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Uncontrolled airway inflammation in lung disease represents a defect in counter-regulatory signaling

Anna Planaguma and Bruce D Levy[†]

Pulmonary & Critical Care Medicine, Brigham & Women's Hospital & Harvard Medical School, 75 Francis St, Boston, MA 02115, USA Tel.: +1 617 525 8362 Fax: +1 617 264 5133
anna_planaguma@yahoo.es

Pulmonary & Critical Care Medicine, Brigham & Women's Hospital & Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA Tel.: +1 617 525 8362 Fax: +1 617 264 5133
blevy@partners.org

Abstract

Counter-regulatory lipid mediators are generated during airway inflammation to promote resolution. Defects in the production of these lipid mediators have now been associated with several diseases of persistent airway inflammation. Lipoxins are the lead members of this class of anti-inflammatory and proresolving chemical mediators. Recently, several new families of fatty acid-derived counter-regulatory mediators have been discovered, including the resolvins and protectins. Diminished formation of these endogenous protective signals would interrupt the natural resolution of inflammation. Here, we review how rapidly emerging information on lipoxins, resolvins and protectins is providing new insights into the pathophysiology of chronic airway inflammation in several common illnesses, including asthma.

Keywords

analogs; asthma; inflammation; lipoxins; mediators; resolution

Chronic diseases of airway inflammation are common conditions that do not have curative therapy and lead to excess morbidity. Examples include asthma, cystic fibrosis and chronic obstructive pulmonary disease. We have become accustomed to thinking of inflammatory diseases as conditions precipitated by an overabundance of provocative stimuli. By contrast, an emerging body of evidence supports a new view that these conditions can also result from defective counter-regulatory signaling. Here, we review the discovery of natural anti-inflammatory and proresolving chemical mediators and their dysregulation in chronic and uncontrolled airway inflammation.

Acute inflammation is a natural host response to injury, noxious stimuli or infection that commonly resolves in healthy individuals [1]. Other potential fates for acute inflammation include chronic inflammation, scarring and exudate formation. Chronic inflammation is a prolonged, dysregulated and ultimately mal-adaptive response that involves active

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[†]Author for correspondence: Pulmonary & Critical Care Medicine, Brigham & Women's Hospital & Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA ■ Tel.: +1 617 525 8362 ■ Fax: +1 617 264 5133 ■ blevy@partners.org.

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inflammation, tissue destruction and failed attempts at tissue repair. During chronic inflammation, there is a multicellular leukocytic infiltrate that interacts with tissue resident cells, including alveolar macrophages and airway epithelial cells. In asthma in particular, persistent airway inflammation is characterized by mucosal infiltration of eosinophils and T lymphocytes and by elaboration of lipid and peptide mediators that regulate the host inflammatory response to inhaled allergens [2]. Chronic disorders of allergy and airway inflammation, such as asthma, are increasing in prevalence, afflicting as many as 25% of the population in the developed world [3].

Asthma is the prototypical example of chronic airway inflammation. Despite several effective therapies to dampen inflammation, broncho-dilate and prevent bronchoconstriction, asthma still has a high morbidity in many parts of the world [2,4]. Of note, no therapies are available to completely resolve asthma. Most patients with persistent asthma have mild to moderate disease. Their symptoms are controlled to a great extent by anti-inflammatory therapies, particularly inhaled corticosteroids. Long-term use of corticosteroids can be associated with significant side effects that highlight the need for additional therapeutics [4]. A minority of asthma patients do not respond to either inhaled or systemic corticosteroids and are classified as having severe asthma. While this group comprises only 5-10% of all asthmatics, it accounts for the majority of the disease-related adverse outcomes, including hospitalizations, emergency room visits and mortality [5]. Severe asthma is characterized by persistent airway inflammation, which can be heterogeneous [6], but often differs from mild and moderate asthma by increases in the number of airway polymorphonuclear neutrophils (PMNs) [7]. Thus, severe asthma has a distinct pathobiology that is not merely an extension of the processes responsible for mild to moderate asthma and in most instances the airway inflammation never resolves. Patients with severe asthma have symptoms that are refractory to current therapy and that constitute an important unmet need in asthma management.

