Human Cathelicidin LL-37 Is a Chemoattractant for Eosinophils and Neutrophils That Acts via Formyl-Peptide Receptors

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Key Words
Eosinophils · Neutrophils · Chemotaxis · Antimicrobial peptides · Innate immunity · Lung inflammation

Abstract
Background: Inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by the presence of eosinophils and neutrophils. However, the mechanisms that mediate the influx of these cells are incompletely understood. Neutrophil products, including neutrophil elastase and antimicrobial peptides such as neutrophil defensins and LL-37, have been demonstrated to display chemotactic activity towards cells from both innate and adaptive immunity. However, chemotactic activity of LL-37 towards eosinophils has not been reported. Therefore, the aim of the present study was to investigate the chemotactic activity of LL-37 for eosinophils and to explore the mechanisms involved in LL-37-mediated attraction of neutrophils and eosinophils. Methods: Neutrophils and eosinophils were obtained from venous blood of healthy donors. Chemotaxis was studied using a modified Boyden chamber technique. Involvement of formyl-peptide receptors (FPRs) was studied using the antagonistic peptide tBoc-MLP. Activation of the mitogen-activated protein kinase (MAPK) ERK1/2 was studied by Western blotting using antibodies directed against phosphorylated ERK1/2. Results: Our results show that LL-37 chemoattracts both eosinophils and neutrophils. The FPR antagonistic peptide tBoc-MLP inhibited LL-37-induced chemotaxis. Whereas the FPR agonist fMLP activated ERK1/2 in neutrophils, LL-37 did not, indicating that fMLP and LL-37 deliver different signals through FPRs. Conclusions: LL-37 displays chemotactic activity for eosinophils and neutrophils, and this activity is mediated via an FPR. These results suggest that LL-37 may play a role in inflammatory lung diseases such as asthma and COPD.

Introduction
Eosinophils and neutrophils play an important role in infection and inflammation, and increased numbers have been demonstrated in various inflammatory diseases, including asthma and chronic obstructive pulmonary disease (COPD). At the site of inflammation, these granulocytes contribute to the inflammatory process by releasing a range of mediators including proteinases, antimicrobial peptides, lipid mediators and reactive oxygen intermediates. In addition to causing tissue injury, granulocyte products may contribute to the recruitment of inflammatory cells. This is illustrated by recent studies demonstrat-
ing that neutrophil-derived antimicrobial peptides like α-defensins and LL-37 mediate chemotaxis of inflammatory cells via activation of chemokine and formyl-peptide receptors (FPRs) [1, 2].

LL-37 is the C-terminal active part of the only human cathelicidin that has been identified to date, hCAP-18 [for recent reviews, see 3, 4]. Cathelicidins are a family of antimicrobial peptides that consist of a conserved N-terminal cathelin domain and a variable C-terminal antimicrobial domain, which can be cleaved off by proteinases [5, 6]. Expression of hCAP-18/LL-37 has been demonstrated in neutrophils [7], monocytes, T cells [8], keratinocytes in inflamed skin [9] and in various squamous epithelia [10]. In the airways, hCAP-18/LL-37 expression was demonstrated in bronchoalveolar lavage fluid [12]. LL-37 was originally identified as an antimicrobial peptide [13], and different studies have suggested that LL-37 plays an important role in host defense [14, 15]. However, additional functions have been described which suggest a regulatory role for LL-37 in inflammatory responses. These include LPS neutralization [16, 17], activation of epithelial cells [18, 19] and chemotactic activity towards neutrophils, monocytes, T cells and mast cells [2, 20]. Collectively, these results show that LL-37 may inhibit inflammatory responses to microbial stimuli, but may also act as a proinflammatory mediator. In addition, its ability to affect differentiation of dendritic cells [21] and chemotaxis of monocytes and T cells [2], its angiogenic activity [22] and its involvement in epithelial wound healing [23] suggest that LL-37 may also be involved in adaptive immunity and wound repair.

