, leading to conversion of acute inflammatory responses to more chronic pathologic inflammation (Schwab et al., 2007), supporting a potential link for defective resolvin signaling to chronic inflammatory diseases. Of interest, there appears to be population heterogeneity in resolution mechanisms. Randomly

Keywords: resolution, resolvins, inflammation, lung, asthma

Table 1

| Table 1 |
|------------------|------------------|
| ALX/FPR2, lipoxin A4 receptor/formyl peptide receptor 2 | AT-RvD1, aspirin-triggered-resolvin D1 (75,8R,17R-trihydroxy-docosa-4Z,8E,11Z,13Z,15E,19Z-hexaenoic acid); AT-RvD2, aspirin-triggered-resolvin D2 (75,8R,17R-trihydroxy-docosa-4Z,8E,10Z,12E,14E,19Z-hexaenoic acid); AT-RvD3, aspirin-triggered-resolvin D3 (45,11,17S-trihydroxy-docosa-5,7E,9E,13Z,15E,19Z-hexaenoic acid); AT-RvD4, aspirin-triggered-resolvin D4 (45,3R,17R-trihydroxy-docosa-6E,8E,10Z,12E,15E,19Z-hexaenoic acid); B4E8, bronchovasculare large fluid; BLT1, LTb4 receptor; CMKLR1, chemokine receptor-like-1; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LC-UV-MS/MS, liquid chromatography-ulaviolet spectrometry-tandem mass spectrometry; LOX, lipoxygenase; LTB4, leukotriene B4 (5S,12R-dihydroxy-eicosa-6Z,8E,10E,14Z-tetraenoic acid); LXA4, lipoxin A4 (5S,6R,15S-trihydroxy-eicosa-7E,9E,11Z,13E-tetraenoic acid); PD1, protectin D1 (10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid); RvD1, resolvin D1 (75,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid); RvD2, resolvin D2 (75,16R,17S-trihydroxy-docosa-4Z,8E,10Z,12E,14E,19Z-hexaenoic acid); RvD3, resolvin D3 (45,11,17S-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid); RvD4, resolvin D4 (45,17S-trihydroxy-6E,8E,10Z,12E,15Z,19Z-docosahexaenoic acid); RvE1, resolvin E1 (5S,12R,16R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid); RvE2, resolvin E2 (5S,18S-dihydroxy-8Z,11Z,14Z,16E-eicosapentaenoic acid); SAA, serum amyloid A; TGF-β, transforming growth factor-beta. |
**Table 1 | Uncontrolled lung inflammation – A defect in specialized pro-resolving mediators.**

<table>
<thead>
<tr>
<th>Pro-resolving mediator</th>
<th>Disease</th>
<th>Finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoxin A₄</td>
<td>Aspirin-exacerbated respiratory disease</td>
<td>Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics</td>
<td>Sarak et al. (2000), Celik et al. (2007), Yanaguchi et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Severe asthma</td>
<td>Diminished lipoxin biosynthesis in severe asthma</td>
<td>Levy et al. (2005), Vachier et al. (2005), Celik et al. (2007), Planaguma et al. (2008), Bhavsar et al. (2010), Wu et al. (2010), Fritscher et al. (2012)</td>
</tr>
<tr>
<td>Bronchoconstriction in asthma</td>
<td>Protocols against bronchoprovocation by either LTC₄ or exercise</td>
<td>Tahan et al. (2008), Christie et al. (1992)</td>
<td></td>
</tr>
<tr>
<td>Asthma exacerbation</td>
<td>Decreased lipoxin levels in exhaled breath during exacerbation</td>
<td>Hasan et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Decreased generation and actions in cystic fibrosis</td>
<td>Karp et al. (2004), Yang et al. (2012), Chiron et al. (2008), Mattoscio et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Scleroderma lung disease</td>
<td>Decreased lipoxin levels in BALFs</td>
<td>Koval-Bielecka et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>Resolvin E₁</td>
<td>Cystic fibrosis</td>
<td>Decreased resolvin E₁ levels in cystic fibrosis with a relationship to lung function</td>
<td>Yang et al. (2012)</td>
</tr>
<tr>
<td>Protectin D₁</td>
<td>Asthma exacerbation</td>
<td>Decreased Protectin D₁ in uncontrolled asthma</td>
<td>Levy et al. (2007)</td>
</tr>
</tbody>
</table>

selected healthy individuals display significant differences in the pace of resolution for acute exudative inflammation with segregation of these apparently healthy subjects into discrete cohorts of rapid and delayed resolvers (Morris et al., 2010) that may be partially explained by genetic variability, which has recently been linked to inflammatory disease (Simiele et al., 2011). In health, the conversion of acute to chronic airway inflammation is prevented by endogenous pro-resolving mechanisms for tissue catabasis.

