Chronic obstructive pulmonary disease (COPD) is a heterogeneous syndrome associated with abnormal inflammatory immune responses of the lung to noxious particles and gases. Cigarette smoke activates innate immune cells such as epithelial cells and macrophages by triggering pattern recognition receptors, either directly or indirectly via the release of damage-associated molecular patterns from stressed or dying cells. Activated dendritic cells induce adaptive immune responses encompassing T helper (Th1 and Th17) CD4+ T cells, CD8+ cytotoxicity, and B-cell responses, which lead to the development of lymphoid follicles on chronic inflammation. Viral and bacterial infections not only cause acute exacerbations of COPD, but also amplify and perpetuate chronic inflammation in stable COPD via pathogen-associated molecular patterns. We discuss the role of autoimmunity (autoantibodies), remodelling, extracellular matrix-derived fragments, impaired innate lung defences, oxidative stress, hypoxia, and dysregulation of microRNAs in the persistence of the pulmonary inflammation despite smoking cessation.

Introduction

Chronic obstructive pulmonary disease (COPD) affects more than 200 million people worldwide and is the fourth leading cause of death.1 Although the major environmental risk factor for COPD is tobacco smoking, only 20% of smokers develop COPD.2 Indoor air pollution from burning biomass fuels is associated with an increased risk of COPD in developing countries.3 Immune dysregulation arises in the peripheral blood of patients with COPD, and might contribute to the pathogenesis of the extrapulmonary effects of the disorder.4 Although overspill of inflammation in the lung into the circulation is suggested to cause systemic inflammation in a subset of patients with COPD, other pathogenic mechanisms such as smoking, ageing, abdominal obesity, and physical inactivity are probably involved.4 We focus on the role of local immune responses in the airways and lungs in the pathogenesis and progression of COPD due to cigarette smoking (figure 1). Table 1 shows characteristics of the innate and adaptive immune system.

Several mechanistic concepts have been implicated in the pathogenesis of COPD. First, the hallmark of COPD is development of exaggerated chronic inflammation in the lung in response to inhalation of cigarette smoke compared with smokers without lung disease.5 Host factors including genetic susceptibility, epigenetic changes, and oxidative stress (webappendix p 2) contribute by amplifying inflammation induced by cigarette smoke. Second, patients with deficiency of α1-antitrypsin, the main inhibitor of neutrophil elastase, develop emphysema early in life,6 due to an imbalance between proteinases and antiproteinases leading to a net increase in proteolytic activity. Third, an imbalance between oxidants and antioxidants in the lungs of patients with COPD, resulting in excessive oxidative stress, not only amplifies airway inflammation in smokers, but also induces cell death of structural cells in the lung (mainly alveolar epithelial and endothelial cells).7 Disruption of the balance between cell death and replenishment of structural cells in the lung contribute to the destruction of alveolar septa, leading to emphysema. Additionally, age-related changes and cellular senescence further impair tissue repair in response to repetitive cigarette smoke-induced injury of the lungs.8 Autoimmunity has been proposed as a late pathogenic event in the progressive course of the disease.9 The diverse mechanisms do not operate separately in the pathogenesis of COPD, but are strongly interrelated. Oxidative stress, for example, contributes to the imbalance between proteinase and antiproteinase by inactivating antiproteinases, whereas an excess of apoptotic cells leads to secondary necrosis and can exaggerate continuing pulmonary inflammation.10,11

Innate immune responses in COPD

To prevent invasion of pathogenic microbes into the lower respiratory tract, the airways and lungs have innate defence mechanisms encompassing the epithelial barrier, mucociliary clearance, humoral factors (eg, antimicrobial peptides, complement proteins, and surfactant proteins) and cells that participate in innate immunity: macrophages, dendritic cells, monocytes, neutrophils, natural killer cells, and mast cells.