Lipoxins & asthma

In asthma and other acute inflammatory responses, prostaglandins and leukotrienes (LTs) are lipid mediators derived from arachidonic acid that are produced in abundance and carry out potent proinflammatory actions [8]. Lipoxins (LXs) are structurally and functionally distinct chemical mediators, also derived from arachidonic acid, that possess anti-inflammatory and proresolving properties [9]. LXs are present in asthmatic lungs [10] and can prevent LT-mediated smooth muscle migration [11] and bronchoconstriction in asthmatic individuals [12]. For leukocytes, LXs are potent 'stop' signals for PMN entry into inflamed tissues and 'go' signals for monocyte locomotion and macrophage phagocytosis of apoptotic PMNs [9]. By blocking further PMN or eosinophil entry and promoting macrophage-mediated clearance of inflamed tissues, LXs promote inflammation resolution.

The pathophysiology of asthma is characterized in part by excess production of the LTs LTB₄ and cysteinyl LTs (CysLTs) [13]. These 5-lipoxygenase (5-LO)-derived lipid mediators display potent proinflammatory and bronchoconstrictive properties [13]. Thus, overproduction of LTs during asthma would increase and perpetuate airway inflammation and airflow obstruction, which are characteristic of this chronic airway disease. Several recent studies have identified defects in LX formation in human subjects with chronic or uncontrolled airway inflammation in a range of respiratory illnesses (Table 1). An imbalance between formation of the provocative LTs and the protective LXs would serve to exacerbate airway inflammation and airflow obstruction typical of the most severe variants of asthma [2].

Lung biopsies and bronchoalveolar lavage fluids from most patients with uncontrolled or severe asthma are notable for increased leukocyte accumulation, particularly eosinophils and PMNs [6,7]. If LXs are such potent regulators of granulocyte activation and trafficking, then

these pathological findings alone suggest a defect in this setting of LX formation or action. To determine LX biosynthetic capacity in asthma, peripheral venous whole blood was obtained from individuals with severe asthma and mild to moderate asthma and exposed to the divalent cation ionophore A23187 at concentrations known to activate phospholipase A₂ and 5-LO [14]. The results uncovered a dramatic difference in the amount of LXs generated *in vitro* [14]. Blood from individuals with mild to moderate asthma displayed a marked increase in LX generation (~2 ng LXA₄/ml) compared with healthy subjects (not detectable), but activated blood from those with severe asthma had significantly lower levels of LXs (~0.5 ng LXA₄/ml) than in milder asthma. To identify the enzyme responsible for decreased LX formation, phospholipase A₂, 5-LO and 15-LO activities were measured by quantitation of LTs and mono-oxygenation products of arachidonic acid (hydroxyeicosatetraenoic acid [HETEs]). In contrast to decrements in LXs, 5-LO-derived products, including LTB₄ and CysLT levels were increased two- and four-fold, respectively, in activated whole blood from severe asthma subjects [14]. 5-HETE was also increased approximately twofold, while 15-HETE levels were decreased approximately threefold in the severe asthma activated whole blood, indicative of increased 5-LO and decreased 15-LO activity. Evidence for the potent airway actions of these eicosanoids was also confirmed, as CysLT levels were inversely correlated and LXA₄ levels positively correlated with lung function (as measured by the forced expiratory volume in 1 s [FEV1] - percent-predicted values). In addition, levels of these mediators were also determined in nonstimulated whole blood [14]. Circulating LXA₄ levels were lower in severe asthma, and normalized values of LXA₄ to CysLTs in non-stimulated whole blood was also correlated with airflow obstruction. Together, these findings were consistent with a fundamental difference in arachidonic acid metabolism in whole blood from individuals with asthma of different severity, and point to 15-LO as a pivotal regulator of LX formation.

To determine if these changes in whole blood were reflective of asthmatic airways, bronchoalveolar lavage fluids (BALFs) were obtained from severe and nonsevere asthma subjects. Compared with healthy subjects, 5-LO-derived CysLTs and 15-LO-derived 15-HETE levels were increased in individuals with asthma independent of severity [15]. Interestingly, levels of LXA₄ were again markedly decreased in severe asthma BALFs (11.2 pg LXA₄/ml BALF) relative to nonsevere asthma (150.1 pg LXA₄/ml BALF). In addition, similar to whole blood, a decreased ratio of BALF LXA₄ to CysLTs sharply distinguished severe from nonsevere asthma. These findings extend to the respiratory tract, the earlier findings of dysregulated LX biosynthesis in severe asthma whole blood.