Intrinsically linked to innate immune responses, the development of adaptive immunity is essential to host defense, but unregulated adaptive inflammation can also lead to disease, including autoimmune disorders, transplant rejection, and allergy. As part of a series of review articles exploring the research theme of “Resolution of inflammation: leukocytes and molecular pathways as potential therapeutic targets,” this article will focus on the regulation of adaptive inflammatory responses by resolvins, in particular shared and distinct counter-regulatory mechanisms for resolvin E₁ and resolvin D₁ in allergic inflammation.

**RESOLVINS AND THEIR RECEPTORS IN THE LUNG**

Eicosapentaenoic acid is an essential fatty acid that can be enzymatically converted to E-series resolvins, including resolvin E₁, resolvin E₂, and resolvin E₃, during inflammation in mammals and fish (Serhan et al., 2000; Isobe et al., 2012). These mediators display stereoselective and cell type specific actions. Resolvin E₁ (5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) can transduce its biological actions by interacting with specific G-protein coupled receptors, namely chemokine-like receptor 1 (CMKL1) and leukotriene B₄ receptor 1 (BLT₁; Arita et al., 2005a; 2007). RvE₁ serves as an agonist for CMKL1, which is expressed in macrophages, dendritic cells (DCs), natural killer (NK) cells, and other T cells, and RvE₁ serves as a receptor antagonist at BLT₁ for leukotriene B₄. BLT₁ is expressed on granulocytes, T cells, and macrophages. Resolvin E₁ and resolvin E₂ (5S, 18R-dihydroxy-6E,8Z,11Z,14Z,16E-eicosapentaenoic acid) are generated via the actions of 5-lipoxygenase (ALOX5) from a common precursor 18-hydroxyeicosapentaenoic acid (18-HEPE) with two parallel stereospecific pathways (Arita et al., 2005a; 2007; Tjonahen et al., 2006; Oh et al., 2012). Resolvin E₃ [17,18(R/S)-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid] is distinct from RvE₁ and RvE₂ because it is generated via the actions of 15-lipoxygenase (ALOX15; Isobe et al., 2012). Since ALOX5 and ALOX12/15 are generally compartmentalized into distinct leukocyte classes, neutrophils (ALOX5) appear to play significant roles in RvE₁ and RvE₂ generation, while eosinophils (ALOX12/15) are significant in RvE₃ biosynthesis.

Docosahexaenoic acid is another essential omega-3 fatty acid that can be enzymatically converted to resolvins. In a lipoxygenase-dependent manner, DHA is transformed to D-series resolvins, including resolvin D₁–D₆, during inflammation (Serhan, 2007). These mediators also display stereoselective and cell type specific actions. Resolvin D₁ (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) transduces its biological actions by interacting with specific G-protein coupled receptors, including the lipoxin A₄ receptor ALX/FPR2 and, in humans, GPR32 (Sun et al., 2007; Krishnamoorthy et al., 2010, 2012). RvD₁ serves as an agonist for both of these receptors. ALX/FPR2 is broadly expressed in many cell types, including most leukocytes as well as structural cells, such as airway epithelial cells (Chiang et al., 2006). GPR32 is expressed on phagocytes (Krishnamoorthy et al., 2010). There are also aspirin-triggered 17R D-series resolvins (AT-RvD₁–4) that are generated in human and murine tissues, including lung, and AT-RvD₁ can also interact with ALX/FPR2 and GPR32 receptors (Krishnamoorthy et al., 2012). Of note, in an aspirin-independent manner, cytochrome P450 enzymes, which are abundant in the lung, can also convert DHA to 17R-hydroxy-DHA that can serve as a precursor for 17R-RvD₁ (i.e., AT-RvD₁), so the presence of aspirin is not required for AT-RvD₁ generation.
Anti-inflammatory pathways involving CMKLR1 expressed by lung is regulated during inflammatory responses. Following acute engagement of CCRL2 on endothelial cells initiates adhesion of CMKLR1-expressing lymphoid cells through an α(4)β(1) integrin/VCAM-1-dependent mechanism. CCR2L2 expression by endothelial cells is regulated by cell activation, so CMKLR1-dependent lymphocyte adhesion to endothelial cells can be targeted to sites of inflammation, including inflamed lung (Monnier et al., 2012).

