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REVIEW

Neutrophils, interleukin-17A and lung disease

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ABSTRACT: It is now established that an excessive and sustained mobilisation of neutrophils is a hallmark of several chronic inflammatory lung disorders, including severe obstructive lung disease. This article reviews evidence that the cytokine interleukin (IL)-17A is a major orchestrator of sustained neutrophilic mobilisation.

Current evidence suggests that IL-17A is produced by T-lymphocytes, and that it exerts an orchestrating effect on the accumulation and associated activity of neutrophils in the bronchoalveolar space indirectly, through an induced release of specific cytokines and colony-stimulating factors in resident lung cells.

Although the involvement of IL-17A in inflammatory lung disorders is supported by several recent studies, its causative role is still uncertain.

However, the unique position of interleukin-17A at the interface between acquired and innate immunity puts this cytokine forward as an important signal for the reinforcement of host defence; it also implies that interleukin-17A may constitute a useful target for pharmacotherapeutic intervention.

KEYWORDS: Cytotoxic T-lymphocyte-associated serine esterase-8, innate immunity, interleukin-17, T-lymphocytes

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The T-lymphocyte cytokine interleukin (IL)-17(A) was discovered in 1993, and, since the first published report on the neutrophil-accumulating effect of IL-17A in the bronchoalveolar space of rats in 1999 [1–3], research in this area has generated a growing body of evidence suggesting that IL-17A plays a central role in host defence within the lungs. IL-17A exerts this effect by orchestrating the local release of neutrophil-mobilising factors in resident cells (table 1). In the present review article, the current evidence that neutrophils are important players in chronic inflammatory lung disorders and that IL-17A is involved in coordinating neutrophil accumulation in these disorders is presented. The review also addresses the potential therapeutic utility of drugs targeting IL-17A and its receptor(s).

NEUTROPHILS AND INNATE IMMUNITY

Neutrophils are believed to have evolved as first-line defenders against a wide range of pathogens, and this is particularly true for the lungs [18–23]. Functionally intact neutrophils are essential for life in mammals, including humans. Patients suffering from defects in neutrophil function, such as their formation in bone marrow, adhesion to inflamed blood vessels, extravasation, accumulation in tissue, or neutrophil oxidant and proteolytic effector functions, suffer from recurrent and severe infections [18–23]. It is noteworthy that neutrophil mobilisation is one of the earliest and most consistent consequences in the immune system after activation of pattern recognition receptors, in particular Toll-like receptors, by bacteria and other invading organisms [23–27]. Given the crucial importance of neutrophils to host defence, it is not surprising that these pathways mediate the expression of a wide range of different neutrophil-mobilising factors, including chemotactic cytokines (IL-8 and other CXC chemokines), activating factors (IL-6) and colony-stimulating factors (granulocyte (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF)) [28, 29].

ACCUMULATION OF NEUTROPHILS IN LUNG DISEASE

Beside its utility in host defence, the potent molecular armature that equips the neutrophil to destroy pathogens is also potentially harmful, if turned against host tissue. In line with such a

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TABLE 1 Functional characteristics of interleukin (IL)-17A in lungs

Cellular source	Primary targets	Synergism	Products released	*In vivo* effects
Lymphocytes [2, 4] **Granulocytes [6, 13]**	Resident cells [3, 5, 6]	TNF-α [3, 7] IL-1β [14] IFN-γ [10] IL-4 [10] IL-13 [10]	CXC chemokines [3, 8–10] IL-6 [6, 8, 9] CSFs [8, 15] Mucin [16] MPO [14] Proteases [14, 17]	Neutrophil accum. [3, 4, 11, 12]

TNF: tumour necrosis factor; accum.: accumulation; IFN: interferon; CSF: colony-stimulating factor; MPO: myeloperoxidase.

destructive action, the accumulation of neutrophils locally in upper and lower airways appears to be linked to the long-term course and exacerbations of several acute and chronic lung disorders, including acute respiratory distress syndrome (ARDS), asthma, bronchiectasis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and chronic lung allograft rejection [30–58]. The accumulation of neutrophils in the airways can also be linked to functional anomalies such as nonspecific bronchial hyperreactivity, hypersecretion and cough, all of which are key characteristics of obstructive lung disease [59, 60]. This evidence provides a solid rationale for focusing research on the role of mechanisms leading to accumulation of neutrophils in severe asthma and COPD. As there are currently no specific means of safely suppressing neutrophils in the lungs of humans, the case for the neutrophil as a pathogenic factor is based largely upon association studies. The evidence includes increased numbers of neutrophils being mainly present in airway tissue, bronchoalveolar lavage fluid or induced sputum.

ACCUMULATION OF NEUTROPHILS AND LUNG FUNCTION

A functional impact of neutrophils is supported by the observation that the number of neutrophils in the bronchoalveolar space correlates with the degree of nonspecific bronchial hyperreactivity in patients with mild, as well as severe persistent, asthma [34, 60]. In severe persistent asthma, the bronchoalveolar content of the neutrophilic enzyme myeloperoxidase (MPO) also correlates with the degree of nonspecific bronchial hyperreactivity [34]. Interestingly, the induced release of MPO from isolated neutrophils originating from the blood of patients with asthma correlates negatively with lung function (forced expiratory volume in one second), suggesting a systemic impact, and that neutrophil products may constitute surrogate biomarkers of more severe disease [61]. By analogy, the number of neutrophils in the bronchoalveolar space and the mucosa, respectively, also correlate negatively with lung function in smokers, with or without chronic bronchitis and COPD [39, 44–46]. In addition, among tobacco smokers, the number of luminal neutrophils correlates positively with the annual decline in lung function [59]. It is also known that neutrophil recruitment is associated with bronchial hyperresponsiveness in guinea pig airways *in vivo* [62]. Interestingly, a recent study on patients with mild stable asthma claimed that treatment with a β-adrenoceptor agonist (but not a glucocorticoid) results in a decrease in the number of neutrophils within the bronchoalveolar space in parallel with a decrease in nonspecific bronchial reactivity and an improvement in subjective symptoms [63]. Taken together, the clinical evidence from patients with obstructive lung disorders suggests that neutrophils constitute an important pathogenic factor, in particular for the long-term course and exacerbations in severe asthma and COPD [30–33, 39–47].

NEUTROPHIL PRODUCTION OF COMPOUNDS WITH PATHOGENIC POTENTIAL

Hypothetically, neutrophils might cause tissue damage through the release of several protein-degrading or cytotoxic compounds.

Neutrophil elastase

There is substantial evidence suggesting that neutrophil elastase degrades elastin, a key structural lung component that prevents small airways from collapsing, and that this leads to an emphysema-like condition in the lungs, and possibly also to remodelling of airway tissue [64–68]. Human neutrophil elastase also augments the fibroblast-mediated contraction of collagen gels *in vitro*, an observation underlining the potential remodelling capacity of this serine proteinase [68, 69]. Interestingly, neutrophil elastase appears to substantially contribute to secretion in airway gland cells from humans and other mammals *in vitro*, as well as in the upper airways of dogs *in vivo* [70–72]. Neutrophil elastase can also cause nonspecific bronchial hyperresponsiveness in guinea pig airways *in vivo* [64, 65]. There is even data from *in vitro* studies demonstrating that peptide fragments from human elastin that has been degraded using human neutrophil elastase cause chemotaxis of human blood monocytes and calf skin fibroblasts; evidence compatible with this elastase causing the recruitment of cells other than neutrophils and thus perpetuating inflammation at sites of neutrophil accumulation [73, 74]. Furthermore, neutrophils also contain another elastolytic enzyme, proteinase-3, which may cause effects similar to those of neutrophil elastase [75–77].

In view of the experimental evidence regarding pathogenically relevant effects exerted by neutrophil elastase, the local detection of this compound in human lung disease is of substantial interest. Thus, in the airway submucosa of patients with severe asthma, neutrophil elastase is detected regardless of whether eosinophils are present or not, thus showing neutrophil activity to be a more consistent factor than eosinophils, in terms of association with severe asthma [35]. The concentration of neutrophil elastase is also increased in the

bronchoalveolar space during severe asthma, even when no bacterial infection is detected [31–33]. In the same compartment, an increased concentration of neutrophil elastase is also present in ARDS, chronic bronchitis, COPD and CF [32, 51, 78–80]. It is noteworthy that several clinical studies demonstrate a correlation between the local concentration of elastase and lung function; these studies include patients with asthma, chronic bronchitis and CF [32, 81].

Matrix metalloproteinases

It is well known that neutrophils can release proteolytic enzymes with tissue-degrading capacity other than neutrophil elastase and proteinase-3. Thus, the two matrix metalloproteinases (MMPs) MMP-8 and -9 are released from human neutrophils upon induced activation *in vitro*, regardless of whether the donors are healthy subjects or patients, with asthma or COPD [82–84]. In the bronchoalveolar space of patients with severe asthma, in particular, the intracellular level of active MMP-8 is increased and seems mainly to be localised in neutrophils [76]. In addition, the concentration of MMP-9 is increased in the bronchoalveolar space of patients with asthma, particularly in patients with severe disease, and the activity of this form of MMP-9 may be less sensitive to a glucocorticoid among patients with asthma than among healthy control subjects [56, 85]. In the same compartment, the concentrations of free soluble MMP-8 and -9 and neutrophil-specific human neutrophil lipocalin are all increased in tobacco smokers with subclinical emphysema [42]. In addition, the intracellular level of MMP-8 tends to be higher in patients with severe asthma than in those with mild-to-moderate asthma [86]. Furthermore, the local concentration or activity of MMP-9 is increased in patients with asthma after allergen challenge, and MMP-9 mediates allergen-induced nonspecific hyperresponsiveness in sensitised mouse airways *in vivo*; these observations are fully in line with this particular proteinase having a functional impact [84, 87–89].