While LL-37 displays chemotactic activity towards different cell types from both innate and adaptive immunity, chemotactic activity towards eosinophils has not been demonstrated yet. In addition, limited data are available on mechanisms mediating LL-37-induced chemotaxis of inflammatory cells. Two receptors have been described that are involved in LL-37-mediated signaling in leukocytes, the FPR-like 1 (FPRL1) [2] and the purinergic P2X7 receptor [24]. Yang et al. [2] suggested that LL-37-induced chemotaxis is mediated by the FPRL1 based on experiments using FPRL1-transfected HEK293 cells and cross-desensitization of LL-37-induced Ca2+ fluxes in monocytes using an FPRL1-specific agonist. In contrast, mast cell chemotaxis was suggested not to be mediated via the FPRL1 [20]. Direct evidence for a role of FPRL1 and related receptors in chemotaxis of granulocytes is missing. The P2X7 receptor has been shown to mediate LL-37 induced processing and release of IL-β, but its involvement in chemotactic responses has not yet been reported.

Therefore, the aim of the present study was to investigate whether LL-37 displays chemotactic activity towards human eosinophils, and to compare the mechanisms involved in eosinophil and neutrophil chemotaxis by LL-37. Our results show that LL-37 chemoattracts both eosinophils and neutrophils via FPRs. In addition, we demonstrated that formoterol, a β2-adrenoceptor agonist widely used in the treatment of inflammatory lung disease, inhibits LL-37-induced chemotaxis. Finally, activation of the mitogen-activated protein kinase (MAPK) ERK1/2 by fMLP and LL-37 was studied. While both fMLP and LL-37 were shown to chemoattract neutrophils and eosinophils, only fMLP induced activation of the MAPK ERK1/2. The results from this study suggest that LL-37 may play a role in inflammatory lung diseases such as asthma and COPD.

Materials and Methods

LL-37 Synthesis

LL-37 (amino acid sequence LLGDFRKSKEKIGKEFKRIVQRKDFLRLNVPRTES) was synthesized by solid-phase peptide synthesis on a TentagelS AC (Rapp, Tübingen, Germany) using 9-fluorenylmethoxycarbonyl (Fmoc)/tBu chemistry, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate/N-methylmorpholine for activation and 20% piperidine in N-methyl-2-pyrrolidone for Fmoc removal. The peptide was cleaved from the resin, deprotected with trifluoroacetic acid/water and purified by reverse-phase high-performance liquid chromatography on a Vydac C18 column. The molecular mass was confirmed by Maldi-Tof mass spectrometry.

Chemotaxis Assay

Granulocytes were isolated from heparinized blood obtained from healthy volunteers by density gradient centrifugation using Percoll (density: 1.082 g/ml) as previously described [25]. Eosinophils were isolated from the mixed granulocyte population by negative selection using anti-CD16 beads [26]. The neutrophil and eosinophil suspensions were at least 95% pure. The chemotactic activity of eosinophils and neutrophils was determined using the modified Boyden chamber technique [27]. Briefly, 26 μl of chemotactic stimuli in assay buffer was added to the lower compartment and 50 μl of cell suspension (2.5 × 106 cells/ml in assay buffer) was added to the upper compartment of a 48-well chamber (Neuroprobe, Cabin John, Md., USA). The assay buffer was composed of 20 mM N-2-hydroxymethylpiperazine-N’-2-ethanesulfonic acid (Hepes buffer; pH 7.4), 132 mM NaCl, 6 mM KCl, 1.2 mM KH2PO4, 1 mM MgSO4, 5.5 mM glucose, 0.1 mM CaCl2 and 0.5% human serum albumin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) diluted 1:1 with serum-free RPMI supplemented with 2 mM L-glutamine, 20 U/ml penicillin and 20 μg/ml streptomycin. The compartments...
were separated by a lower filter with a pore width of 0.45 μm (Millipore Products, Bedford, Mass., USA) and an upper filter with a pore width of 8 μm (Sartorius Filter, San Francisco, Calif., USA). After incubation (90 min for neutrophils and 120 min for eosinophils) at 37°C, the filters were removed, fixed in ethanol-butanol (80:20, v/v), and stained with Weigert solution. Migrated cells were counted in six random high-power fields (×400). As a positive control, chemotactic activity of N-formyl-methionyl-leucyl-phenylalanine (fMLP; Sigma-Aldrich, St. Louis, Mo., USA) was used. The FPR antagonistic peptide tert-butoxy-carbonyl-methionyl-leucyl-phenylalanine (tBoc-MLP) [28] was obtained from Sigma (St. Louis, Mo., USA), the long-acting β2-adrenoceptor agonist formoterol was provided by Sigma, and the monoclonal anti-LL-37 antibody 1.10C8 was generated in our laboratory [18].