EXPRESSION OF CMKLR1 – CELL TYPE, LUNG TISSUE, ASTHMA

CMKLR1 is highly expressed in immature plasmacytoid DCs and at lower levels in myeloid DCs, macrophages, and NK cells (Arita et al., 2005a; Parolini et al., 2007). The CMKLR1 signaling pathway is structurally and functionally conserved between human and mouse. In a model of zymosan induced peritonitis, CMKLR1 deficient mice, exhibit increased inflammation, indicating that this receptor is important for counter-regulatory signaling (Cash et al., 2008). Both RvE1 and chemerin can interact with CMKLR1 and display potent anti-inflammatory properties in LPS-induced acute lung inflammation in mice, reducing neutrophil infiltration and inflammatory cytokine release in a CMKLR1-dependent manner (Luangsay et al., 2009).

The expression of CMKLR1 in plasmacytoid DCs suggests an important role in anti-viral immunity. When wild-type and CMKLR1 knock-out mice are infected by pneumonia virus of mice, the CMKLR1 deficient mice display higher mortality and morbidity, alteration of lung function, delayed viral clearance and increased neutrophil infiltration. The CMKLR1 deficient mice have a lower recruitment of plasmacytoid DCs and a reduction in type I interferon production. Recruitment of plasmacytoid DCs via CMKLR1 contributes to adaptive immune responses and viral clearance, but also enhances the inflammatory response. Anti-inflammatory pathways involving CMKLR1 expressed by non-leukocytic cells in the lung also contribute to the increased morbidity/mortality in CMKLR1 deficient mice (Bondeau et al., 2011).

Of interest, CMKLR1 signaling in acute lung inflammation appears context dependent. In a separate model of acute lung inflammation, cigarette smoke-induced lung inflammation was attenuated in CMKLR1 deficient mice with decreased levels of inflammatory chemokines and inflammatory cells. In addition, the infiltration of leukocytes persists for 14 days after cessation of smoke exposure in this model in wild-type mice, but the CMKLR1 deficient mice have a marked decrease in lung T cells at this time point (Demoor et al., 2011). Together, these findings indicate that the RvE1 receptor CMKLR1 is expressed in the lung by both leukocytes and structural cells and CMKLR1 signaling plays pivotal roles in the regulation of innate and adaptive immune cell activation in the lung.

The recruitment of CMKLR1-expressing leukocytes to the lung is regulated during inflammatory responses. Following acute LPS-induced lung inflammation, NK cells expressing CMKLR1 are recruited to the airways in a CCR2L2-dependent manner. Engagement of CCR2L2 on endothelial cells initiates adhesion of CMKLR1-expressing lymphoid cells through an α(4)β(1) integrin/VCAM-1-dependent mechanism. CCR2L2 expression by endothelial cells is regulated by cell activation, so CMKLR1-dependent lymphocyte adhesion to endothelial cells can be targeted to sites of inflammation, including inflamed lung (Monnier et al., 2012).