Reactive oxygen species

The production of cytotoxic reactive oxygen species by neutrophils is increased in patients with inflammatory lung diseases [90–93]. This is of interest as there is experimental evidence in mammals that reactive oxygen species can induce the transcription of the mRNA encoding neutrophil-recruiting cytokines, such as the functionally important neutrophil chemoattractant IL-8, leading to subsequent release of IL-8 and more neutrophil recruitment [94, 95]. Hypothetically, these events might contribute to altered lung function, since reactive oxygen species contribute to nonspecific bronchial hyperresponsiveness and this phenomenon may involve neutrophils in the lungs *in vivo*, as indicated by studies in cats, dogs and guinea pigs [64, 96–99]. Conditioned medium from stimulated human neutrophils also increases the reactivity of human bronchial smooth muscle *in vitro*, even though it is unclear whether or not reactive oxygen species can account for such transferable effects, given their short half-lives [100].

Neutrophil-accumulating factors

Interestingly, neutrophils can produce and release the neutrophil-accumulating compounds leukotriene B_4, tumour necrosis factor (TNF)-α and IL-8 [38, 101–109]. Thus, neutrophils themselves have the capacity to recruit even more neutrophils; this mechanism probably normally serves to mobilise large numbers of neutrophils to sites of infection. TNF-α probably also causes additional neutrophil recruitment *via* the stimulation of IL-8 release from bronchial epithelial cells [94, 110, 111]. In addition, there is evidence that IL-8 prolongs neutrophil survival, by delaying cell death through apoptosis [112, 113]. These events may have a functional impact, since there are data compatible with TNF-α and IL-8 causing bronchial hyperresponsiveness in guinea pig and mouse airways *in vivo* [95, 114, 115]. There is also evidence that the bronchoalveolar concentration of IL-8 correlates with the degree of nonspecific bronchial hyperreactivity in mild asthma [34].

Summary

In summary, there is now convincing evidence that neutrophils contribute to bronchoconstriction, nonspecific bronchial hyperreactivity, hypersecretion in glands and lung tissue destruction. For these reasons, the endogenous mechanisms orchestrating the accumulation and activation of neutrophils constitute potential targets for novel pharmacotherapy against lung disease associated with excessive neutrophil accumulation.

ORCHESTRATION OF NEUTROPHIL MOBILISATION IN THE LUNGS

Despite recent advances in chemokine and eicosanoid biology, current understanding of the orchestration of neutrophil mobilisation remains weak; this is particularly true for the sustained mobilisation of neutrophils in chronic lung disease [23].

Cellular sources of interleukin-8

A number of different cell types in the bronchial wall, bronchoalveolar space and post-capillary venule can release potent chemoattractant signals to neutrophils. Thus, bronchial epithelial cells, bronchial smooth muscle cells, fibroblasts, monocyte-derived cells, neutrophils and even eosinophils bear the potential of contributing to neutrophil recruitment by releasing IL-8 and other CXC chemokines in diseases such as asthma and chronic bronchitis [5, 94, 110, 111, 116–120].

Effect of glucocorticoids on interleukin-8

It remains a paradox that many experimental studies indicate that glucocorticoids inhibit IL-8 production in bronchial epithelial cells, bronchial smooth-muscle cells, fibroblasts, monocytes and venous endothelial cells, whereas these normally anti-inflammatory compounds do not inhibit the accumulation and activity of airway neutrophils in patients with COPD or very severe asthma [31, 35, 78, 121–125]. In line with this lack of effect on neutrophil mobilisation in certain patients, glucocorticoids do not inhibit the neutrophil chemoattractant IL-8 in severe asthma or COPD [34, 78]. This observation, together with the proven ability of glucocorticoids to directly increase neutrophil survival, probably contributes to the weak effect of glucocorticoids on neutrophil mobilisation in COPD and severe asthma [126, 127]. In light of these facts, it appears feasible that mechanisms other than the glucocorticoid-sensitive ones are important in orchestrating the local accumulation and activation of neutrophils through various mobilising factors in COPD and severe asthma.

T-LYMPHOCYTES AND NEUTROPHIL ACCUMULATION

T-lymphocytes and eosinophil mobilisation

It has been recognised since the early 1970s that T-lymphocytes can orchestrate the sustained local accumulation and activation of eosinophils in the airways [128], through soluble factors subsequently identified as T-helper cell (Th) type 2 cytokines, including IL-3 and IL-5, as well as the growth factor GM-CSF [129–131]. Strangely, the contribution of T-lymphocytes to the corresponding mobilisation of neutrophils has not received as much attention in the literature.

T-lymphocytes and neutrophil mobilisation

The acute and sustained phases of neutrophil mobilisation are key components of innate immunity contributing to host defence [18–23]. The discovery of the endotoxin recognition system in a number of mammalian species, a system that potently and rapidly recruits and activates neutrophils, has underlined the potential importance of neutrophil-mobilising mechanisms in particular [18–23]. Interestingly, there is now growing evidence that T-lymphocytes are involved in orchestrating the sustained mobilisation of neutrophils.

In airway tissue from patients with newly diagnosed asthma, the number of lymphocytes, eosinophils and neutrophils are increased [132]. Specific blockade of lymphocytes, with an antibody directed against either CD4 or the IL-2 receptor, prevents allergen-induced recruitment of eosinophils and neutrophils in the bronchoalveolar space of sensitised mice and rats *in vivo* [133, 134]. Importantly, in the airway tissue of certain patients with COPD, there is accumulation of both CD4+ and CD8+ lymphocytes, which is associated with accumulation of neutrophils [135, 136]. Remarkably, there may even also be an association between the accumulation of CD8+ lymphocytes and neutrophils in the nasal mucosa of patients with COPD [58]. Furthermore, exposure to cigarette smoke causes accumulation of CD4+ lymphocytes and neutrophils in the bronchoalveolar space of guinea pigs *in vivo* [137]. There is also accumulation of CD4+ lymphocytes and neutrophils in airway tissue from patients with bronchiectasis [36]. The accumulation of CD4+ lymphocytes is even associated with functional changes in the lungs; the local presence of CD4+ lymphocytes is related to nonspecific bronchial hyperreactivity in humans, mice and rats *in vivo*, even though it is not known whether this phenomenon is linked to the orchestration of neutrophils or eosinophils [129, 130, 138–140]. It is of special interest that the mediator mechanisms linking CD4+ or CD8+ lymphocytes to the accumulation of neutrophils have been unclear; IL-17A now emerges as a candidate for linking activated T-lymphocytes to sustained local accumulation of neutrophils in the lungs.

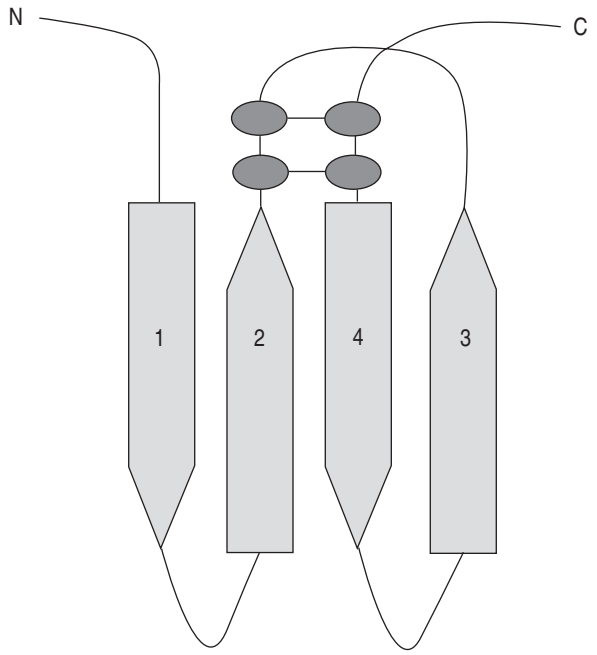

FIGURE 1. Generic structure of monomer of the interleukin-17 family of homodimeric cytokines. The typical canonical cysteine knot fold of β-strands (■) 1–4 is shown, including two disulphide linkages (horizontal bars between cysteine residues (●)). N: N-terminus; C: C-terminus. (Modified from [141].)

THE INTERLEUKIN-17 FAMILY

Shared molecular characteristics

At present, the IL-17 family of cytokines comprises six unique homodimeric glycoproteins that share a high degree of structural homology (table 2), including disulphide linkage, a relatively similar C-terminal amino acid sequence and cysteine knot folds when crystallised as monomers (fig. 1) [141–143]. The molecular size of the homodimeric proteins ranges 35–52 kDa, their monomers consist of ~150–200 amino acids and their chromosomal gene localisation is widely distributed (table 2).

TABLE 2 Molecular characteristics of human interleukin-17 family of cytokines

Subtype	Mr kDa	Length AA residues	Chromosomal location (GeneID#)	Common structural characteristics
A	35 [1, 2]	155 [1, 2]	2 (3605) [1, 2]	Cysteine residues [1, 2, 145–149]
B	41 [150]	180 [145]	5 (55540) [145]	Disulphide linkages [1, 2, 145–149]
C	40 [145]	197 [145]	16 (27189) [145]	Glycoproteins [1, 2, 145–149]
D	52 [146]	202 [146]	13 (53342) [146]	Homodimers [1, 2, 145–149]
E	34 [148]	161 [147, 148]	14 (64806) [148]	
F	44 [149]	153 [149]	6 (112744) [149, 151]	

Mr: relative molecular mass; AA: amino acid; GeneID: gene identifier. #: further gene and protein information in [144].

Interleukin-17A

IL-17A (previously named IL-17 or cytotoxic T-lymphocyte-associated serine esterase-8) constitutes the prototype member of the IL-17 family of cytokines [1, 2]. This particular glycoprotein consists of 155 amino acids and the molecular mass of the homodimer is 30–35 kDa [1, 2]. Interestingly, mouse and rat IL-17A both display structural homology with human IL-17A; the glycosylation site appears to be highly conserved, a fact compatible with IL-17A playing an important role in the mammalian immune system [1, 2, 142].