Cellular Lysates

Neutrophils were suspended in RPMI medium and incubated at 37°C. After 15 min cells were lysed by adding 1 vol lysis buffer (5 mM Tris, 100 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 1% (v/v) Triton X-100, 2 mM Na3VO4 and protease inhibitors (mini complete protease inhibitor cocktail; Boehringer Mannheim, Basel, Switzerland) in washing buffer), and incubated for 15 min on ice. After centrifugation for 5 min at 13,000 rpm at 4°C, the supernatant was collected and stored at −20°C. Protein concentrations of the lysates were measured by the bicinchoninic acid protein assay system (Pierce, Rockford, Ill., USA).

Gel Electrophoresis and Western Blotting

Gel electrophoresis and Western blotting were performed using Bio-Rad systems (Hercules, Calif., USA) according to the manufacturer’s instructions. Samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 10% glycine-based gel, and dissolved proteins were transferred to a polyvinylidene difluoride membrane. Non-specific binding sites were blocked, and the blots were incubated with rabbit polyclonal antibodies against phosphorylated ERK1/2 (New England Biolabs, Beverly, Mass., USA), and secondary horseradish peroxidase-conjugated anti-rabbit IgG antibodies. The enhanced chemoluminescent Western blotting detection system (Amersham Pharmacia Biotech, Upsala, Sweden) was used to reveal immunoreactivity.

Results

LL-37 Induces Chemotaxis of Eosinophils and Neutrophils

To study the effects of LL-37 on chemotaxis of eosinophils and neutrophils, chemotactic activity of various LL-37 concentrations or the FPR ligand fMLP (as a positive control) towards human peripheral blood eosinophils and neutrophils was evaluated using a modified Boyden chamber technique. LL-37 was shown to induce a dose-dependent chemotaxis of eosinophils and neutrophils (fig. 1). When LL-37 or fMLP was added to the upper compartment or both compartments, no increased migration was observed (table 1). These results show that LL-37 displays chemotactic rather than chemokinetic activity for eosinophils and neutrophils.

Monoclonal Anti-LL-37 Antibody Inhibits LL-37-Induced Chemotaxis

To study the specificity of LL-37-induced chemotaxis, LL-37 was preincubated for 2 h with a monoclonal anti-LL-37 antibody before neutrophil chemotaxis assay in the
Table 1. Checkerboard analysis of LL-37-induced chemotaxis of eosinophils and neutrophils

<table>
<thead>
<tr>
<th>Lower compartment</th>
<th>Upper compartment</th>
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<tbody>
<tr>
<td></td>
<td>medium</td>
<td>LL-37</td>
<td>fMLP</td>
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<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LL-37</td>
<td>23</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>fMLP</td>
<td>23</td>
<td>ND</td>
<td>13</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>29</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>LL-37</td>
<td>54</td>
<td>28</td>
<td>ND</td>
</tr>
<tr>
<td>fMLP</td>
<td>61</td>
<td>ND</td>
<td>20</td>
</tr>
</tbody>
</table>

Chemotaxis was determined using the modified Boyden chamber technique with cells present in the upper compartment. LL-37 was used at $0.8 \times 10^{-5}$ M, whereas fMLP was used at $10^{-8}$ M. ND = Not determined. Results are from a single experiment (cell count in 6 high-power fields); similar results were obtained using cells from another donor in a separate experiment.