EXPRESSION OF ALX/FPR2 – CELL TYPE, LUNG TISSUE, ASTHMA

ALX/FPR2 is expressed in several types of leukocytes (Chiang et al., 2006), including neutrophils (Fiore et al., 1992, 1994), monocytes (Maddox and Serhan, 1996), eosinophils (Levy et al., 2002), myeloid progenitors (Stenke et al., 1991), NK cells (Ramstedt et al., 1987; Haworth et al., 2011), and activated T cells (Ariel et al., 2003), as well as resident cells such as macrophages (Godson et al., 2000), synovial fibroblasts (Sodin-Semrl et al., 2000), and intestinal epithelial cells (Gronert et al., 1998). ALX/FPR2 is expressed in murine and human lung (Planaguma et al., 2008; Rogerio et al., 2012), airway epithelial cells (Bonnans et al., 2003, 2006), and alveolar macrophages (Rogerio et al., 2012). As early as 2 h after acute lung injury or inflammation, ALX/FPR2 expression increases in mucosal epithelial cells (Bonnans et al., 2006). Counter-regulatory signaling via ALX/FPR2 has been demonstrated in vivo using ALX/FPR2 deficient mice (Dufton et al., 2010) and transgenic mice that express human ALX/FPR2 directed by a component of the myeloid CD11b promoter (Devchand et al., 2003). ALX/FPR2 deficient mice have more marked inflammatory responses with increased leukocyte adherence and emigration into inflamed tissue after ischemia-reperfusion injury and after carrageenan-induced paw edema. In addition, ALX/FPR2 knock-out mice display increased sensitivity to arthrogenic serum and fail to resolve from this chronic inflammatory arthritis (Dufton et al., 2010). Also of note, human ALX/FPR2-transgenic mice have decreased inflammatory responses and are protected from the development of allergic airway inflammation with markedly decreased eosinophil activation and tissue accumulation (Levy et al., 2002). In asthma, ALX/FPR2 receptor expression is regulated in a cell type specific manner with decreases in peripheral blood neutrophil and eosinophil expression in this chronic inflammatory condition (Planaguma et al., 2008).

Recently, in subjects with chronic obstructive pulmonary disease, serum amyloid A (SAA) was identified as a biomarker for acute exacerbations (Bozinovski et al., 2008). SAA can also interact with ALX/FPR2 receptors, and unlike RvD1 or LXA4, the SAA-ALX/FPR2 interactions are pro-inflammatory (Bozinovski et al., 2012). Because plasma levels of SAA are at least two-log orders higher than LXA4 during acute exacerbations (Bozinovski et al., 2012), the pro-inflammatory SAA-ALX/FPR2 signaling can overwhelm the pro-resolving mediator protective signaling at this receptor. The balance of ALX/FPR2 ligands during asthma and the influence of corticosteroids is a subject of on-going investigation.

ALLERGIC AIRWAY RESPONSES – AN EXPERIMENTAL MODEL OF ADAPTIVE IMMUNITY AND ASTHMA

Animal models have not been developed that fully resemble human asthma, but they are quite useful for investigation of adaptive immunity and asthma traits. To model allergic airway...
inflammation, animals are first sensitized to an allergen and then challenged by respiratory tract exposure to the same allergen (Kipsky et al., 2003; Corry and Irvin, 2006; Pichavant et al., 2007; Zosky and Sly, 2007). Roles for representative family members of D-series resolins and E-series resolins have been determined using a model in which chicken ovalbumin (OVA) serves as an allergen for in-bred mice. The animals are sensitized by intraperitoneal injection of OVA combined with the adjuvant aluminum hydroxide to initiate a strong Th2 phenotype (Aoki et al., 2008; Haworth et al., 2008, 2011; Bilal et al., 2011; Rogerio et al., 2012).

In sensitized mice, OVA aerosol challenge on four consecutive days leads to adaptive inflammation consisting of predominantly eosinophils and T-lymphocytes, in particular in small airways and alveoli (Levy, 2010). There is also perivascular inflammation. Antigen-induced responses also increase airway mucus metaplasia and hyper-responsiveness (Levy, 2010). To determine the extent of the airway hyper-responsiveness, methacholine is administered via inhalation while the mice are intubated and sedated on a ventilator circuit. A dose response curve is constructed for methacholine-initiated changes in lung resistance.