Search strategy and selection criteria

We searched Medline for the past 10 years with the search terms “COPD” or “emphysema” in combination with one of the following terms: “immunity”, “macrophages”, “neutrophils”, “mast cells”, “natural killer cells”, “T-cells (Th1, Th2, Th17, or Treg)”, “B-cells” or “(auto)antibodies”. We largely selected publications in the past 5 years, but did not exclude commonly referenced and highly regarded older publications. The date of the last search was April 26, 2011. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles are cited to provide readers with more details and more references than this Review has room for.
The first step towards the induction of innate immune responses is recognition of different microbes by identification of molecules that are exclusive to microbes—pathogen-associated molecular patterns (PAMPs). This recognition is achieved by several families of pattern recognition receptors (PRRs) expressed in alveolar macrophages, dendritic cells, and epithelial cells, which first contact microbial pathogens (webappendix p 2). The PRRs include transmembrane Toll-like receptors (TLRs), cytosolic NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs). In addition to PAMPs, PRRs are activated by specific endogenous molecules, which are normally intracellular, but are released after cell damage. After release from injured or dying cells, these endogenous molecules are called damage-associated molecular patterns (DAMPs). The recognition of PAMPs and DAMPs by PRRs is crucial in mediation of inflammatory responses to infection and sterile tissue damage, respectively (figure 2).

Acute exposure to cigarette smoke leads to activation of several PRRs, either directly by individual components of cigarette smoke, or indirectly by causing injury to epithelial cells, which release DAMPs (table 2). Several mechanisms, including inhalation of toxic agents and irritants, oxidative stress, infections, and tissue hypoxia, lead to the release of DAMPs by injured airways after cigarette smoke exposure. Concentrations of high-mobility group box 1 (HMGB1), uric acid, and extracellular ATP are increased in bronchoalveolar lavage fluid of patients with COPD compared with smokers without COPD. Moreover, expression of receptor for advanced glycation end products (RAGE), which binds HMGB1, is raised in airway epithelium of patients with COPD. Activation of PRRs such as TLRs and RAGE leads to increased expression of pro-interleukin 1β, which is subsequently cleaved into mature interleukin 1β by NLR family, pyrin domain-containing (NLRP) inflammasomes (figure 2). IL-1R knock-out mice showed attenuated pulmonary inflammation after acute exposure to cigarette smoke and were significantly protected against emphysema after chronic exposure to cigarette smoke. Moreover, inhibition of P2 purinergic receptors—which bind extracellular ATP—prevented the development of smoke-induced lung injury and emphysema in a COPD model.

In lung cells, another general inducible response to injury resulting from exposure to cigarette smoke and oxidative stress is autophagy (webappendix p 3). In lung tissue from patients with COPD, as early as stage 0 on the global initiative of obstructive lung disease scale, autophagy...
is increased as indicated by increased autophagic vacuoles (autophagosomes and autolysosomes) on electronic microscopic analysis and by increased activation of autophagic proteins such as Atg4B, Atg5-Atg12, and Atg7. In human pulmonary epithelial cells, exposure to cigarette smoke extract in vitro rapidly induces autophagy. The autophagic protein microtubule-associated protein 1 light chain 3B (LC3B) has a pivotal role not only in autophagy, but also in apoptosis since it associates with the extrinsic apoptotic factor Fas. LC3B knock-out mice have significantly decreased rates of apoptosis in the lungs after exposure to cigarette smoke and display resistance to cigarette-smoke-induced emphysema in vivo. The transcription factor early growth response-1 (Egr-1) is believed to regulate LC3B and promote autophagy and apoptosis in response to cigarette smoke, because activation of autophagic proteins and apoptotic factors is attenuated in Egr-1 knock-out mice that are exposed to cigarette smoke.

In the effector phase of innate immune responses, cigarette-smoke-induced release of proinflammatory cytokines and chemokines—such as tumour necrosis factor α (TNFα) and chemokine (C-X-C motif) ligand (CXCL)8—by airway epithelial cells and alveolar macrophages elicits the expression of adhesion molecules on endothelial cells and the recruitment of neutrophils and inflammatory monocytes to the lungs. Numbers of neutrophils and macrophages are increased in the lungs of smokers and patients with COPD. At sites of sterile inflammation neutrophils contribute to wound healing, but can cause tissue damage. Activated neutrophils and macrophages cause lung destruction through the release of oxygen radicals and proteolytic enzymes such as neutrophil elastase and matrix metalloproteinases (MMPs), including MMP-8, MMP-9, and MMP-12 (formerly called macrophage elastase). CD8+ T cells and natural killer cells contribute to cytotoxicity of lung tissue cells through the release of the proteolytic enzymes perforin and granzyme B. In addition to its ability to breakdown extracellular matrix, neutrophil elastase is a potent stimulator of mucin production and secretion. Neutrophil elastase-induced production of mucin occurs via proteolytic cleavage of transforming growth factor α (TGFα), a ligand of epidermal growth factor receptor. Excessive mucous production and impaired mucociliary clearance contribute to airway obstruction in patients with COPD.