**Fig. 2.** Effect of a monoclonal anti-LL-37 antibody on LL-37-induced chemotaxis of neutrophils. LL-37 was preincubated with the monoclonal anti-LL-37 antibody 1.10C8 and neutrophil chemotaxis by LL-37 ($0.8 \times 10^{-5}$ M) was determined using a modified Boyden chamber technique. Chemotaxis was assessed by counting cells in 6 high-power fields (HPF) and expressed as mean ± SEM of three individual experiments. Data were analyzed for statistical differences by Student’s t test for paired samples. LL-37 significantly increased chemotaxis as compared to the control. * Significant effect of anti-LL-37 monoclonal antibody on LL-37-induced chemotaxis, p < 0.05.

The presence of the monoclonal antibody. While LL-37 induced chemotaxis of neutrophils, the monoclonal anti-LL-37 antibody inhibited LL-37-induced chemotaxis (fig. 2). This confirms that the observed chemotaxis is caused by LL-37 and not by a contaminant in the LL-37 preparation used.

**LL-37-Induced Chemotaxis of Eosinophils and Neutrophils Is Mediated via Formyl-Peptide Receptors**

LL-37-induced chemotaxis of neutrophils, monocytes, and T cells was suggested to be mediated via the FPR, based on studies using FPR1-transfected HEK293 cells [2]. However, involvement of FPR1 in LL-37-induced chemotaxis of inflammatory cells was not formally demonstrated. Therefore, we studied the effect of pertussis toxin, which acts as a G-protein inhibitor by causing ADP-ribosylation of the α-subunit of G-proteins, and the FPR antagonistic peptide tBoc-MLP on LL-37-induced chemotaxis of eosinophils and neutrophils (fig. 3). Inhibition of LL-37-induced chemotaxis by pertussis toxin did not reach statistical significance (eosinophils: p = 0.0554; neutrophils: p = 0.1085). However, tBoc-MLP did significantly inhibit LL-37-induced chemotaxis, suggesting that LL-37-induced chemotaxis of both eosinophils and neutrophils is mediated via a FPR. As expected, fMLP-induced chemotaxis was fully inhibited by both pertussis toxin and tBoc-MLP.

**LL-37-Induced Chemotaxis Is Inhibited by the Long-Acting β2-Adrenoceptor Agonist Formentorol**

To study the effect of β2-agonists on LL-37-induced chemotaxis of human eosinophils and neutrophils, cells were preincubated with formoterol and LL-37-induced chemotaxis was determined using a modified Boyden chamber assay. Formoterol inhibited both LL-37- and fMLP-induced chemotaxis (fig. 4).

**Combination of LL-37 and fMLP Inhibits Neutrophil Chemotaxis**

To study the combined effect of LL-37 and fMLP on chemotaxis of neutrophils, chemotaxis assays were performed using LL-37, fMLP and a combination of fMLP and LL-37. While both LL-37 and fMLP separately increased chemotaxis of neutrophils, the combination of LL-37 and fMLP decreased chemotaxis (fig. 5).

**LL-37 Does Not Activate Mitogen-Activated Protein Kinases in Human Neutrophils**

MAPKs have been demonstrated to be involved in a variety of cellular processes, including chemotaxis. Therefore, the effect of LL-37 on MAPKs activation in human neutrophils was studied. Cells were stimulated with LL-37 or fMLP for 15 min, and activation of the MAPK
**Fig. 3.** Effect of pertussis toxin and tBoc-MLP on LL-37-induced chemotaxis of human peripheral blood eosinophils and neutrophils. Cells were preincubated with pertussis toxin (160 ng/ml) or tBoc-MLP (50 µM) and chemotaxis by LL-37 (0.8 × 10^{-5} M) and fMLP (10^{-8} M) was determined using a modified Boyden chamber technique. Cells in 6 high-power fields (HPF) were counted and expressed as mean ± SEM of three individual experiments. Data were analyzed for statistical differences by Student’s t test for paired samples. fMLP and LL-37 increased chemotaxis of both eosinophils and neutrophils as compared to the control, while only LL-37-induced chemotaxis of eosinophils did not reach statistical significance (p value 0.0821). * p < 0.05.

