

Review article

Neutrophilic airway inflammation and IL-17

A. Lindén¹, M. Adachi²

¹Department of Allergology and Respiratory Medicine, Institute of Internal Medicine, Sahlgrenska Academy, Göteborg University, Sweden; ²First Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan

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Anders Lindén, MD, PhD
C/o Lung Pharmacology Group
Department of Respiratory Medicine & Allergology
Institute of Internal Medicine
Göteborg University
Guldhedsgatan 10A
SE-413 46 Gothenburg
Sweden

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Neutrophilic airway inflammation

There is evidence from clinical studies that an exaggerated recruitment and activation of neutrophilic granulocytes in the airways is linked to the clinical course of several inflammatory diseases in the airways and lungs, such as asthma (1–6,82), nonspecific bronchial hyperreactivity (BHR) (7), chronic bronchitis (3,8–10), chronic obstructive pulmonary disease (COPD) (3,11–13), cystic fibrosis (14–17), and acute respiratory distress syndrome (18–20). The referred evidence has been gathered analysing cellular contents in bronchial tissue, in bronchoalveolar lavage (BAL) fluid, in bronchial biopsies, in induced sputum and in peripheral blood.

Pathogenetic potential of neutrophils

It is known that the number of neutrophils in the airways correlates negatively with bronchial reactivity in patients with mild asthma and with lung function in patients with COPD (5,8). Neutrophils may thus have functional impact on airways and lungs. Also, it is a fact that neutrophils are capable of releasing bioactive products that can actively contribute to the

pathogenesis of inflammatory diseases in the airways and in the lungs.

Neutrophils can release cytokines that perpetuate additional neutrophil recruitment, such as tumour necrosis factor- α (TNF- α) and the potent neutrophil chemoattractant interleukin (IL)-8, both cytokines being likely to cause bronchial hyperresponsiveness in rodent airways (21–26). It is possible that TNF- α stimulates additional neutrophil recruitment in part via stimulation of bronchial epithelial cells and subsequent release of IL-8 itself (27,28). Furthermore, the concentration of IL-8 correlates negatively with bronchial reactivity in the airways of patients with mild asthma (5), supporting a functional impact of neutrophils in the airways and lungs.

Oxygen free radicals can also be produced by neutrophils. These radicals can increase the transcription of IL-8 mRNA, leading to IL-8 protein production, subsequent neutrophil recruitment and bronchial hyperresponsiveness in canine airways (29–31). This also supports the idea that neutrophils have a functional impact in the airways and lungs.

Elastase released from neutrophils is a very potent secretagogue in airway gland cells (32,33). In addition, this specific indicator of neutrophil activity can cause bronchial hyperresponsiveness and tissue remodelling in

rodent airways *in vivo* and in collagen gels *in vitro* (34–38), thus providing additional evidence for functional impact of neutrophils in the airways and lungs. In fact, neutrophil elastase can be detected in several diseases of the airways and lungs. In severe asthma, for example, it can be detected in the submucosa of bronchi, even when no eosinophils are present, underlining the possibility that the neutrophil itself is more pivotal for developing severe asthma than is the eosinophil (6). The concentration of neutrophil elastase within the airway lumen is also increased in severe asthma, even when there is no detectable airway infection present (2–4). An increase in neutrophil elastase is also detected in the airways in chronic bronchitis and in COPD (3,39). The same is the case for cystic fibrosis and ARDS (17,19,40). Interestingly, the concentration of neutrophil elastase in the airway correlates negatively with lung function in asthma and in chronic bronchitis (3), once again supporting functional impact of neutrophils in the airways and lungs.

Taken together, the reviewed evidence thus suggest that the neutrophil may mechanistically account for gland hypersecretion, constriction and hyperreactivity of bronchial smooth muscle and for remodelling in the airways and in the lungs. The neutrophil may also perpetuate additional neutrophilic inflammation, and there is evidence that the presence and activity of the neutrophil correlates negatively with lung function in patients with inflammatory disease in the airways and lungs. Because of these facts, the endogenous mechanisms controlling neutrophil recruitment and activation constitute potentially important targets for new pharmacotherapy.