In most instances, the airway inflammation of asthma in humans does not resolve completely; however, in healthy airways, inhalation of potential allergens or provocative stimuli leads to an acute inflammatory response that is self-limited. Several classes of natural anti-inflammatory mediators, including resolins, have been identified in inflamed airways (Bilal et al., 2011; Eickmeier et al., 2012). Because the clinical presentation of asthma is after the disease has already developed, more recent research has focused on the natural factors that promote resolution of allergic airway responses and identification of potential disease mechanisms that counter these endogenous, protective signals to perpetuate inflammation and potentially maladaptive airway responses. In the murine model of allergic airway responses described above, the cessation of OVA aerosol challenge leads to self-limited lung inflammation with resolution of the adaptive immune responses within 1–2 weeks (Haworth et al., 2008). Investigation of the resolution phase of allergic airway responses has uncovered several pro-resolving molecular and cellular mechanisms for adaptive airway inflammation (Levy et al., 2007; Haworth et al., 2008, 2011; Rogerio et al., 2012).

NK cells were recently assigned important roles for clearance of antigen-specific T cells. During the natural resolution phase of allergic airway inflammation, eosinophils, and T cells decrease markedly concomitant with an increase in the numbers of NK cells in the lung and associated mediastinal lymph nodes (Haworth et al., 2011). These resolution NK cells also acquire cell surface markers, including NKG2D, consistent with NK cell activation (Haworth et al., 2011). The timely resolution of allergic airway inflammation is prolonged when NK cells are depleted, blocked from interacting with target cells, or inhibited from migrating to the lung, leading to a persistence of airway eosinophils and antigen-specific CD4 (Haworth et al., 2011) T cells (Haworth et al., 2011). Lung macrophages also serve important pro-resolving roles in the clearance of allergic airway inflammation. After the respiratory tract is exposed to allergen, lung macrophages display a significant capacity for clearance of airway antigen that was introduced during aerosol challenge (Rogerio et al., 2012). These recent findings identify important pro-resolving roles for innate lung tissue lymphocytes and macrophages in the regulation of adaptive immune responses.

**ACTIONS OF RvD1 AND RvE1 IN ALLERGIC AIRWAY INFLAMMATION**

LC-MS/MS-based lipido-metabolomic analyses of inflamed murine lung reveals picogram quantities of RvD1 and RvE1 that can be increased several fold with increased substrate availability (Bilal et al., 2011; Eickmeier et al., 2012). In asthma, airway mucosal epithelial cells have depleted stores of docosahexaenoic acid (Freedman et al., 2004) and lower levels of 17-hydroxy-DHA and protectin D1 in exhaled breath condensates compared with healthy control subjects (Levy et al., 2007). Recently, the potential beneficial actions of resolins in experimental models of airway mucosal inflammation have been reported.

Lung expression of the RvD1 receptor ALX/FPR2 is induced in vivo in allergic airway inflammation (Levy et al., 2002). When RvD1 is given to OVA-sensitized mice just prior to OVA aerosol challenge, the development of allergic airway responses is significantly decreased (Rogerio et al., 2012). In particular, RvD1 markedly decreases eosinophils and levels of IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluids (BALFs), consistent with a dominant effect of the mediator on the development of Th2 adaptive inflammation. Regulation of these cytokines by RvD1 is associated with a significant increase in lung IκBα, suggesting decreased activation of NF-κB. Airway mucus metaplasia is also decreased by RvD1 with a more modest effect on airway hyper-responsiveness to methacholine. BALF levels of the counter-regulatory mediators IL-10 and LXA4 are not increased by RvD1 administration, indicating non-redundant anti-inflammatory signaling circuits for these mediators.

Potent regulation by RvD1 and AT-RvD1 of the allergen-driven accumulation of eosinophils was linked to significant decrements in BALF IL-5, eosin, and LTβ (Rogerio et al., 2012). In many asthmatics, a Th2 cytokine gene expression signature is induced (Woodruff et al., 2007), including IL-5, which is an important cytokine for the recruitment and activation of eosinophils, especially in conjunction with eotaxins (Busse and Lemanske, 2001). IL-5, eosin, NF-κB activation (Yang et al., 1998), and LTβ (Terawaki et al., 2005) can all increase airway eosinophilia. In murine allergic airway inflammation, RvD1 and AT-RvD1 decreased each of these mediators of eosinophil activation and accumulation. Eosinophils may also play important roles in airway remodeling and can generate the pro-fibrotic growth factor TGF-β1 (Wong et al., 1991). The RvD1 and AT-RvD1 mediated decrease in eosinophils and TGF-β levels (Rogerio et al., 2012) suggest additional beneficial actions for these mediators in preventing chronic airway remodeling. Further study in models of chronic inflammation is needed to address this potential tissue protective role for these D-series resolins.