Unlike CysLTs or 15-HETE, LX biosynthesis requires the actions of more than one LO (FIGURE 1). Transcellular LX biosynthesis occurs most efficiently during cell-cell interactions and decreases when direct cell contact is prevented [16]. 5-LO activity is consistently increased in asthma and successful therapeutics have been developed to inhibit 5-LO or CysLT signaling [13]. By contrast, 15-LO is a key enzyme capable both of initiating LX biosynthesis as well as converting 5-LO-derived LTA₄ to LXs [9]. No therapies currently exist to modulate 15-LO activity. Increased expression of 15-LO in experimental animal systems and human subjects attenuates inflammation, partly via increased LX formation [17, 18].

Altered eicosanoid metabolism in severe asthma is related, in part, to changes in expression of LX biosynthetic genes [15]. Materials obtained from whole blood, endobronchial biopsy (large airways) and BAL cells (peripheral airways) were analyzed by quantitative PCR for 5-LO and both 15-LO isoforms to explore LX biosynthetic gene expression in different anatomical compartments in addition to asthma severity. Of note, expression of 15-LO-1 mRNA was decreased in BAL cells in severe asthma [15]. In this report, BALF levels of LXA₄ were decreased 15-fold in severe asthma, yet changes in the expression of 15-LO-1 and 15-LO-2 combined in BAL cells revealed only a sixfold decrease, indicating the involvement of post-

transcriptional factors to the decreased LXA₄ generation in severe asthma airways. Unlike 15-HETE, LX biosynthesis requires both 15-LO and 5-LO activity. 5-LO-derived CysLTs in BALF were increased in both severe and nonsevere asthma subjects, correlating with the 5-LO gene expression data. Since biosynthetic intermediates are shared for LTs and LXs, there is a reciprocal relationship between LT and LX formation that can be influenced by redox state, as well as several other factors [19]. Thus, altered LT and LX generation in severe asthma appears to relate to changes in eicosanoid biosynthetic gene expression, cell-cell interactions and redox state; any one of which can disrupt LX biosynthesis without also concurrently decreasing 15-HETE or LT production.

Patients with severe asthma differ from nonsevere cohorts by exposure to larger amounts of systemic and inhaled corticosteroids [5,14]. Relationships between corticosteroids and either 15-LO expression or LXA₄ have not been identified in severe asthma. While not seen in the respiratory compartments sampled or with oral corticosteroids, there was a significant correlation in nonsevere asthma whole blood between inhaled corticosteroids and decreased 15-LO-1 and 15-LO-2 expression [15]; this relationship is potentially explained by corticosteroid-induced decreases in viable circulating leukocytes, particularly eosinophils and cytokine-primed monocytes that can express 15-LO [20]. Together, these findings raise the possibility that corticosteroid therapy may adversely impact LX biosynthesis.

In addition to 5-LO and 15-LOs, COX-2 is also pivotal to resolution of airway injury and inflammation, partly via prostaglandin-mediated induction of 15-LO-1 and LX formation (FIGURE 1) [21,22]. Of note, COX-2 expression was downregulated in all samples from subjects with severe asthma [15]. Since COX-2 expression is sensitive to glucocorticoids [23], an indirect effect of steroids on LX production by decreased COX-2 expression is also possible.

Induced sputum from individuals with severe asthma also contains lower concentrations of LXA₄ than do samples from mild or moderate asthmatic subjects (Table 1) [24], indicating similar changes in LX production in both upper and lower airways. Many individuals with severe asthma are aspirin-sensitive [5] and, similar to the larger severe asthma cohort, activated whole blood from carefully phenotyped aspirin-intolerant asthmatics displays relative decrements in LXA₄ biosynthesis (Table 1) [25]. Recently, lower levels of LXA₄ were also associated with exercise-induced bronchoconstriction in asthma [26], raising the possibility that these changes in arachidonate metabolism may also be operative during asthma exacerbations. Together, analyses of patient-derived materials from cohorts with asthma from diverse cultures on three continents have all confirmed a defect in LX generation in uncontrolled and severe asthma [14,15,24,27].