Interleukin-17B–F

Among the additional members of the IL-17 family, IL-17F probably constitutes the one most similar to IL-17A, with 50% sequence homology over its 163 amino acids [141, 151]. In contrast, IL-17B, -C, -D and -E (now renamed IL-25) all display variation in their biological profiles, in terms of both cellular sources and action [141, 143, 145–151]. In line with the substantial variability among their biological profiles, their amino acid sequence homology with IL-17A is as low as 16% [145]. At present, knowledge regarding the various roles of IL-17B–F in innate immunity or lung disease in humans is very limited. For this reason, this article focuses mainly on IL-17A.

CELLULAR SOURCES OF IL-17A

T-lymphocytes

Importantly, T-lymphocytes of the CD4+ and CD8+ subset that have been harvested from the spleen or blood of humans, mice or rats, most probably constituting memory (CD45RO) cells, produce and release IL-17A upon activation *in vitro* [2, 4, 142, 152].

Of specific interest for the potential role of IL-17A in the lungs, it was recently demonstrated that activated CD3+ lymphocytes, isolated from mouse lung tissue, release the free soluble form of IL-17A *in vitro* [4]. As indicated for synovial CD4+ T-lymphocytes from patients with rheumatoid arthritis, the Th0 and Th1 subsets, but not the Th2 subset, produce IL-17A *in vitro* [153]. However, there is also evidence from mice spleen CD4+ T-lymphocytes *in vitro*, showing that IL-17A can be produced in parallel with GM-CSF as well as TNF-α, but not with the classic Th1 and Th2 cytokines, interferon (IFN)-γ and IL-4, respectively [154]. These observations question the concept that IL-17A production follows a typical Th1 or Th2 pathway. Additional *in vivo* evidence in favour of T-lymphocytes constituting an important source of IL-17A was recently published; separate systemic depletion of the CD4+ and CD8+ subsets of lymphocytes, respectively, results in a decrease in the concentration of IL-17A in the bronchoalveolar space of mice exposed to Gram-negative bacteria *in vivo* [155]. Interestingly, there is now evidence from the human bronchoalveolar space favouring the idea that substantial amounts of IL-17A are not released under physiological conditions [156].

There is very little knowledge about the promoter and crucial transcription factors for IL-17A in lung lymphocytes. However, there is *in vitro* data on human blood T-lymphocytes of the CD8+ subset, indicating a cyclic AMP-sensitive mechanism and involvement of phosphokinase A in the transcription of IL-17A mRNA [157]. There has also been a recent study on genetically engineered mice, indicating that the transcription factor signal transducer and activator of transcription-4 is involved in production of IL-17A, during formation of peritoneal abscesses after abdominal Gram-negative infection *in vivo* [158].

There is now evidence from mouse cells *in vitro* that the release of IL-17A in response to endotoxin from Gram-negative bacteria requires the presence of macrophage-like cells [4]. As judged by another recent study, it seems as though the IL-17A response to Toll-like receptor-4 stimulation by live Gram-negative bacteria is triggered by IL-23, a recently described cytokine that shares a subunit with IL-12, and, just like IL-12, is produced by macrophage-like cells [155]. This IL-23-mediated phenomenon does not appear to require cell-to-cell contact, and puts IL-23 forward as a key upstream regulator of IL-17A [155].

Granulocytes

Two studies have claimed that granulocytes produce IL-17A [6, 13]. Thus immunoreactivity for intracellular IL-17A protein was detected in eosinophils harvested from the bronchoalveolar fluid, induced sputum and peripheral blood of patients with asthma [6]. Utilising *in situ* hybridisation, IL-17A mRNA was present in the same eosinophils [6]. The evidence promoting neutrophils as a source of IL-17A also includes detectable concentrations of IL-17A protein in bronchoalveolar fluid from endotoxin-exposed mice claiming to be lacking T-lymphocytes [13]. There is also evidence of IL-17A mRNA in bronchoalveolar neutrophils, after stimulation with endotoxin or the neutrophilic cytokine IL-15 *in vitro* [13]. It is worthy of note, though, that the cultured neutrophils were dead at the time that the extracellular IL-17A protein was detected, and that the data were referred to but not shown in the publication. Thus, it remains to be proven that live human eosinophils or neutrophils can release free soluble IL-17A upon activation *in vitro*.

RECEPTORS IN LUNGS

The general understanding of the receptor biology of the IL-17 family of cytokines is weak and this is also true for the lungs. At present, the most solid information relates to two subtypes of IL-17 receptors; these are the IL-17 receptor (IL-17R) and the IL-17B receptor (IL-17BR), the latter subsequently renamed IL-17 receptor homologue 1 (IL-17Rh1) [2, 141, 142, 145, 159–161].

Interleukin-17 receptor

In contrast to its protein ligands, the structure of the human variant of IL-17R displays a relatively common structure; it constitutes a type I membrane protein containing a 293-amino acid extracellular domain, a 21-amino acid transmembrane domain and a 525-amino acid cytoplasmic tail, totalling 739 amino acids [159]. Interestingly, and also in contrast to its protein ligands, the cellular distribution of human IL-17 mRNA is very broad [159, 161]. Thus, cells bearing the IL-17R include epithelial cells, fibroblasts, B- and T-lymphocytes, and myelomonocytic cells. In line with these findings, the organ distribution of the mRNA encoding mouse and rat IL-17R is also broad; this mRNA is detected in lungs, kidneys, liver and spleen [159]. Mouse and rat cells, such as fibroblasts, epithelial cells and various cells related to T-lymphocytes, also express IL-17R mRNA [159]. As revealed indirectly by immunoreactivity, IL-17R is also present in peripheral blood T-lymphocytes and vascular endothelial cells from humans [159,

161]. It is of principal immunological interest that many of these cells seem to be ready for an immediate response to IL-17A under physiological conditions, since they express IL-17R constitutively. However, in terms of a general understanding of the specific receptor for IL-17A, the existing data are probably incomplete. This is because IL-17A is approximately 10 times more potent in causing secondary cytokine release than it is in specifically activating IL-17R [159]. In view of this, the existence of additional facilitating subunits of IL-17R seems likely [141].

Interleukin-17 receptor homologue 1

Similarly to IL-17R, IL-17Rh1 constitutes a type I transmembrane protein, but is believed to be a smaller molecule, consisting of ~400–500 amino acids, depending on individual report [147, 150]. It displays <30% amino acid sequence homology to human IL-17R [147, 150]. Expression of its mRNA appears more restricted than that of IL-17R, with weak or no expression in human lungs and almost no expression in human blood leukocytes [150]. As indicated by its original name (IL-17BR), IL-17Rh1 was initially believed to specifically bind IL-17B, but a recent study has indicated that it also binds IL-17E (now renamed IL-25 [147, 150]).

ACTION ON NEUTROPHIL-MOBILISING FACTORS IN AIRWAY EPITHELIAL CELLS

Interleukin-8 and other CXC chemokines

Several studies have now demonstrated that stimulation of human airway epithelial cells with IL-17A *in vitro* causes the production and release of IL-8 [3, 8–10, 162, 163]. This IL-8 release is functionally significant for neutrophil recruitment; conditioned medium from IL-17A-stimulated human bronchial epithelial cells causes chemotaxis of human neutrophils, and this effect is blocked when the medium is pretreated with an antibody directed against IL-8 [3]. It is also noteworthy that this release of IL-8 is specific to IL-17A, since pretreatment of the recombinant IL-17A protein with a specific neutralising antibody attenuates its effect.

Stimulation with IL-17A also releases additional CXC chemokines, such as growth-related oncogene (GRO)-α and granulocyte chemotactic protein (GCP)-2 in human airway epithelial cells [162, 163]. The induced mRNA expression, as well as IL-8 and GRO-α release, is of a similar order of magnitude to that after stimulation with the pro-inflammatory cytokine TNF-α, even though the absolute concentration needed to produce these responses is higher for IL-17A [8, 9]. At present, there are no published studies on the involvement of nuclear factor (NF)-κB in IL-17A-induced chemokine release from human lung cells. However, as indicated in a rat intestinal epithelial cell line *in vitro*, IL-17A induces release of the CXC chemokine cytokine-induced neutrophil chemoattractant *via* a NF-κB-inducing kinase, in a TNF receptor-associated factor (TRAF)-6-dependent manner [164]. The fact that NF-κB is involved in the production of IL-8 in response to a wide range of pro-inflammatory stimuli other than IL-17A in airway epithelial cells also makes an involvement of this generic transcription factor likely [165–169]. Mitogen-activated protein (MAP) kinases are clearly involved as mediators of IL-17A-induced release of CXC chemokines in airway epithelial cells [9, 10, 162]. Specifically, p38 and extracellular signal-regulated kinase (ERK) appear to be the most important MAP kinases in this context, even though the involvement of ERK has not yet been confirmed in primary human airway epithelial cells *in vitro*. In certain airway epithelial cells, the release of IL-8, GRO-α and GCP-2 caused by IL-17A display low sensitivity to a glucocorticoid, even though this may be related to the specific conditions in the *in vitro* model [162, 163]. Of functional importance, it seems as though human IL-17 *per se* does not cause neutrophil chemotaxis, as indicated in blood neutrophils from humans stimulated *in vitro* [3].

Interleukin-6

Interestingly, human airway epithelial cells cultured *in vitro* respond to IL-17A by releasing cytokine IL-6, a cytokine known to increase elastase release from human neutrophils *in vitro* [8–10, 170]. However, the involvement of the particular MAP kinase p38 remains unclear, whereas the involvement of ERK has been confirmed in primary human airway epithelial cells [9, 10]. The sensitivity of this IL-6 release to a glucocorticoid is not currently known.