When administered after acute airway inflammation is established, RvD1 significantly and rapidly decreases the allergic lung inflammation within 1 h (Rogerio et al., 2012). Because RvD1 is subject to rapid inactivation in the lung, it is impact is transient (Rogerio et al., 2012), so when RvD1 is given daily for three consecutive days, there is only a modest decrease in the BALF response.
Current anti-inflammatory therapeutic strategies for asthma include corticosteroids, CysLT1 receptor antagonists and anti-IgE antibody (Fanta, 2009). While little is known regarding the influence of these approaches on the actions of resolvins, their independent signaling pathways suggest that the resolvins...
would complement existing therapeutics. Corticosteroids share the resolvins’ anti-inflammatory actions on eosinophils and T cells; however, they do not share resolvins’ pro-resolving actions for macrophages and NK cells. CysLT1 receptor antagonists and anti-IgE antibody target specific pro-phlogistic pathways and would not be expected to interfere with the resolvins’ mechanisms of action. Regarding pharmacological considerations for the resolvins, these mediators carry potent actions in sub-nanomolar concentrations in vitro (reviewed in Serhan, 2007) and at doses of \( \sim 0.005 \text{mg/kg} \) in vivo (Haworth et al., 2008; Rogerio et al., 2012). As with the Fat-1 transgenic mouse (Bilal et al., 2011), increasing the tissue levels of omega-3 fatty acids can increase resolin formation and offer protection from allergic airway responses; however, dietary supplementation is less potent than direct administration of resolvins (Seki et al., 2010).

### SHARED AND DISTINCT PRO-RESOLVING MECHANISMS FOR RvE1 AND RvD1/AT-RvD1 IN ADAPTIVE INFLAMMATION

RvE1 and RvD1/AT-RvD1 are agonists at distinct pro-resolving receptors, yet share many similar properties in the regulation of adaptive inflammation in the lung (Figure 1, Table 2). These mediators decrease recruitment of lung eosinophils, lymphocytes, and macrophages during adaptive immune responses and decrease allergic airway responses, including mucus metaplasia and hyper-responsiveness to methacholine. In addition, these specialized pro-resolving mediators share many similarities in the regulation of lung inflammatory peptide and lipid mediators. Despite these commonalities, the RvE1 and RvD1/AT-RvD1 pro-resolving pathways are not entirely redundant. In addition to their distinct

### Table 2 | Shared and distinct pro-resolving mechanisms for RvE1 and RvD1/AT-RvD1 during murine adaptive inflammation.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>RvE1</th>
<th>RvD1/AT-RvD1</th>
<th>LXA(_4) analog</th>
</tr>
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<tr>
<td><strong>ALLERGIC AIRWAY RESPONSES</strong></td>
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<td></td>
<td></td>
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<td>Eosinophils</td>
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<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Lymphocytes</td>
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<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>NK Cells</td>
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<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Decreased</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
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<td>Mucus metaplasia</td>
<td>Decreased</td>
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<td>Decreased</td>
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<tr>
<td>MCh ED200</td>
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<td>Increased</td>
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<tr>
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<td><strong>LIPID MEDIATORS</strong></td>
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<tr>
<td>LTB(_4)</td>
<td>Decreased</td>
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<tr>
<td>CysLTs</td>
<td>No change</td>
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<td>LXA(_4)</td>
<td>Increased</td>
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**SHARED AND DISTINCT PRO-RESOLVING MECHANISMS FOR RvE1 AND RvD1/AT-RvD1 IN ADAPTIVE INFLAMMATION**