Alterations in arachidonic acid metabolism have also been observed in other chronic inflammatory airway diseases. Low levels of LXs and a defect in LX signaling have been linked to excess PMN-rich inflammation in the airways of patients with cystic fibrosis (Table 1) [28]. In addition, lower LX levels are related to persistent inflammation during the progression of scleroderma lung disease (Table 1) [29]. In scleroderma lung disease, BALF LTB₄ and LTC₄ levels are increased disproportionately to LXA₄ levels, which are decreased [29,30]. Overproduction of LTB₄ with under-production of LXs in scleroderma lung disease would lead to a proinflammatory airway typical of fibrosing alveolitis.

Together, these recent results suggest that uncontrolled and more severe variants of asthma may result from a defect in LX-mediated counter-regulatory signaling, which is present in whole blood and large and small airways. The genesis of the decreased LX generation is likely multi-factorial, with some of the defect attributable to changes in gene expression and some reflective of post-transcriptional regulation, perhaps by microenvironmental factors in the lung

or current therapeutic agents. A defect in the generation of LXs in severe asthma would lead to unregulated airway inflammation and relatively unopposed LT-mediated bronchoconstriction, findings characteristic of the disease.

Omega-3 fatty acids & asthma

Epidemiological evidence supports a beneficial influence of diets with increased amounts of cold water fish (enriched in omega-3 fatty acids) on the development of asthma [31]. Recently, bioactive counter-regulatory chemical mediators, which are enzymatically derived from the two major omega-3 fatty acids in fish oils, eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), have been identified in spontaneously resolving exudates and human whole blood [9]. These families of compounds have been termed E-series resolvins (from EPA), D-series resolvins (from DHA) and protectins. Recent results in murine model systems of asthma have identified potent actions for resolvin E1 in blocking the development of experimental asthma and facilitating its resolution, in part, by increasing LXA₄ generation [32]. Human data for this compound in asthma are not available at present. Protectin D1 is a 15-LO-derived product of DHA that is generated in human asthma and displays potent inhibitory actions for both airway inflammation and hyperresponsiveness in experimental asthma [33]. Of note, protectin D1 is present in exhaled breath condensates from volunteer human subjects and levels of protectin D1 are lower during asthma exacerbations (Table 1) [33]. This suggests that, together with the LX decrements in severe asthma, an overall decrease in the capacity to generate 15-LO-derived counter-regulatory lipid mediators during uncontrolled asthma may occur.

Counter-regulatory lipid mediators in experimental models of lung inflammation

Experimental models of asthma have provided several important insights into the impact of LX signaling on the regulation of allergic airway inflammation and hyper-responsiveness. Using chicken ovalbumin as an allergen, mice can be sensitized and then aerosol challenged to direct the allergic response to the airway. In this setting, LX analogs that resist metabolic inactivation display potent inhibition of the development of airway inflammation and hyper-responsiveness to methacholine [34,35]. Both eosinophil and T-cell accumulation are dampened by the LX analog and levels of the important Th2 cytokines IL-5 and IL-13 are markedly decreased. [34,35]. LX analogs can be administered by intravenous, intraperitoneal and oral routes and display an IC₅₀ of approximately 0.05 mg/kg [34,35]. In a distinct murine model of asthma based on a clinically relevant allergen for human asthma, cockroach antigen can be used to sensitize and airway challenge mice to generate a robust peribronchial allergic inflammatory infiltrate and airway hyper-responsiveness [35]. LX analogs are similarly potent regulators of this model [35]. LXs and their stable analogs also prevent PMN-mediated tissue injury (reactive oxygen species), enhance bronchial epithelial cell proliferation in response to acid injury, and block TNF- α signaling for the release of proinflammatory cytokines from epithelial cells [21,36].

When animals are no longer exposed to allergen, there is a natural resolution of the allergic airway inflammation, which is notable for increased endogenous LXA₄ generation in the airways [32]. Interestingly, when animals are given a bioactive LX analog at peak inflammation, resolution of the allergic airway responses is accelerated, in part, by inhibition of IL-17 [32]. In several experimental model systems, disruption of LX biosynthesis resolution is 'toxic' for acute inflammation [21,37], consistent with pivotal roles for LXs in the natural restitution of airway homeostasis.