**Fig. 4.** Effect of formoterol on LL-37-induced chemotaxis of human peripheral blood eosinophils and neutrophils. Cells were preincubated with formoterol (10^{-4} and 10^{-6}M) and chemotaxis by LL-37 (0.8 × 10^{-5} M) and fMLP (10^{-8} M) was determined using the modified Boyden chamber technique. Cells in 6 high-power fields (HPF) were counted and expressed as mean ± SEM of three individual experiments. Data were analyzed for statistical differences by Student’s t test for paired samples. fMLP and LL-37 increased chemotaxis of both eosinophils and neutrophils as compared to the control, while only fMLP-induced chemotaxis of neutrophils did not reach statistical significance (p value 0.0687). * p < 0.05.
ERK1/2 was determined using Western blot analysis. LL-37 did not induce MAPKs activation in neutrophils, while fMLP activated ERK1/2 (fig. 6). This suggests that LL-37 and fMLP activate different cellular pathways in neutrophils.

**Discussion**

In this study, we demonstrate that LL-37 induces chemotaxis of human eosinophils and neutrophils. This effect was inhibited by the FPR antagonistic TBoc-MLP peptide, indicating the involvement of an FPR. Furthermore, the long-acting β2-adrenoceptor agonist formoterol inhibited LL-37-induced chemotaxis of both eosinophils and neutrophils, suggesting that β2-agonists may inhibit LL-37-mediated inflammatory responses. Finally, LL-37 and fMLP appeared to activate different cellular pathways, since in contrast to fMLP, LL-37 did not activate ERK1/2 in human neutrophils. The results from this study suggest that LL-37 may contribute to inflammation in inflammatory lung diseases such as asthma and COPD.

Chemotactic activity of antimicrobial peptides has been suggested to be mediated via chemokine and FPRs [29]. Human β-defensins, that are mainly produced by a variety of epithelial cells, chemotactically immature dendritic cells and memory T cells via the human CC-chemokine receptor 6 [1], while the receptor involved in neutrophil α-defensin-induced chemotaxis of T cells and immature dendritic cells has not yet been identified [30]. Cathepsin G, a serine proteinase with antimicrobial activity which is abundantly present in the azurophilic granules of neutrophils, has been demonstrated to chemotactically monocytes and neutrophils [31, 32]. Chemotactic responses to cathepsin G were shown to be mediated via the high-affinity FPR, which was supported by transfection studies using FPR-transfected cell lines [32]. In addition to the FPR, two homologues of this receptor have been described, namely FPRL1 and FPRL2 [33]. In contrast to the low-affinity FPRL1, the FPRL2 does not bind fMLP. LL-37-induced chemotaxis of neutrophils, monocytes and T cells was suggested to be mediated by the low-affinity FPRL1 [2]. This conclusion was based on experiments showing that FPRL1-transfected HEK293 cells migrate in response to LL-37 whereas ETFR cells expressing FPR do not respond. Furthermore, the authors showed cross-desensitization of LL-37-induced Ca²⁺ mobilization in monocytes by the FPRL1-specific agonist Su-peptide. In the present study the involvement of FPRs in LL-37-induced chemotaxis of human eosinophils and neutrophils was demonstrated using the FPR antagonis-

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**Fig. 5.** Effect of LL-37, fMLP and the combination of LL-37 and fMLP on chemotaxis of neutrophils. Cells were incubated with LL-37 (0.8 × 10⁻⁵ M), fMLP (10⁻⁸ M) or the combination of LL-37 (0.8 × 10⁻⁵ M) and fMLP (10⁻⁸ M), and chemotaxis was determined using the modified Boyden chamber technique. Cells in 6 high-power fields (HPF) were counted and expressed as mean ± SEM of three individual experiments. Data were analyzed for statistical differences by Student’s t test for paired samples. * p < 0.05.