Colony-stimulating factors

There is evidence that IL-17A stimulates the production and release of colony-stimulating factors in human airway epithelial cells. Thus, IL-17A causes the release of G-CSF as well as GM-CSF in these cells *in vitro*, these two colony-stimulating factors being powerful anti-apoptotic survival factors for neutrophils [15, 127, 163, 171, 172]. Interestingly, this IL-17A-induced release is also of a similar order of magnitude to that obtained after stimulation with TNF-α, as is the case for CXC chemokines, but, again, a higher absolute concentration of IL-17A is needed to produce this response. Measured as gene expression in human airway epithelial cells *in vitro*, IL-17A also increases G-CSF levels at a similar order of magnitude as for TNF-α [163]. IL-17A may also exert a functionally significant effect on the release of GM-CSF *in vitro*, a release that increases the survival of human neutrophils *in vitro* [15]. At least under these *in vitro* conditions, this GM-CSF release is sensitive to a glucocorticoid, but the mechanisms behind this sensitivity remain unknown.

Mucin

Among the interleukins, IL-1–18, only IL-6 and -17A are capable of directly increasing levels of mRNA encoding mucin, including MUC5AC and MUC5B, as demonstrated in primary human airway epithelial cells *in vitro* [16]. This is also true for mucin protein itself. For IL-17A, this effect is in part mediated by IL-6, and, for MUC5B specifically, the intracellular MAP kinase ERK is involved.

Functional interactions with other cytokines

It is probably pathogenically relevant that IL-17A can interact with other pro-inflammatory cytokines when causing secondary cytokine release in local lung cells. For example, co-stimulation of human airway epithelial cells with the pro-inflammatory cytokine TNF-α enhances IL-17A-induced release of two CXC chemokines *in vitro* [3, 164]. In a similar way, co-stimulation with the classic Th1 cytokine IFN-γ also enhances IL-17A-induced IL-8 release in human airway epithelial cells [10]. Somewhat remarkably, co-stimulation with the classic Th2 cytokines IL-4 and -13, respectively, also

enhances the IL-8 response to IL-17A in the same epithelial cells [10]. In addition, the release of G-CSF and GM-CSF are enhanced by co-stimulation with TNF-α and IL-17A in human airway epithelial cells *in vitro* [15, 164]. Finally, co-stimulation of human airway epithelial cells *in vitro* with human IL-17A plus IFN-γ markedly enhances intercellular adhesion molecule-1 levels, and this is also the case for co-stimulation with IL-4 and -13, respectively [10]. Taken together, these observations are compatible with IL-17A exerting synergistic effects in Th1 as well as Th2 settings.

ACTION ON NEUTROPHIL-MOBILISING FACTORS IN NON-EPITHELIAL LUNG CELLS

Several studies now indicate that IL-17A increases the release of neutrophil-mobilising factors in local lung cells other than epithelial cells. Of particular interest, human venous endothelial cells, cells that constitute a crucial barrier for the extravasation of neutrophils, release IL-8 in response to IL-17A *in vitro*. This release is sensitive to a glucocorticoid, although once again, the molecular mechanisms remain unknown [3]. Of additional interest, primary human lung fibroblasts, cells that possess the potential to communicate with neutrophils in local tissue, also respond to IL-17A by producing and releasing IL-6 and IL-8 *in vitro* [6]. Similarly, in lung fibroblasts from mice, IL-17A can induce the release of the mouse CXC chemokines macrophage inflammatory protein (MIP)-2 and keratinocyte cytokine [7]. By analogy, IL-17A also stimulates the release of IL-6 in embryonic lung fibroblasts [8]. Again, this response to IL-17A seems to be sensitive to a glucocorticoid, even though the molecular mechanisms remain largely unknown [6]. As indicated for mouse embryonic fibroblasts *in vitro*, the IL-17A-induced transcription and release of IL-6 is mediated *via* NF-κB and requires TRAF-6, but it is not known whether these mechanisms are involved in setting the sensitivity to a glucocorticoid [173]. There is now also a published study on isolated primary airway smooth muscle cells from patients with bronchiolitis obliterans, which claims that IL-17A stimulates IL-8 release and that this response is not sensitive to a glucocorticoid [5]. In the same type of cells, IL-17A also induces the release of 8-isoprostane, a metabolite from the peroxidation of arachidonic acid that is regarded as a marker of oxidative stress [174]. Unfortunately, there are no studies on neutrophil accumulation or activity evaluating the functional significance of these *in vitro* findings.

At present, no thorough molecular characterisation of the mechanisms determining sensitivity to glucocorticoids of the effects of IL-17A in non-epithelial or epithelial lung cells has been published. Such a characterisation is well warranted. Data from chondrocytes suggest that IL-17A exerts its effect on secondary mediator production in its target cells *via* certain MAP kinases, concomitantly with the transcription factor NF-κB [170]. In these chondrocytes, a glucocorticoid blunted the activation of MAP kinases, but, again, the molecular basis of this repression remains unknown [175].

ACCUMULATION OF NEUTROPHILS IN THE LUNGS

In contrast to its lack of a direct effect on neutrophil chemotaxis *in vitro*, IL-17A from mice, rats and humans causes substantial accumulation of neutrophils when administered locally into the airways of mice or rats *in vivo* [3, 14, 17, 155, 175]. Interestingly, in the very first experiments in rats, conducted at a time when no rat or mouse IL-17A was commercially available, human IL-17A was administered intratracheally, and this *in vivo* response to human IL-17A was attenuated when the human IL-17A was pretreated with a neutralising antibody directed against hIL-17 [3]. This argues that, just like the IL-8 response *in vitro*, the *in vivo* response to human IL-17A is specific [3]. Systemic pretreatment with a glucocorticoid attenuates this effect of IL-17A on neutrophil accumulation in the rat model, even though the molecular mechanisms remain unknown.

Local stimulation with IL-17A increases the concentration of the CXC chemokine MIP-2 in the bronchoalveolar space of rats *in vivo* [3]. It also appears as though the IL-17A-induced release of keratinocyte cytokine, another CXC chemokine, and IL-6, respectively, also play a role in neutrophil accumulation in mouse lungs [13, 155]. Interestingly, GM-CSF may also be involved in this type of neutrophil accumulation, even though normally considered to be mainly a colony-stimulating factor [15]. Endogenous tachykinins enhance the referred IL-17A-induced neutrophil accumulation *via* neurokinin-1 receptors in rat lungs, drawing attention to a putative link to neurogenic inflammation, at least in this particular species [176].

ACTIVITY OF NEUTROPHILS IN THE LUNGS

There is evidence supporting the idea that IL-17A activates accumulated neutrophils in the bronchoalveolar space; local administration of recombinant human IL-17A causes an increase in neutrophil elastase and MPO activity in the bronchoalveolar space of rats *in vivo* [14]. However, no corresponding effect is observed in rat neutrophils from blood after stimulation with human IL-17A *in vitro*, and, therefore, if a true activation effect exists, then this effect is probably mediated mainly through indirect mechanisms. No increased elastase or MPO activity is observed in the bronchoalveolar space of rats *in vivo* after local stimulation with recombinant rat IL-1β, using a dose equally as effective as IL-17A in terms of neutrophil accumulation [14]. In spite of this, local co-stimulation with rat IL-1β plus human IL-17 displays substantially more elastase and MPO activity compared to stimulation with human IL-17A alone, thus revealing a true synergistic effect. It remains unclear whether the activating effect of human IL-17A on neutrophil elastase and MPO activity is a selective one; species heterology may be an issue for these particular findings. This would be consistent with the recent observation that local administration of recombinant mouse IL-17A increases the concentration of MMP-9 and the corresponding gelatinase activity in the bronchoalveolar space of mice *in vivo*, even though this increase does not cause any more MMP-9 or gelatinase activity per neutrophil [17]. Thus, even though the precise mechanisms remain unclear, it seems as though the net proteolytic load in the bronchoalveolar space is increased after local stimulation with IL-17A [14, 17].

INTERLEUKIN-17A AND HOST DEFENCE IN THE LUNGS

There is a growing body of evidence, from mice in particular, in favour of IL-17A signalling playing a central role in host defence within the lungs. Thus, mice lacking IL-17R die due to lung infection caused by *Klebsiella pneumoniae* more frequently than do wild-type mice [11]. Interestingly, the increased death

rate is paralleled by weakened mobilisation of neutrophils, as well as weakened clearance of bacteria locally [177]. In addition, the local increase in IL-17A concentration parallels bacterial growth in this mouse model of Gram-negative lung infection [177]. In line with a specific role in host defence, local administration of endotoxin from *Escherichia coli*, another Gram-negative bacteria, requires the presence of endogenous IL-17A, not for the early phase, but for the sustained phase of neutrophil accumulation in the bronchoalveolar space of mice *in vivo* [4, 13]. It is also clear that local administration of this endotoxin increases the relative amount of IL-17A mRNA in the lungs and the concentration of IL-17A in the bronchoalveolar space of mice *in viv*o [4, 178]. In addition, adenovirus-mediated overexpression of IL-17A stimulates granulopoiesis in mice *in vivo* in a stem cell factor- and G-CSF-dependent manner [179, 180]. All of these findings are compatible with the T-lymphocyte cytokine IL-17A constituting a crucial factor in the orchestration of the neutrophil component in host defence. However, importantly, corresponding evidence from studies in human lungs is still lacking.

INTERLEUKIN-17A IN LUNG DISEASE

The causative role of IL-17A in human lung disease is very much unknown. A substantial increase in the concentration of free soluble IL-17 within the bronchoalveolar space has been demonstrated in healthy human volunteers, after exposure to organic dust in a swine confinement area [156]. This local increase in IL-17A parallels a substantial local accumulation of neutrophils and a more moderate accumulation of lymphocytes. However, at present, it is not known how this increase in IL-17A relates to the increased morbidity in lung disease observed among swine farmers with regular exposure to organic dust [181].