Lipoxin A₄ receptors

Lipoxin A₄ and bioactive LX stable analogs can interact with several classes of receptors, including the LXA₄ cognate receptor, termed ALX [38]. This receptor is a 7-transmembrane G-protein-coupled receptor that binds LXA₄ with high affinity ($K_D = 1.7$ nM) [38]. ALX is expressed on several cell types, including human PMNs, eosinophils, monocytes/macrophages, enterocytes, synovial fibroblasts and airway epithelium [38], with IL-13 and IFN- γ able to dramatically induce ALX expression *in vitro* in epithelial cells [39]. Transgenic expression in mice of the human ALX gene coupled to a component of the *CD11b* promoter blocks both allergic airway inflammation and acute airway injury from acid aspiration [21,34].

COX-2-derived eicosanoids, particularly PGE₂, regulate ALX expression in airway epithelial cells (FIGURE 1) [36]. Interestingly, ALX expression is also decreased in granulocytes from severe asthmatic whole blood [15]. Recently, we have demonstrated that ALX gene expression was decreased by 50 and 75% in nonsevere and severe asthma subjects, respectively, when compared with healthy individuals [15]. In addition, significant differences were also present for ALX expression by quantitative PCR and FACS analyses in asthma compared with healthy whole blood [15]. ALX expression is also decreased in eosinophils from subjects with both severe asthma and nonsevere asthma compared with healthy individuals. These findings indicate that ALX-receptor expression is downregulated in granulocytes in severe asthma peripheral blood and suggest a defect in LX signaling in addition to LX biosynthesis in this condition.

Lipoxin A₄ stable analogs

As LXA₄ is rapidly metabolized, LX stable analogs have been prepared to probe *in vivo* mechanisms for LX signaling and provide more potent pharmacological actions than the native compound [40]. The first generation of LXA₄ analogs were designed to resist metabolic inactivation at carbon 15 and displayed potent bioactivity *in vivo* in several murine model systems of acute inflammation [40]. For example, a bioactive LX analog administered to ovalbumin-sensitized mice prior to ovalbumin aerosol challenge dampened the development of allergic airway inflammation at low doses [34]. With further recognition of *in vivo* LX metabolism, a second generation of LXA₄ stable analogs, the 3-oxa-LXA₄ analogs, were developed to resist *in vivo* β -oxidation [41]. These 3-oxa-LXA₄ analogs also display similar anti-inflammatory and proresolving properties *in vivo* in several murine models of acute inflammation, including allergic airway inflammation [35].

Conclusion

This review highlights several new insights into the pathophysiology of persistent airway inflammation in disease. Decreased levels of counter-regulatory lipid mediators together with a defect in the expression of the related biosynthetic genes and receptors are linked to the more severe variants of asthma. The LX stable analogs represent development of pharmacological resolution agonists and provide excitement for the potential of new therapeutic strategies for future treatment of chronic and uncontrolled diseases of airway inflammation.

Future perspective

Identification of several families of counter-regulatory lipid mediators has already provided insights into the natural resolution of acute inflammation and host mechanisms for catabasis. However, with these recent discoveries come many important, and as yet unanswered, questions related to the roles of these chemical mediators in physiological and pathophysiological processes. First, bioactive counter-regulatory lipid mediators have been

identified from arachidonic acid (C20:4, C20:5 and C22:6), but how do these mediators relate to each other in terms of:

- Time course to formation:
- Inter-relationships in stimulating their production, since biosynthetic enzymes are shared in some cases (e.g., LXA₄ and protectin D1);
- Identification of additional sites of action;
- Signaling mechanisms for their cell-type specific actions.

Second, the natural resolution of inflammation is not associated with an increase in susceptibility to infections. Thus, these mechanisms would not be predicted to be immunosuppressive during physiological responses. With regard to this point, LXA₄ actually enhances host defense in gastrointestinal mucosa by inducing the expression of bactericidal/permeability increasing protein [42]. Moreover, adversely affecting the capacity of the host to generate LXs can lead to mortal consequences from pathogen-mediated inflammation in the setting of infectious challenge [43,44]. More work is needed in order to fully understand the potential mechanisms for augmenting host defense with counter-regulatory lipid mediators.