Control of neutrophil recruitment in the airways

Several different cell types in the bronchial wall, in the airway lumen and in the postcapillary venule can produce chemotactic signals to neutrophils by releasing C-X-C-chemokines, such as the potent neutrophil chemoattractant IL-8. In asthma, the bronchial epithelial cell, the macrophage, the neutrophil and even the eosinophil bear the potential of contributing to neutrophil recruitment by producing IL-8 (41–45). In chronic bronchitis, the neutrophil itself may be an important producer of IL-8 (45).

It remains a paradox that IL-8 production in bronchial epithelial cells, bronchial smooth muscle cells, fibroblasts, monocytes and in venous endothelial cells is inhibited by glucocorticoids *in vitro*, whereas the number and activity of airway neutrophils in patients with severe asthma and COPD, respectively, is not (5,39,46–51). Nor is IL-8 inhibited by a glucocorticoid in patients with COPD (39). For these reasons, it seems likely that other mechanisms than those previously characterized *in vitro* are important in controlling neutrophil recruitment in airway and lung disease.

T-lymphocytes and neutrophilic airway inflammation

There is evidence that T-lymphocytes may orchestrate the recruitment and activation of granulocytes in the airways and lungs; in particular there is such evidence for CD4⁺ lymphocytes and eosinophilic granulocytes. It is well known that the total number of lymphocytes as well as eosinophils and neutrophils is increased in the airways of patients with newly diagnosed asthma (52). Furthermore, specific blockade of CD4⁺ lymphocytes, either with an anti-CD4⁺ antibody or an anti-IL-2 receptor antibody, prevents allergen-induced recruitment of both eosinophils and neutrophils in sensitized murine airways *in vivo* (53,54). The number of CD4⁺ lymphocytes is also increased in parallel with the number of neutrophils in the airways of patients with COPD (55,56) and exposure to cigarette smoke causes recruitment of neutrophils and CD4⁺ lymphocytes in rodent airways (57). A recent study shows an increased number of CD4⁺ lymphocytes and neutrophils in bronchial tissue from patients with bronchiectasis as well (58). There is also evidence that CD4⁺ lymphocytes have a functional impact; the presence of CD4⁺ lymphocytes is related to bronchial reactivity in murine and in human airways *in vivo*, but it is not known whether this phenomenon depends upon orchestration of eosinophils or neutrophils or both (59–61). Furthermore, the mechanisms linking CD4⁺ lymphocytes to neutrophil recruitment and activation as well as to functional alterations, have remained unknown. This lack of knowledge has been in sharp contrast to the link between CD4⁺ lymphocytes and eosinophil recruitment; where for example the Th₂ cytokine IL-5 probably plays an important role (62–65).

T-lymphocytes can produce IL-17

CD4⁺ lymphocytes are capable of producing numerous cytokines. Of particular interest in the context of neutrophilic airway inflammation is the homodimer interleukin-17 (IL-17), that is constituted by 155 amino acids and has a molecular weight ranging from 15 to 22 kDa (66). Murine (m) IL-17 displays a high degree of structural homology with human (h) IL-17. The glycosylation site is highly conserved (66), a fact that is compatible with IL-17 playing an important role in the mammalian immune system.

Human and murine CD4⁺ lymphocytes can produce and subsequently release IL-17 protein when activated *in vitro* (66–68). It may be that IL-17 is produced mainly by the Th₀ and Th₁ subset of lymphocytes *in vitro*, as suggested by findings in synovial CD4⁺ cells from patients with rheumatoid arthritis (69). It may also be that CD8⁺ as well as the CD4⁺ memory T lymphocytes (CD45RO) express IL-17 mRNA under certain conditions, as suggested in isolated human peripheral blood mononuclear cells *in vitro* (70). However, the

CD4⁺ lymphocyte appears to be a more important producer of IL-17 protein than does the CD8⁺ lymphocyte from a quantitative point-of-view (67). IL-17 does not appear to be expressed under physiological conditions (66).