RvE1 and RvD1/AT-RvD1 are agonists at distinct pro-resolving receptors, yet share many similar properties in the regulation of adaptive inflammation in the lung (Figure 1, Table 2). These mediators decrease recruitment of lung eosinophils, lymphocytes, and macrophages during adaptive immune responses and decrease allergic airway responses, including mucus metaplasia and hyper-responsiveness to methacholine. In addition, these specialized pro-resolving mediators share many similarities in the regulation of lung inflammatory peptide and lipid mediators. Despite these commonalities, the RvE1 and RvD1/AT-RvD1 pro-resolving pathways are not entirely redundant. In addition to their distinct independent signaling pathways initiated via interactions with distinct receptors, yet they share many overlapping properties at the cellular and tissue levels.
receptors, there are clear differences between RvE1 and RvD1/AT-RvD1 in the regulation of BALF levels of IL-5, IFN-γ, and LXA₄ during resolution (Haworth et al., 2008, 2011; Rogerio et al., 2012). While there is no data on concomitant administration of RvE1 and RvD1 in this model, co-administration of RvE1 and a bioactive LXA₄ analog, which, like RvD1/AT-RvD1, interacts with ALX/FPR2 receptors, provides additive pro-resolving actions, yet there are important differences in their mechanisms. Both RvE1 and the LXA₄ analog decrease BALF levels of IL-17, but distinct from RvE1, the LXA₄ analog does not inhibit IL-23 production or increase IFN-γ levels (Haworth et al., 2008). These findings of shared and distinct points of counter-regulation for RvE1 and the LXA₄ stable analog indicate the presence of independent pro-resolving signaling circuits, likely mediated by CMKLR1 and ALX/FPR2 respectively, which in this model of adaptive immunity converge on the regulation of IL-17 to promote catabasis (Table 2). Of interest, when administered during the upstroke of allergic inflammation, both RvD1 and RvE1 are also potent regulators of the development of airway hyper-responsiveness to methacholine, mucus metaplasia, eosinophil accumulation, and T₁h2 cytokine mediator release (e.g., IL-13; Haworth et al., 2008, 2011). Regulation of IL-13 by resolvins during induction of adaptive inflammation is distinct from their actions when given during resolution. When given after the adaptive inflammation is already established, these resolvins do not lead to significant changes in BALF levels of IL-13; however, both RvD1 and RvE1 lead to marked decreases in BALF levels of IL-17. While the mechanisms that induce adaptive inflammation (i.e., Th2 cytokines) are distinct from those linked to persistent mucosal inflammation (i.e., IL-17 and IL-23), the protective actions of resolvins include regulation of both of these important families of inflammatory mediators. Inhibition of IL-17 production appears to be a common point of regulation for RvE1 and RvD1 for resolution of allergic airway responses (Serhan et al., 2000; Haworth et al., 2011).

**SUMMARY AND CONCLUSIONS**

The recent discovery of resolvins, endogenously generated from the essential fatty acids docosahexaenoic acid and eicosapentaenoic acid, has uncovered molecular and cellular mechanisms for the resolution of acute and adaptive inflammation. These specialized and stereosepecific pro-resolving chemical mediators play important roles in limiting allergic airway responses and promoting catabasis of the inflamed lung. In the lung, resolvins are enzymatically generated during cell–cell interactions, often between leukocytes and structural cells. Their interactions with specific receptors establish resolution circuits with cell type specific functional responses. While RvD1 and RvE1 share some cellular targets and pro-resolving actions, there is accumulating evidence for distinct counter-regulatory signaling pathways, including distinct receptors. In response to acute inflammation, these endogenous mediators blunt the inflammatory response by inhibiting aberrant neutrophil trafficking and activation, stimulating efferocytosis of apoptotic neutrophils and promoting anti-angiogenic, anti-fibrotic, and anti-infective actions. In allergic immune responses, resolvins enlist NK cells to facilitate clearance of activated T cells and activate macrophages (in a non-phlogistic manner) for phagocytic removal of allergen deposited in inflamed lung. Rapidly formed during inflammatory responses, these autacoids are also rapidly inactivated by eicosanoid oxidoreductases. In the setting of chronic inflammatory lung disease, airway levels of omega-3 fatty acids and pro-resolving mediators are decreased. With no curative therapy currently available for asthma or several other chronic inflammatory diseases, the development of resolvin stable analogs is leading to exciting new potential therapeutic approaches in acute and adaptive chronic inflammation that emphasize these natural homeostatic pathways.

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**Conflict of Interest Statement:** Mediators (resolvins and protectins) used and evaluated in this study have been licensed by the Brigham and Women’s Hospital (BWH) to Resolvyx. Bruce D. Levy has an equity interest in Resolvyx and receives a share of licensing income through BWH.

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