Certain patients with mild asthma may display a modest increase in the concentration of free soluble IL-17A within the bronchoalveolar space [6]. This IL-17A concentration increase is associated with a more pronounced increase in indirect immunoreactivity for intracellular IL-17A in inflammatory cells from the bronchoalveolar space. However, one recent study on induced sputum claims that there is no major difference in the concentration of IL-17A in patients with moderate asthma and healthy control subjects [182]. Even though the number of included subjects was limited, the data from the very same study suggest that the local concentration of IL-17A is higher in asthma than in moderate COPD. In this particular study, a correlation between the local concentration of IL-17A and the degree of nonspecific bronchial hyperreactivity was reported but this report was based upon an analysis of pooled material originating from healthy control subjects, as well as from patients with asthma or COPD. The study included no analysis of the association of local IL-17A with inflammatory cell subtypes. At present, there are no further studies on IL-17A in human lung disease, even though there is a recently published clinical study showing increased expression of IL-17A in the nasal mucosa of patient with nasal polyps, compatible with IL-17A being linked to atopic airway disease [183].

Potentially important clues regarding the potential role of IL-17A in lung disease are also provided by studies on sensitised mice. It has now been shown that allergen challenge is followed by transcription of IL-17A in mouse lungs [12]. The indicated *de novo* synthesis of endogenous IL-17A also seems to parallel allergen-induced neutrophil accumulation in the bronchoalveolar space [12]. Furthermore, inhibition of endogenous IL-17A even increases allergen-induced eosinophil accumulation and elevates levels of the Th2 cytokine IL-5 in the same compartment, suggesting that IL-17A may have an impact on the balance between eosinophilic and neutrophilic inflammation in mouse lungs. Another study on sensitised mice has provided data compatible with endogenous IL-17A also mediating allergen-induced nonspecific bronchial hyperresponsiveness, possibly by contributing to the activation of T-lymphocytes [184].

CONCLUSIONS AND FUTURE QUESTIONS

Recent studies promote IL-17A as a candidate cytokine for linking the activation of T-lymphocytes to the sustained accumulation and activity of neutrophils locally in the lungs. IL-17A seems to be most important not for the early phase but for the sustained phase of neutrophil mobilisation in host defence in the lungs. It is, therefore, possible that certain T-lymphocytes play both complementary and supplementary roles to well-known neutrophil-mobilising cells, such as the bronchial epithelium and bronchoalveolar macrophages. IL-17A may thus be uniquely positioned in the interface between acquired and innate immunity, as a reinforcing signal. It remains an open question as to whether inflammatory cells other than subsets of T-lymphocytes also release IL-17A in the lungs. Regardless of its cellular origin, IL-17A appears to exert its effects on the sustained mobilisation of neutrophils primarily *via* an induced release of neutrophil-mobilising cytokines from local cells in the lungs. Whether IL-17A is mainly important for host defence, or whether this molecule also plays a pathogenic role, in particular in chronic lung disease associated with excessive neutrophil activity, is not yet certain.

Future studies should further evaluate the therapeutic utility of drugs targeting interleukin-17A and its receptor(s), a strategy that has now also been discussed for organs other than the lungs [185–187].

REFERENCES

1 Rouvier E, Luciani MF, Mattei MG, Denizof F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpes virus saimiri gene. *J Immunol* 1993; 150: 5445–5456.

2 Yao Z, Painter SL, Fanslow WC, *et al.* Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995; 155: 5483–5486.

3 Laan M, Cui ZH, Hoshino H, *et al.* Neutrophil recruitment by human IL-17 *via* C-X-C chemokine release in the airways. *J Immunol* 1999; 162: 2347–2352.

4 Miyamoto M, Prause O, Laan M, Sjöstrand M, Lötvall J, Lindén A. Endogenous IL-17 mediates endotoxin-induced airway neutrophilia in mice *in vivo*. *J Immunol* 2003; 170: 4665–4672.

5 Vanaudenaerde BM, Wuyts WA, Dupont LJ, Van Raemdonck DE, Demedts MM, Verleden GM.

Interleukin-17 stimulates release of interleukin-8 by human airway smooth muscle cells *in vitro*: a potential role for interleukin-17 and airway smooth muscle cells in bronchiolitis obliterans syndrome. *J Heart Lung Transplant* 2003; 22: 1280–1283.

6 Molet S, Hamid QA, Davoine F, *et al.* Interleukin-17 is increased in asthmatic airways and induces human fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001; 108: 103–109.

7 Numasaki M, Lotze MT, Sasaki H. Interleukin-17 augments tumour necrosis factor-α-induced elaboration of proangiogenic factors from fibroblasts. *Immunol Lett* 2004; 93: 39–43.

8 Fossiez F, Djossou O, Chomarat P, *et al.* T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med* 1996; 183: 2593–2603.

9 Laan M, Lötvall J, Chung KF, Lindén A. IL-17-induced cytokine release in human bronchial epithelial cells *in vitro*: role of mitogen-activated protein (MAP) kinases. *Br J Pharmacol* 2001; 133: 200–206.

10 Kawaguchi M, Kokubu F, Kuga H, *et al.* Modulation of bronchial epithelial cells by IL-17. *J Allergy Clin Immunol* 2001; 108: 804–809.

11 Ye P, Rodriguez FH, Kanaly S, *et al.* Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001; 194: 519–527.

12 Hellings PW, Kasran A, Liu Z, *et al.* Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Crit Care Med* 2003; 28: 42–50.

13 Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol* 2003; 170: 2106–2112.

14 Hoshino H, Laan M, Sjöstrand M, Lötvall J, Skoogh BE, Lindén A. Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways *in vivo*. *J Allergy Clin Immunol* 2000; 105: 143–149.

15 Laan M, Prause O, Miyamoto M, *et al.* A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-α. *Eur Respir J* 2003; 21: 1–7.

16 Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003; 278: 17036–17043.

17 Prause O, Bozinovski S, Anderson GP, Lindén A. Increased matrix metalloproteinase-9 concentration and activity after stimulation with interleukin-17 in mouse airways. *Thorax* 2004; 59: 313–317.

18 Ibbotson GC, Doig C, Kaur J, *et al.* Functional α_4-integrin: a newly identified pathway of neutrophil recruitment in critically ill septic patients. *Nature Med* 2001; 7: 465–470.

19 Shiohara M, Gombart AF, Sekiguchi Y, *et al.* Phenotypic and functional alterations of peripheral blood monocytes in neutrophil-specific granule deficiency. *J Leukoc Biol* 2004; 75: 190–197.

20 Terregino CA, Lubkin CLL, Thom SR. Impaired neutrophil adherence as an early marker of systemic inflammatory response syndrome and severe sepsis. *Ann Emerg Med* 1997; 29: 400–403.

21 Meddows-Taylor S, Pendle S. Altered expression of CD88 and associated impairment of complement 5a-induced neutrophil responses in human immunodeficiency virus type 1-infected patients with and without pulmonary tuberculosis. *J Infect Dis* 2001; 183: 662–665.

22 Link H, Böhme A, Cornely OA, *et al.* Antimicrobial therapy of unexplained fever in neutropenic patients. *Ann Hematol* 2003; 82: Suppl. 2, S105–S117.

23 Zhang P, Summer WR, Bagby GJ, Nelson S. Innate immunity and pulmonary host defense. *Immunol Rev* 2000; 173: 39–51.

24 Schwartz DA. The genetics of innate immunity. *Chest* 2002; 121: 62S–68S.

25 Paterson HM, Murphy TJ, Purcell EJ, *et al.* Injury primes the innate system for enhanced Toll-like receptor reactivity. *J Immunol* 2003; 171: 1473–1483.

26 Calkins CM, Barsness K, Bensard DD, *et al.* Toll-like receptor-4 signaling mediates pulmonary neutrophil sequestration in response to Gram-positive bacterial enterotoxin. *J Surg Res* 2002; 104: 124–130.

27 Droemann D, Goldmann T, Branschield D, *et al.* Toll-like receptor 2 is expressed by alveolar epithelial cells type II and macrophages in the human lung. *Histochem Cell Biol* 2003; 119: 103–108.

28 Escotte S, Daniel C, Gaillard D, *et al.* Fluticasone propionate inhibits lipopolysaccharide-induced proinflammatory response in human cystic fibrosis airway grafts. *J Pharmacol Exp Ther* 2002; 302: 1151–1157.

29 Dinakar C, Malur A, Raychaudhuri B, *et al.* Differential regulation of human blood monocyte and alveolar macrophage inflammatory cytokine production by nitric oxide. *Ann Allergy Asthma Immunol* 1999; 82: 217–222.

30 Fahy JV, Kim KW, Liu JJ, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995; 95: 843–852.

31 Ordonez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma. Clinical and biological significance. *Am J Respir Crit Care Med* 2000; 161: 1185–1160.

32 Vignola AM, Bonanno A, Mirabella A, *et al.* Increased level of elastase and alpha 1-antitrypsin in sputum of asthmatic patients. *Am J Respir Crit Care Med* 1998; 157: 505–511.

33 Lamblin C, Gosset P, Tillie-Leblond I, *et al.* Bronchial neutrophilia in patients with noninfectious status asthmaticus. *Am J Respir Crit Care Med* 1998; 157: 394–402.

34 Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999; 160: 1532–1539.

35 Wenzel SE, Schwartz LB, Langmack EL, *et al.* Evidence that severe asthma can be divided into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; 160: 1001–1008.