Lung receptors for IL-17

IL-17 receptors display a unique structure and their mRNA is expressed in a wide range of human cell types, including lung epithelial cells, foreskin fibroblasts, B cells, myelomonocytic cells as well as embryonal kidney cells (66,68,71,72). The hIL-17 receptor protein is often expressed constitutively in these cell types. Furthermore, mRNA for mIL-17 receptors is present in murine lungs and in cells such as fibroblasts, intestinal epithelial cells and T lymphocyte cell lines (72).

Stimulation with IL-17 causes expression of neutrophil-mobilizing cytokines and adhesion molecules

Human bronchial epithelial cell lines respond to stimulation with hIL-17 by releasing IL-8, after *de novo* synthesis of this specific and potent neutrophil chemo-attractant (46,73,74). This IL-8 production in response to hIL-17 is probably specific, because it is attenuated if the hIL-17 protein is co-incubated with an anti-hIL-17 antibody (46). It is noteworthy that the glucocorticoid hydrocortisone attenuates the increase in IL-8 protein caused by hIL-17 in bronchial epithelial cell lines *in vitro* (46). In these cells, co-stimulation with the Th₁ cytokine human tumour necrosis factor (hTNF)- α and hIL-17 substantially potentiates IL-8 release (46).

Human bronchial epithelial and lung fibroblast cell lines also respond to stimulation with hIL-17 by releasing the neutrophil-activating cytokine interleukin-6 (IL-6) *in vitro* (73,74). The intracellular pathways leading to this type of response appear to involve MAP kinases, even though there is conflicting data on the specific type of MAP kinase involved in human bronchial epithelial cells (74,75). Of particular interest in the context of neutrophilic airway inflammation is the fact that hIL-17 stimulates human venous endothelial cells, bronchial fibroblasts and macrophages to release IL-6 and IL-8 as well, even though the functional significance of these effects remains to be proven (46,76,77).

Finally, recent evidence suggest that whereas hIL-17 alone does not increase expression of intracellular adhesion molecule (ICAM)-1 in human bronchial epithelial cells *in vitro*, co-stimulation with hIL-17 plus the Th₁ cytokine human interferon (hIFN)- γ does augment ICAM-1 expression (75).

Neutrophil recruitment caused by IL-17

It has been shown that conditioned cell medium from human bronchial epithelial cells, that have been stimulated with hIL-17, does cause neutrophil chemotaxis *in vitro* (46). The major part of this effect is mediated via release of the C-X-C-chemokine IL-8, because pre-incubation of this medium with an anti-IL-8 antibody blocks its chemotactic activity and IL-17 *per se* does not stimulate neutrophil chemotaxis *in vitro* (46).

Human IL-17 and mIL-17 causes similar and substantial neutrophil recruitment, that lasts for at least 8 h after intratracheal administration with hIL-17 in murine airways *in vivo* (46,76,77). Pretreatment of the IL-17 protein with a neutralizing anti-hIL-17 antibody attenuates this neutrophil recruitment *in vivo*, thus proving the specific effect of IL-17 *in vivo* as well (46). The recruiting effect of IL-17 on neutrophils is as specific as that of the Th₁ cytokine murine interleukin (mIL)-1 β (76). Of potential clinical relevance is the finding that the glucocorticoid dexamethasone attenuates the effect of hIL-17 on neutrophil recruitment in murine airways *in vivo* (46).

One murine IL-8 correlate, macrophage inflammatory protein (MIP)-2, is increased in murine airways *in vivo* after stimulation with IL-17, adding evidence that an induced release of C-X-C-chemokines mediates the effect of IL-17 on neutrophils (46). In line with this, pretreatment with a neutralizing anti-MIP-2 antibody does attenuate hIL-17-induced neutrophil recruitment in murine airways *in vivo* (46).