**Fig. 6.** Effect of fMLP and LL-37 on ERK1/2 activation in neutrophils isolated from human peripheral blood. Cells were incubated for 15 min with various concentrations of fMLP or LL-37, and lysed in lysis buffer. Activation of ERK1/2 was determined by assessment of phosphorylated ERK1/2 (pERK1/2) in cellular lysates using Western blot analysis.
tic peptide tBoc-MLP. However, based on these results, we cannot discriminate to which FPR(s) LL-37 binds. In addition to chemotaxis, also LL-37-induced endothelial cell proliferation [22], but not LL-37-induced activation of airway epithelial cells [18] and human monocytes [34] have been suggested to be mediated via an FPR. A recent study shows that LL-37 increases processing and release of IL-1β in LPS-primed monocytes via the P2X7 receptor [24]. Furthermore, we demonstrate that while both LL-37 and fMLP induce chemotaxis of eosinophils and neutrophils via an FPR, only fMLP activates the MAPK ERK1/2 in human eosinophils and neutrophils. This suggests that LL-37 and fMLP activate different FPRs, or that they differentially signal through the same FPR. Differential signaling via the FPRL1 by multiple ligands was already demonstrated by Bae et al. [35, 36], suggesting that the proinflammatory activity of ligands of FPRs may differ and that FPR-mediated chemotaxis is associated with additional cellular activities. In addition, other receptors than FPRs may be involved in MAPK activation. Finally, we observed that the combination of LL-37 and fMLP was less effective in inducing neutrophil chemotaxis than LL-37 or fMLP alone. Whether this is explained by competition for binding sites on the same receptor, activation of different signaling cascades by both components, a parallel inhibitory effect of LL-37 on chemotaxis that is independent of FPRs, or other mechanisms, remains to be established.

The antimicrobial peptide LL-37 may affect the inflammatory process by regulating recruitment of granulocytes to inflammatory sites [2], by induction of release of the chemokine IL-8 from lung epithelial cells [18, 19] and by induction of cell death as demonstrated in airway epithelial cells and Jurkat T cells [Aarbiou, Tjabringa et al., manuscript in preparation]. Recruitment of inflammatory cells to sites of inflammation may result in a circle of inflammation via release of chemokines and antimicrobial peptides (fig. 7). Various in vivo studies have demonstrated regulation of hCAP-18/LL-37 levels in infection and inflammation. Both hCAP-18/LL-37 gene and protein expression were shown to be increased in keratinocytes from inflamed skin from psoriasis patients, and psoriatic scales were shown to contain hCAP-18/LL-37 [9]. In addition, tracheal aspirates derived from mechanically ventilated newborns with pulmonary or systemic infections were demonstrated to contain increased levels of hCAP-18/LL-37 as compared to non-infected newborns [37]. Furthermore, hCAP-18/LL-37 was found to be increased in chronic nasal inflammatory disease [38], and in cultured pharyngeal cells after Haemophilus influenzae infection [39]. In contrast, Islam et al. [40] demonstrated that Shigella spp. decreased LL-37 expression in colon epithelial cells both in vitro and in vivo, a process which was suggested to be mediated via cellular signaling through bacterial DNA. Bals et al. [14] showed that gene transfer of hCAP-18/LL-37 in a cystic fibrosis xenograft model restored bacterial killing to normal levels, suggesting that antimicrobial peptides protect against bacterial infection ex vivo. Therefore, LL-37 was suggested to be involved in infection and inflammatory diseases.