36 Gaga M, Bentely AM, Humbert M, *et al.* Increases in CD4+ T lymphocytes, macrophages, neutrophils and

interleukin-8 positive cells in the airways of patients with bronchiectasis. *Thorax* 1998; 53: 685–691.
37 Angrill J, Augusti C, De Celis R, *et al.* Bronchial inflammation and colonisation in patients with clinically stable bronchiectasis. *Am J Respir Crit Care Med* 2001; 164: 1628–1632.
38 Zeng L, Shum H, Tipoe GL, *et al.* Macrophages, neutrophils and tumour necrosis factor-α expression in bronchiectatic airways *in vivo*. *Respir Med* 2001; 95: 792–798.
39 Saetta M, Turato G, Facchini F, *et al.* Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 1997; 156: 1633–1639.
40 Riise GC, Ahlstedt S, Larsson S, *et al.* Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. *Thorax* 1995; 50: 360–365.
41 Balbi B, Bason C, Balleari E, *et al.* Increased bronchoalveolar granulocytes and granulocyte/colony-stimulating factor during exacerbations of chronic bronchitis. *Eur Respir J* 1997; 10: 846–850.
42 Betsuyaku T, Nishimura M, Takeyabu K, *et al.* Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Am J Respir Crit Care Med* 1999; 159: 1985–1991.
43 Aaron SD, Angel JB, Lunau M, *et al.* Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163: 349–355.
44 Balzano G, Stefanelli F, Irorio C, *et al.* Eosinophilic inflammation in stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160: 1486–1492.
45 Di Stefano A, Capelli A, Lusardi M, *et al.* Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1999; 158: 1277–1285.
46 Soler N, Ewig S, Torres A, Filella X, Gonzales J, Zaubet A. Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. *Eur Respir J* 1999; 14: 1015–1022.
47 Noguera A, Batle S, Miralles C, *et al.* Enhanced neutrophil response in chronic obstructive pulmonary disease. *Thorax* 2001; 56: 432–437.
48 Koller DY, Urbanek R, Götz M. Increased degranulation of eosinophil and neutrophil granulocytes in cystic fibrosis. *Am J Respir Crit Care Med* 1995; 152: 629–633.
49 Dakin CJ, Numa AH, Wang H, Morton JR, Vertzyas CC, Henry LL. Infection, inflammation and pulmonary function in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 2002; 165: 904–910.
50 Muhlebach MS, Noah TL. Endotoxin activity and inflammatory markers in the airways of young patients with cystic fibrosis. *Am J Respir Crit Care Med* 2002; 165: 911–915.
51 Sagel SD, Kapsner R, Osberg I, Sontag MK, Accurso FJ. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. *Am J Respir Crit Care Med* 2001; 164: 1425–1431.
52 Riise GC, Kjellström C, Ryd W, *et al.* Inflammatory cells and activation markers in BAL during acute rejection and infection in lung transplant recipients: a prospective, longitudinal study. *Eur Respir J* 1997; 10: 1742–1746.
53 Riise GC, Andersson BA, Kjellström C, *et al.* Persistent high BAL fluid granulocyte activation marker levels as early indicators of bronchiolitis obliterans after lung transplant. *Eur Respir J* 1999; 14: 1123–1130.
54 Baugham R, Gunther KL, Rashkin MC, Keeton DA, Pattishal EN. Changes in the inflammatory response of the lung during acute respiratory distress syndrome: prognostic indicators. *Am J Respir Crit Care Med* 1996; 154: 76–81.
55 Geerts L, Jorens PG, Willems J, De Ley M, Slegers H. Natural inhibitors of neutrophil function in acute respiratory distress syndrome. *Crit Care Med* 2001; 29: 1920–1924.
56 Cundall M, Sun Y, Miranda C, Trudeau JB, Barnes S, Wenzel SE. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. *J Allergy Clin Immunol* 2003; 112: 1064–1071.
57 Baraldo S, Turato G, Badin C, *et al.* Neutrophilic infiltration within the airway smooth muscle in patients with COPD. *Thorax* 2003; 59: 308–312.
58 Vachier I, Vignola AM, Chiappara G, *et al.* Inflammatory features of nasal mucosa in smokers with and without COPD. *Thorax* 2004; 59: 303–307.
59 Stanescu D, Sanna A, Kostianev S, Calgagni PG, Fabbri LM, Maestrelli P. Airway obstruction, chronic expectoration, and rapid decline in FEV_1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996; 51: 267–271.
60 Betz R, Kohlhaufl M, Kassner G, *et al.* Increased sputum IL-8 and IL-5 in asymptomatic non-specific hyperresponsiveness. *Lung* 2001; 179: 119–133.
61 Monteseirin J, Bonilla I, Camacho J, Conde J, Sobrino F. Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol* 2001; 107: 623–626.
62 Fujimura M, Xiu Q, Tsujiura M, *et al.* Role of leukotriene B_4 in bronchial hyperresponsiveness induced by interleukin-8. *Eur Respir J* 1998; 11: 306–311.
63 Jeffrey PK, Venge P, Gizycki MJ, Egerod I, Dahl R, Faurschou P. Effects of salmeterol on mucosal inflammation in asthma: a placebo-controlled study. *Eur Respir J* 2002; 20: 1378–1385.
64 Matsumoto K, Aizawa H, Inoue H, Koto H, Nakano H, Hara N. Role of neutrophil elastase in ozone-induced airway responses in guinea-pigs. *Eur Respir J* 1999; 14: 1088–1094.
65 Suzuki T, Wang W, Lin JT, Shirato K, Mitsuhashi H, Inoue H. Aerosolised human neutrophil elastase induces airway constriction and hyper-responsiveness with protection by intravenous pretreatment with half-length secretory leukoprotease inhibitor. *Am J Respir Crit Care Med* 1996; 153: 1405–1411.
66 Chen XJ, Hedlund LW, Moller HE, Chawla MS, Maronpot RR. Detection of emphysema in rat lungs by using magnetic resonance measurements of ^3He diffusion. *Proc Natl Acad Sci USA* 2000; 10: 11478–11481.
67 Janoff A, Sloan B, Weinbaum G, *et al.* Experimental emphysema induced with purified human neutrophil

elastase: tissue localization of the instilled protease. *Am Rev Respir Dis* 1977; 115: 461–478.
68 Sköld CM, Liu X, Umino T, *et al.* Human neutrophil elastase augments fibroblast-mediated contraction of released collagen gels. *Am J Respir Crit Care Med* 1999; 159: 1138–1146.
69 Zhu YK, Liu XD, Skold CM, *et al.* Synergistic neutrophil elastase–cytokine interaction degrades collagen in three-dimensional culture. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L868–L878.
70 Sommerhof CP, Nadel JA, Basbaum C, Caughey G. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland cells. *J Clin Invest* 2000; 85: 682–689.
71 Schuster A, Ueki I, Nadel JA. Neutrophil elastase stimulates tracheal submucosal gland secretion that is inhibited by ICI 200,355. *Am J Physiol* 1992; 262: L86–L91.
72 Cardell LO, Agusti C, Takeyama K, Stjärne P, Nadel JA. LTB_4-induced nasal gland serous secretion mediated by neutrophil elastase. *Am J Respir Crit Care Med* 1999; 160: 411–414.
73 Senior RM, Griffin GL, Mecham RP. Chemotactic activity of elastin-derived peptides. *J Clin Invest* 1980; 66: 859–862.
74 Senior RM, Griffin GL, Mecham RP. Chemotactic responses of fibroblasts to tropoelastin and elastin-derived peptides. *J Clin Invest* 1982; 70: 614–618.
75 Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR. Proteinase 3: distinct human polymorphonuclear leukocyte proteinase that produces emphysema in hamsters. *J Clin Invest* 1988; 82: 1963–1973.
76 Campanelli D, Melchior M, Fu Y, *et al.* Cloning of cDNA for proteinase 3: a serine protease, antibiotic and autoantigen from human neutrophils. *J Exp Med* 1990; 172: 1709–1715.
77 Fruh H, Kostoulas G, Michael BA, Baici A. Human myeloblastin (leukocyte proteinase 3): reactions with substrates, inactivators and activators in comparison with leukocyte elastase. *J Biol Chem* 1996; 377: 579–586.
78 Culpitt AV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, Barnes PJ. Effect of high dose inhaled steroid on cells, cytokines and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160: 1635–1639.
79 Adams JM, Hauser CJ, Livingston DH, Lavery RF, Fekete Z, Dietch EA. Early trauma polymorphonuclear neutrophil responses to chemokines are associated with development of sepsis and organ failure. *J Trauma* 2001; 51: 452–456.
80 Ishizaka A, Watanabe M, Yamashita T, *et al.* New bronchoscopic microsample probe to measure the biochemical constituents in epithelial lining fluid of patients with acute respiratory distress syndrome. *Crit Care Med* 2001; 29: 896–898.
81 Sagel SD, Sontag MK, Wagener JS, Kapsner RK, Osberg I, Accurso FJ. Induced sputum inflammatory measures correlate with lung function in children with cystic fibrosis. *J Pediatr* 2002; 141: 811–817.
82 Takafuji S, Ishida A, Miyakuni Y, Nakagawa T. Matrix metalloproteinase-9 release from human leukocytes. *J Investig Allergol Clin Immunol* 2003; 13: 50–55.
83 Claesson R, Johansson A, Belibasakis G, Hänström L, Kalfas S. Release and activation of matrix metalloproteinase 8 from human neutrophils triggered by the leukotoxin of *Actinobacillus actinomycetemcomitans*. *J Periodontal Res* 2002; 37: 353–359.
84 Cataldo DD, Bettiol J, Noel A, Bartsch P, Foidart JM, Louis R. Matrix metalloproteinase-9, but not tissue inhibitor of matrix metalloproteinase-1, increases in the sputum from allergic asthmatic patients after allergen challenge. *Chest* 2002; 122: 1553–1559.
85 Wenzel SE, Balzar SB, Cundall M, Chu HW. Subepithelial basement membrane imunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunol* 2003; 111: 1345–1352.
86 Prikk K, Maisis P, Pirila E, *et al.* Airway obstruction correlates with collagenase-2 (MMP-8) expression and activation in bronchial asthma. *Lab Invest* 2002; 82: 1534–1545.
87 Cataldo DD, Tournoy KG, Vermaelen K, *et al.* Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation. *Am J Pathol* 2002; 161: 491–498.
88 Mattos W, Lim S, Russell R, Jatakanon A, Chung KF, Barnes PJ. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. *Chest* 2002; 122: 1543–1552.
89 Lee YC, Lee HB, Rhee YK, Song CH. The involvement of matrix metalloproteinase-9 in airway inflammation of patients with acute asthma. *Clin Exp Allergy* 2001; 31: 1623–1630.
90 Cataldo D, Munuat C, Noel A, *et al.* MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 2000; 123: 259–267.
91 Watanabe M, Kaihatsu T, Miwa M, Maeda T. Ca^{2+}/calmodulin-dependent protein kinase II inhibitors potentiate superoxide production in polymorphonuclear leukocytes. *J Pharm Pharmacol* 1999; 51: 295–300.
92 Cortijo J, Villagrasa V, Pons R, *et al.* Bronchodilator and anti-inflammatory activities of glaucine: *in vitro* studies on human airway smooth muscle and polymorphonuclear leukocytes. *Br J Pharmacol* 1999; 127: 1641–1651.
93 Sustiel AM, Joseph B, Rocklin RE, Borish L. Asthmatic patients have neutrophils that exhibit diminished responsiveness to adenosine. *Am Rev Respir Dis* 1989; 140: 1556–1561.
94 Massion P, Lindén A, Inoue H, Mathy M, Grattan KM, Nadel JA. Dimethyl sulfoxide decreases interleukin-8-mediated neutrophil recruitment in the airways. *Am J Physiol* 1996; 271: L838–L843.
95 Inoue H, Aizawa H, Nakano H, *et al.* Nitric oxide synthase inhibitors attenuate ozone-induced airway inflammation in guinea pigs. Possible role of interleukin-8. *Am J Respir Crit Care Med* 2000; 161: 249–256.
96 Stevens WH, Inman MD, Wattie J, O'Byrne PM. Allergen-induced oxygen radical release from bronchoalveolar lavage cells and airway hyperresponsiveness in dogs. *Am J Respir Crit Care Med* 1995; 151: 1526–1531.