Lastly, the findings of defective generation of LXs during severe asthma and related conditions of airway inflammation and fibrosis are a sobering reminder of the importance of both pro- and anti-inflammatory signals in order to generate a controlled, self-limited response to provocative stimuli. Mechanisms underlying aberrant LX generation in airway disease are still being explored. Furthermore, early results suggest a potential for corticosteroids to impact the biosynthesis of these natural mediators of counter-regulation. Elucidation of the endogenous and pharmacological factors impinging on LX, resolvin and protectin D1 formation in severe and uncontrolled asthma are crucial areas for future investigation.

Executive summary

Lipoxins & asthma

- Lipoxins (LXs) are products of arachidonic acid metabolism that are structurally and functionally distinct from prostaglandins and leukotrienes.
- LXs are anti-inflammatory and promote resolution.
- LX production is decreased in severe asthma secondary to dysregulated expression of biosynthetic genes plus post-transcriptional factors.

Omega-3 fatty acids & asthma

- New bioactive lipid mediators derived from the omega-3 fatty acids eicosapentaenoic and docosahexaenoic acid have been identified and termed resolvins and protectins for their counter-regulatory actions.
- Protectin D1 is present in human exhaled breath condensates and its levels are decreased during asthma exacerbation.

Counter-regulatory lipid mediators in experimental models of lung inflammation

- LXA₄, LX-stable analogs, resolvin E1 and protectin D1 all block allergic airway inflammation and hyper-responsiveness in murine experimental models of asthma and can promote resolution of these asthma phenotypes.

Lipoxin A₄ receptors

- Several receptors have been identified for LXA₄ and bioactive LX-stable analogs, including LXA₄'s cognate receptor ALX.

Lipoxin A₄-stable analogs

- Elucidation of LXA₄'s metabolism has enabled the preparation of LX-stable analogs that carry potent anti-inflammatory and proresolving properties in several *in vivo* model systems.

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Figure 1. Airway lipoxin biosynthesis

When an inflammatory stimulus occurs in the airway, circulating leukocytes traffic to the inflammatory site and interact with resident airway epithelial cells. In the process of transcellular eicosanoid biosynthesis, arachidonic acid is released for enzymatic conversion to bioactive products or their biosynthetic intermediates. Airway epithelial cells prominently express 15-LO-1 and 15-LO-2, and in many cases COX-2. Airway epithelial 15-LO-1/2 can initiate LX biosynthesis by generating the intermediate 15S-H(p)ETE for export and subsequent conversion by leukocyte 5-LO to LXs. The reduced form (i.e., 15(S)-HETE) can also serve as a 5-LO substrate. In addition to these enzymatic activities, the epithelial cells can donate arachidonic acid to neighboring leukocytes for conversion by 5-LO to LTs. The 5-LO-derived LTA₄ is a pivotal intermediate capable of enzymatic conversion to LTB₄, LTC₄ or LXA₄. COX-2-derived PGE₂ can also influence LX signaling via the induction of both 15-LO-1 and ALX expression. 15S-H(p)ETE: 15S-hydroperoxy-eicosatetraenoic acid; 5-LO: 5-lipoxygenase; LT: Leukotriene; LX: Lipoxins; PGE₂: Prostaglandin E₂.

Table 1

Defective counter-regulatory signaling in lung diseases.

Lipid mediator	Disease	Biological materials	Ref.
LXA ₄	Aspirin-intolerant asthma	Activated whole blood	[25]
LXA ₄	Severe asthma	Activated whole blood BALF	[14,15]
LXA ₄	Severe asthma	Induced sputum	[24]
LXA ₄	Exercise-induced asthma	Plasma	[26]
LXA ₄	Cystic fibrosis	BALF	[28]
LXA ₄	Scleroderma lung disease	BALF	[29]
PD1	Asthma exacerbations	EBC	[33]

BALF: Bronchoalveolar lavage fluid; EBC: Exhaled breath condensate; LXA₄: Lipoxin A₄; PD1: Protectin D1.