Neutrophil recruitment by IL-17 and tachykinins

It appears as if endogenous tachykinins can modulate IL-17-induced neutrophil recruitment *in vivo*, at least in murine airways. Thus, pretreatment with peptidase inhibitors increases hIL-17-induced neutrophil recruitment in these airways (76). The magnitude of this effect is similar to that on rIL-1 β -induced neutrophil recruitment in murine airways, pointing out a universal effect of tachykinins on neutrophil recruitment (76). The endogenous enzyme responsible for this tachykinin-related modulation is most likely to be neutral endopeptidase (NEP). This is because the NEP inhibitor phosphoramidon, but not the angiotensin-converting enzyme inhibitor captopril, increases hIL-17-induced neutrophil recruitment in murine airways *in vivo* (76). It appears as if the NK-1 receptor is mediating the facilitating effect of endogenous tachykinins on neutrophil recruitment induced by hIL-17 in murine airways (76).

Neutrophil activation by IL-17

There is evidence that IL-17 can activate airway neutrophils, mainly through indirect mechanisms. Local

administration of the Th₁ cytokine rIL-1 β causes neutrophil recruitment but not an increase in elastase activity in murine airways *in vivo* (77). In contrast to rIL-1 β , local administration of hIL-17 causes neutrophil recruitment and an increase in elastase activity in the same airway model, when administered in an equally effective dose in terms of neutrophil recruitment (77). Of additional interest, local administration of a threshold dose of rIL-1 β plus hIL-17 substantially enhances the mentioned increase in elastase activity without altering neutrophil recruitment markedly (77).

Just as for elastase, local administration of hIL-17 causes an increase in myeloperoxidase (MPO) activity in murine airways *in vivo*, whereas IL-1 β does not, and there is a similar synergistic effect of rIL-1 β plus hIL-17 in terms of MPO activity (77). The referred elastase and MPO activities are likely to originate from airway neutrophils mainly for two reasons. First, the elastase activity is sensitive to a specific neutrophil elastase inhibitor and, second, the activities of elastase and MPO, respectively, correlate strongly with one another and the number of neutrophils in the airways and lungs (77).

Finally, stimulation of isolated murine neutrophils *in vitro* with hIL-17 does not cause any increase in MPO activity, a finding that is compatible with IL-17 exerting its activating effect on neutrophils through indirect mechanisms, in analogy with its indirect effect on neutrophil recruitment (77).

IL-17 in airway and lung disease

The role of IL-17 in airway and lung diseases in man remains to be established. At present, there is very limited knowledge on the expression of IL-17 in airway and lung disease in man. One recent study on a Th₁-like condition demonstrates that exposure to organic dust causes an intense neutrophilic inflammation, accompanied by an increased number of lymphocytes, in parallel with a substantial increase in IL-17 protein in the airways of previously healthy human volunteers (78). Yet another recent study on a Th₂-like condition claims an increase in immunoreactivity for hIL-17 in the airways of patients

with asthma and that eosinophils may express hIL-17 protein in these patients (79). It is of particular interest that the effect of IL-17 on airway neutrophils is indeed potentiated by pro-inflammatory cytokines such as TNF- α and IL-1 β , because the release of both these cytokines can indeed be stimulated by the very same hIL-17 in macrophages (80). Furthermore, both TNF- α and IL-1 β , just like the number of macrophages and neutrophils, are increased in the airways of patients with asthma (81).

Concluding remarks

In conclusion, there is substantial evidence that neutrophilic airway inflammation plays an active, pathogenetic role in airway and lung diseases in man. Furthermore, even though the pathogenetic role of IL-17 in airway and lung disease remains to be established, the reviewed studies support the idea that the CD4⁺ cytokine IL-17 can play a local, pro-inflammatory role in neutrophilic airway inflammation. This role is probably due to IL-17's stimulatory effects on neutrophil-mobilizing cytokines such as IL-6 and IL-8. Hypothetically, any disease characterized by activated CD4⁺ cells and mobilization of neutrophils in the airways and/or lungs may therefore involve IL-17 as a key controlling mediator, capable of orchestrating neutrophilic inflammation locally. The two published studies on IL-17 in human airway disease are compatible with IL-17 playing a role in Th₁- as well as Th₂-like airway inflammation. However, there is a clear need for more documentation on the putative presence of IL-17 in airway and lung disease characterized by neutrophilic inflammation. This type of clinical research may reveal novel targets for pharmacotherapeutical intervention against an exaggerated recruitment and activation of neutrophils in the airways and lungs.

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