97 Takahashi T, Miura M, Katsumata U, *et al.* Involvement of superoxide in ozone-induced airway hyperresponsiveness in anesthetized cats. *Am Rev Respir Dis* 1993; 148: 103–106.

98 Nakano H, Aizawa H, Matsumoto K, Fukuyama S, Inoue H, Hara N. Cyclooxygenase-2 participates in the late phase of airway hyperresponsiveness after ozone exposure in guinea pigs. *Eur J Pharmacol* 2000; 403: 267–275.

99 Matsui S, Jones GL, Woolley MJ, Lane CC, Gontovnick LS, O'Byrne PM. The effect of antioxidants on ozone-induced airway hyperresponsiveness in dogs. *Am Rev Respir Dis* 1991; 144: 1287–1290.

100 Hallahan AR, Armour CL, Black JL. Products of neutrophils and eosinophils increase the responsiveness of human isolated bronchial tissue. *Eur Respir J* 1990; 3: 554–558.

101 Volcano M, Alves Rosa MF, Minnucci FS, Chernavsky AC, Isturiz MA. *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) inhibits tumour necrosis factor-α (TNF-α) production in lipopolysaccharide (LPS)-stimulated human neutrophils. *Clin Exp Immunol* 1998; 113: 39–47.

102 Page SM, Gleich GJ, Roebuck KA, Thomas LL. Stimulation of neutrophil interleukin-8 production by eosinophil granule major basic protein. *Am J Respir Cell Mol Biol* 1999; 21: 230–237.

103 Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumour necrosis factor-α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996; 153: 530–534.

104 Sibelius U, Hattar K, Hoffman S, *et al.* Distinct pathways of lipopolysaccharide priming of human neutrophil respiratory burst: role of lipid mediator synthesis and sensitivity to interleukin-10. *Crit Care Med* 2002; 30: 2306–2312.

105 Gambero A, Landucci EC, Toyama MH, *et al.* Human neutrophil migration *in vitro* induced by secretory phospholipases A_2: a role for cell surface glycosaminoglycans. *Biochem Pharmacol* 2002; 63: 65–72.

106 Falt J, Dahlgren C, Ridell M. Difference in neutrophil cytokine production induced by pathogenic and non-pathogenic mycobacteria. *APMIS* 2002; 110: 593–600.

107 Kuhns DB, Nelson EL, Alvord WG, Gallin JI. Fibrinogen induces IL-8 synthesis in human neutrophils stimulated with formyl-methionyl-leucyl-phenylalanine or leukotriene B_4. *J Immunol* 2001; 167: 2869–2878.

108 Koller M, Wick M, Muhr G. Decreased leukotriene release from neutrophils after severe trauma: role of immature cells. *Inflammation* 2001; 25: 53–59.

109 Hattar K, Sibelius U, Bickenbach A, Csernock E, Seeger W, Grimminger F. Subthreshold concentrations of anti-proteinase 3 antibodies (c-ANCA) specifically prime human neutrophils for fMLP-induced leukotriene synthesis and chemotaxis. *J Leukoc Biol* 2001; 69: 89–97.

110 Lindén A. Increased interleukin-8 release by β-adrenoceptor activation in human transformed bronchial epithelial cells. *Br J Pharmacol* 1996; 119: 402–406.

111 Jorens PG, Richman-Eisenstadt JBY, Housset BP, *et al.* Interleukin-8 induces neutrophil accumulation but not protease secretion in the canine trachea. *Am J Physiol* 1992; 263: L708–L713.

112 Leuenroth S, Lee C, Grutkoski P, Keeping H, Simms HH. Interleukin-8-induced suppression of polymorphonuclear leukocyte apoptosis is mediated by suppressing CD95 (Fas/Apo-1) Fas-1 interactions. *Surgery* 1998; 124: 409–417.

113 Dunican AL, Leuenroth SJ, Grutkoski P, Ayala A, Simms HH. TNFα-induced suppression of apoptosis is mediated through interleukin-8 production. *Shock* 2000; 14: 284–288.

114 Xiu Q, Fujimura M, Nomura M, *et al.* Bronchial hyperresponsiveness and airway neutrophil accumulation induced by interleukin-8 and the effect of the thromboxane A_2 antagonist S-1452 in guinea-pigs. *Clin Exp Allergy* 1995; 25: 51–59.

115 Hessel EM, Van Oosterhout AJ, Van Ark I, *et al.* Development of airway hyperresponsiveness is dependent on interferon-gamma and independent of eosinophil infiltration. *Am J Respir Crit Care Med* 1997; 160: 325–334.

116 Amin K, Ludviksdottir D, Janson C, *et al.* Inflammation and structural changes in the airways of patients with atopic and non-atopic asthma. *Am J Respir Crit Care Med* 2000; 162: 2295–2301.

117 Mazarella G, Grella E, D'Auria D, *et al.* Phenotypic features of alveolar monocytes/macrophages and IL-8 gene activation by IL-1 and TNF-α in asthmatic patients. *Allergy* 2000; 55: 36–41.

118 Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992; 89: 1001–1009.

119 Yousefi S, Hemmann S, Weber M, *et al.* IL-8 is expressed by human peripheral blood eosinophils: evidence for increased secretion in asthma. *J Immunol* 1995; 154: 5481–5490.

120 Hoshi H, Ohno M, Hinma M, *et al.* IL-5, IL-8 and GM-CSF immunostaining of sputum cells in bronchial asthma and chronic bronchitis. *Clin Exp Allergy* 1995; 25: 720–728.

121 Korn SH, Jerre A, Brattsand R. Effects of formoterol and budesonide on GM-CSF and IL-8 secretion by triggered human bronchial epithelial cells. *Eur Respir J* 2001; 17: 1070–1077.

122 Pang L, Knox AJ. Synergistic inhibition by β_2-agonists and corticosteroids on tumour necrosis factor α-induced interleukin-8 release from cultured human airway smooth-muscle cells. *Am J Respir Cell Mol Biol* 2000; 23: 79–85.

123 Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF. Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. *Blood* 1992; 79: 45–51.

124 Lukacs BW, Strieter RM, Elner V, Evanoff HL, Burdick MD, Kunkel SL. Production of chemokines, interleukin-8 and monocyte chemoattractant protein-1, during monocyte: endothelial cell interactions. *Blood* 1995; 86: 2767–2773.

125 Pizzichini MMM, Pizzichini E, Clelland L, *et al.* Prednisone-dependent asthma: inflammatory indices in induced sputum. *Eur Respir J* 1999; 13: 15–21.

126 Liles WC, Dale DC, Klebanoff SJ. Glucocorticoids inhibit apoptosis of human neutrophils. *Blood* 1995; 86: 3181–3188.

127 Yamasawa H, Oshikawa K, Ohno S, Sugiyama Y. Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating GM-CSF release. *Am J Respir Cell Mol Biol* 2004; 30: 569–575.

128 Torisu M, Yoshida T, Ward PA, Cohen S. Lymphocyte-derived eosinophil chemotactic factor. II. Studies on the mechanism of activation of the precursor substance by immune complexes. *J Immunol* 1973; 111: 1450–1458.

129 O'Byrne PM, Inman MD, Adelroth E. Reassessing the Th2 cytokine basis of asthma. *Trends Pharmacol Sci* 2004; 25: 244–248.

130 Bochner BS, Busse WW. Advances in mechanisms of allergy. *J Allergy Clin Immunol* 2004; 113: 868–875.

131 Tomaki M, Zhao LL, Sjostrand M, Lindén A, Ichinose M, Lötvall J. Comparison of effects of anti-IL-3, IL-5 and GM-CSF treatments on eosinophilopoiesis and airway eosinophilia induced by allergen. *Pulm Pharmacol Ther* 2002; 15: 161–168.

132 Gavett SH, Chen X, Finkelman F, Wills-Karp M. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994; 10: 587–593.

133 Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993; 147: 697–704.

134 Renzi PM, Yang JP, Diamantstein T, Martin JG. Effects of depletion of cells bearing the interleukin-2 receptor on immunoglobulin production and allergic airway responses in the rat. *Am J Respir Crit Care Med* 1996; 153: 1214–1221.

135 Rutgers SR, Postma DS, ten Hacken NH, *et al.* Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax* 2000; 55: 12–18.