In the present study, we demonstrate that LL-37, which is abundantly present in neutrophils, chemotaxtacts both eosinophils and neutrophils. This suggests that LL-37 may play a role in both neutrophil- and eosinophil-dominated diseases including the inflammatory lung diseases COPD and asthma, which are characterized by an influx of neutrophils and eosinophils respectively. Various studies demonstrated mixed neutrophil-eosinophil
infiltration in these lung diseases. While in patients with chronic bronchitis neutrophil influx is accompanied by eosinophilic infiltration [41], in asthma not only eosinophil but also neutrophil numbers in bronchoalveolar lavage or induced sputum increase following experimental allergen exposure [42, 43] and during an exacerbation [44]. Furthermore, interactions between neutrophils and eosinophils were suggested in inflammatory lung diseases, as in a study demonstrating a more extensive eosinophil degranulation in children with acute severe asthma with a combined neutrophil/eosinophil airway inflammation, compared to patients with isolated eosinophilic inflammation [45]. In addition, eosinophil migration across layers of cultured epithelial cells was enhanced by neutrophils, suggesting involvement of neutrophil-eosinophil interactions [46]. A mechanistic basis for interactions between neutrophils and eosinophils was provided by studies suggesting that neutrophil elastase [47–49] and eosinophilic major basic protein [50, 51] may mediate these interactions. Although extrapolation of in vitro studies to the situation in vivo is difficult, our results demonstrating that neutrophil-derived LL-37 chemoattracts eosinophils suggest that LL-37 acts as a mediator in the interaction between neutrophils and eosinophils. We used relatively high concentrations of LL-37 to induce chemotaxis. However, these concentrations can be reached in the close vicinity of degranulating neutrophils. In addition, seminal plasma has been found to contain high concentrations (up to 80 µg/ml) of hCAP-18 [52]. Furthermore, as previously suggested by Yang et al. [2], the high concentrations required for LL-37 to induce chemotaxis indicate that the interaction between LL-37 and its FPR is a low-affinity interaction.

Current therapy for inflammatory lung diseases includes treatment with corticosteroids and β2-agonists like formoterol. Also MAPKs, which play an important role in various cellular processes including chemotaxis, have been evaluated as target for drug therapy. β2-Agonists act on a variety of cell types, resulting in e.g. smooth muscle cell relaxation, reduced epithelial cytokine expression and reduced infiltration of inflammatory cells [53]. The inhibitory activity of β2-agonists is not restricted to responses mediated by FPRs. In the present study, we demonstrate that the β2-adrenoceptor agonist formoterol inhibits LL-37-induced chemotaxis of both eosinophils and neutrophils, suggesting that therapeutic intervention with β2-agonists in both COPD and asthma may reduce the proinflammatory effects mediated by LL-37. Previously, formoterol has been shown to inhibit fMLP-induced chemotaxis of eosinophils [54–56]. It is likely that formoterol inhibits chemotaxis via an increase in intracellular cyclic AMP (cAMP), since other cAMP-elevating agents such as phosphodiesterase inhibitors and dibutryl cAMP also block chemotaxis [57, 58]. Since LL-37 does not activate the MAPKs ERK1/2, p38 or JNK, this suggests that therapy targeting MAPKs does not affect LL-37-induced chemotaxis of eosinophils and neutrophils.

In conclusion, we demonstrate that LL-37 chemoattracts both eosinophils and neutrophils via activation of an FPR. This suggests that LL-37 may regulate the inflammatory response by selectively attracting inflammatory cells. Since both eosinophils and neutrophils are chemoattracted by LL-37, LL-37 may be involved in both eosinophil- and neutrophil-dominated diseases. Furthermore, formoterol inhibits LL-37-induced chemotaxis of granulocytes, suggesting that therapeutic intervention of inflammatory diseases with β2-agonists may affect LL-37-mediated inflammatory processes. This study suggests an interaction of eosinophils and neutrophils via the antimicrobial peptide LL-37.

Acknowledgements

This study was supported by a grant from The Netherlands Asthma Foundation (grant 98.46) and by a research grant from AstraZeneca (Lund, Sweden).
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