136 Turato G, Zuin R, Miniati M, *et al.* Airway inflammation in severe chronic obstructive pulmonary disease: relationship with lung function and radiologic emphysema. *Am J Respir Crit Care Med* 2002; 166: 105–110.

137 Meshi B, Vitalis TZ, Ionescu D, *et al.* Emphysemateous lung destruction by cigarette smoke. The effects of latent adenoviral infection on the lung inflammatory response. *Am J Respir Cell Mol Biol* 2002; 26: 52–57.

138 Walker C, Kaegi MK, Braun P, Blaser K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991; 88: 935–942.

139 Mishima H, Hojo M, Watanabe A, Hamid QA, Martin JG. CD4+ T cells can induce airway hyperresponsiveness to allergen challenge in the Brown Norway rat. *Am J Respir Crit Care Med* 1998; 158: 1863–1870.

140 De Sanctis GT, Itoh A, Green FHY, *et al.* T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice. *Nature Med* 1997; 4: 460–462.

141 Hymowitz SG, Filvaroff EH, Yin JP, *et al.* IL-17s adopt a cysteine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *EMBO J* 2001; 20: 5332–5341.

142 Yao Z, Fanslow FC, Seldin MF, *et al.* Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 1995; 3: 811–821.

143 Hurst SD, Muchamuel T, Gorman DM, *et al.* New IL-17 family members promote Th1 or Th2 responses in the lung: *in vivo* function of the novel cytokine IL-25. *J Immunol* 2002; 169: 443–453.

144 National Center for Biotechnology Information. Entrez Gene. www. ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Search&DB=gene. Date last updated for IL-17: September 2 2004. Date last accessed: July 20 2004.

145 Li H, Chen J, Huang A, *et al.* Cloning and characterization of IL-17B and IL-17C: two new members of the IL-17 cytokine family. *Proc Natl Acad Sci USA* 2000; 97: 773–778.

146 Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol* 2002; 169: 642–646.

147 Lee J, Ho W-H, Maruoka M, *et al.* IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J Biol Chem* 2001; 276: 1660–1664.

148 Kim MR, Manoukian R, Yeh R, *et al.* Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. *Blood* 2002; 100: 2330–2340.

149 Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. *J Immunol* 2001; 167: 4137–4140.

150 Shi Y, Ullrich SJ, Zhang J, *et al.* A novel cytokine receptor–ligand pair: identification, molecular characterization, and *in vivo* immunomodulatory activity. *J Biol Chem* 2000; 275: 19167–19176.

151 Kawaguchi M, Onuchic LF, Li X-D, *et al.* Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. *J Immunol* 2001; 167: 4430–4435.

152 Shin H, Benbernou N, Esnault S, Guenounou M. Expression of IL-17 in human memory CD45RO+ T lymphocytes and its regulation by protein kinase A pathway. *Cytokine* 1999; 11: 257–266.

153 Aarvak T, Chabaud M, Miossec P, Natvig JB. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J Immunol* 1999; 162: 1246–1251.

154 Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol* 2000; 165: 6107–6115.

155 Happel KI, Zheng M, Young E, *et al.* Cutting edge: roles of Toll-like receptor 4 and IL-23 in IL-17 expression in response to *Klebsiella pneumoniae* infection. *J Immunol* 2003; 170: 4432–4436.

156 Laan M, Palmberg L, Larsson K, Linden A. Free, soluble interleukin-17 protein during severe inflammation in human airways. *Eur Respir J* 2002; 19: 534–537.

157 Shin HC, Benbernou N, Fekkar H, Esnault S, Guenounou M. Regulation of IL-17, IFN-γ and IL-10 in human CD8(+) cells by cyclic AMP-dependent signal transduction pathway. *Cytokine* 1998; 10: 841–850.

158 Chung DR, Kasper DL, Panzo RJ, *et al.* CD4+ T cells mediate abscess formation in intra-abdominal sepsis by

an IL-17-dependent mechanism. *J Immunol* 2003; 170: 1958–1963.

159 Yao Z, Spriggs M, Derry J, *et al.* Molecular characterisation of the human interleukin (IL)-17 receptor. *Cytokine* 1997; 9: 794–800.

160 Keehlen A, Thiele K, Rieman D, Langner J. Expression, modulation and signalling of IL-17 receptor in fibroblast-like synoviocytes of patients with rheumatoid arthritis. *Clin Exp Immunol* 2002; 127: 539–546.

161 Honorati MC, Meliconi R, Pulsatelli L, Cane S, Frizziero L, Facchini A. High *in vivo* expression of interleukin-17 receptor in synovial endothelial cells and chondrocytes from arthritis patients. *Rheumatology* 2001; 40: 522–527.

162 Prause O, Laan M, Lötvall J, Lindén A. Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-α- and interleukin-8 release in human bronchial epithelial cells. *Eur J Pharmacol* 2003; 462: 193–198.

163 Jones CE, Chan K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-α, and granulocyte-colony-stimulating factor by human airway epithelial cells. *Am J Respir Cell Mol Biol* 2002; 26: 748–753.

164 Awane M, Andres PG, Li DJ, Reinecker HC. NF-κB-inducing kinase is a common mediator of IL-17-, TNF-α-, and IL-1β-induced chemokine promoter activation in intestinal epithelial cells. *J Immunol* 1999; 162: 5337–5344.

165 Mori N, Oishi K, Sar B, *et al.* Essential role of transcription factor nuclear factor-kappa B in regulation of interleukin-8 gene expression by nitrite reductase from *Pseudomonas aeruginosa* in respiratory epithelial cells. *Infect Immun* 1999; 67: 3872–3878.

166 Harper R, Wu K, Chang MM, *et al.* Activation of nuclear factor-kappa B in airway epithelial cells by thioredoxin but not *N*-acetylcysteine and glutathione. *Am J Respir Cell Mol Biol* 2001; 25: 178–185.

167 Chang MM, Harper R, Hyde DM, Wu R. A novel mechanism of retinoic acid-enhanced interleukin-8 gene expression in airway epithelium. *Am J Respir Cell Mol Biol* 2000; 22: 502–510.

168 Kennedy T, Ghio AJ, Reed W, *et al.* Copper-dependent inflammation and nuclear factor-kappa B activation by particulate air pollution. *Am J Respir Cell Mol Biol* 1998; 19: 366–378.

169 Mastronarde JG, Monick MM, Mukaida N, Matsushima K, Hunninghake GW. Activator protein-1 is the preferred transcription factor for cooperative interaction with nuclear factor-kappa B in respiratory syncytial virus-induced interleukin-8 gene expression in airway epithelium. *J Infect Dis* 1998; 177: 1275–1281.

170 Bank U, Reinhold D, Kunz D, *et al.* Effects of interleukin-6 (IL-6) and transforming growth factor β (TGF-β) on neutrophil elastase release. *Inflammation* 1995; 19: 83–89.

171 Ottonello L, Frumento G, Arduino N, *et al.* Differential regulation of spontaneous and immune complex-induced neutrophil apoptosis by proinflammatory cytokines. Role of oxidants, Bax and caspase-3. *J Leukoc Biol* 2002; 72: 125–132.

172 Saba S, Soong G, Greenberg S, Prince A. Bacterial stimulation of epithelial G-CSF and GM-CSF expression promotes PMN survival in CF airways. *Am J Respir Cell Mol Biol* 2002; 27: 561–567.

173 Schwander R, Yamaguchi K, Zhaodan C. Requirement of tumour necrosis factor receptor-associated factor (TRAF)-6 in interleukin-17 signal transduction. *J Exp Med* 2000; 191: 1233–1239.

174 Wuyts WA, Vanaudanaerde BM, Dupont LJ, Van Raemdock DE, Demedts MG, Verleden GM. *N*-acetylcysteine inhibits interleukin-17-induced interleukin-8 production from human airway smooth muscle cells: a possible role for anti-oxidative treatment in chronic lung rejection? *J Heart Lung Transplant* 2004; 23: 122–127.

175 Shalom-Barak T, Quach J, Lotz M. Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NFκB. *J Biol Chem* 1998; 273: 27467–27473.

176 Hoshino H, Lötvall J, Skoogh BE, Lindén A. Neutrophil recruitment by IL-17 into rat airways *in vivo*: role of tachykinins. *Am J Respir Crit Care Med* 1999; 159: 1423–1428.

177 Ye P, Garvey PB, Zhang P, *et al.* Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. *Am J Respir Crit Care Med* 2001; 25: 335–340.

178 Larsson R, Rocksén D, Lillienhook B, Jonsson A, Bucht A. Dose-dependent activation of lymphocytes in endotoxin-induced airway inflammation. *Infect Immun* 2000; 68: 6962–6969.

179 Schwarzenberger P, La Russa V, Miller A, *et al.* IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for *in vivo* evaluation of cytokines. *J Immunol* 1998; 161: 6383–6389.

180 Schwarzenberger P, Huang W, Ye P, *et al.* Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. *J Immunol* 2000; 164: 4783–4789.

181 Reynolds SJ, Donham KJ, Whitten P, Merchant JA, Burmeister LF, Popendorf WJ. Longitudinal evaluation of dose–response relationships for environmental exposures and pulmonary function in swine production workers. *Am J Ind Med* 1996; 29: 33–40.

182 Barzyk A, Pierzcha W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* 2003; 97: 726–733.

183 Molet SM, Hamid QA, Hamilos DL. IL-11 and IL-17 expression in nasal polyps: relationship to collagen disposition and suppression by intranasal fluticasone propionate. *Laryngoscope* 2003; 113: 1803–1812.

184 Nakae S, Komiyama Y, Nambu A, *et al.* Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002; 17: 375–387.

185 Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *J Leukoc Biol* 2002; 71: 1–8.

186 Dumont F. IL-17 cytokine/receptor families: emerging targets for the modulation of inflammatory responses. *Expert Opin Ther Patents* 2003; 13: 287–303.

187 Witovski J, Ksiazek K, Jorres A. Interleukin-17: a mediator of inflammatory responses. *Cell Mol Life Sci* 2004; 61: 567–579.