In models of COPD, chronic exposure of mice to cigarette smoke for 6 months induces several pathological hallmarks of the disorder, including pulmonary inflammation, airway remodelling and airspace enlargement due to destruction of alveolar walls (ie, emphysema). In much the same way as differences in genetic susceptibility in human smokers affect development of COPD, the induction of emphysema in mice is strain dependent. Importantly, in immuno-deficient mice lacking B cells and T cells, the innate immune system suffices to develop cigarette-smoke-induced inflammation and emphysema.

Although mast cells have been traditionally associated with the pathophysiological mechanisms of allergic asthma, evidence suggests that mast cells could be implicated in the pathogenesis of COPD. Histological studies of human lungs have shown that as COPD progresses to its severe stages mast cell populations undergo changes in density, morphology, and distribution, including an increase in the number of luminal mast cells. Although natural killer cells are classified as lymphocytes on the basis of their morphology, the expression of lymphoid markers, and their origin from a common lymphoid progenitor cell, they are deemed components of innate immune defence because they lack antigen-specific cell surface receptors. Natural killer cells act as cytolytic effector lymphocytes, which can directly induce the death not only of virus-infected cells and tumour cells, but also of structural cells in the lungs, which die by apoptosis or necrosis from damage caused by perforin and granzyme B. Increased proportions of natural killer cells that are CD3− and CD56+ and increased cytotoxic activity of natural killer cells expressing both perforin and granzyme B, have been shown in induced sputum of patients with COPD compared with healthy smokers. Additionally, natural killer cells cross-talk with dendritic cells, promoting the maturation of dendritic cells by producing interferon γ and TNFα.

### Dendritic cells and innate and adaptive immunity

Dendritic cells are specialised antigen-presenting cells that link innate and adaptive immune responses (figure 1 and webappendix p 3). By the integration of many signals of the microenvironment dendritic cells promote CD4+ T helper (Th) cell differentiation and CD8+...
Cigarette smoke activates epithelial cells, macrophages, and neutrophils via oxidative stress and by triggering pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and purinergic receptors (eg, P2X, and P2Y), either directly by cigarette components or indirectly via the release of damage-associated molecular patterns (DAMPs) by dying—autophagic death, apoptotic or necrotic—cells. Viral and bacterial respiratory tract infections amplify the chronic inflammation in COPD by triggering PRRs via pathogen-associated molecular patterns (PAMPs). On activation, the innate immune cells release pro-inflammatory cytokines and chemokines, reactive oxygen species (ROS) and proteolytic enzymes (neutrophil elastase [NE] and matrix metalloproteinases [MMPs]). More research is needed to show the importance of NOD-like receptor family, pyrin domain containing (NLRP) inflammasomes in chronic obstructive pulmonary disease. HSP=heat shock protein. HMGB1=high-mobility group box 1. HA=hyaluronic acid. NF-κB=nuclear factor κB. CXCL=chemokine (c-x-c motif) ligand. RAGE=Receptor for Advanced Glycation End products.

Figure 2: Afferent and efferent part of innate immune responses in chronic obstructive pulmonary disease

Cigarette smoke activates epithelial cells, macrophages, and neutrophils via oxidative stress and by triggering pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and purinergic receptors (eg, P2X, and P2Y), either directly by cigarette components or indirectly via the release of damage-associated molecular patterns (DAMPs) by dying—autophagic death, apoptotic or necrotic—cells. Viral and bacterial respiratory tract infections amplify the chronic inflammation in COPD by triggering PRRs via pathogen-associated molecular patterns (PAMPs). On activation, the innate immune cells release pro-inflammatory cytokines and chemokines, reactive oxygen species (ROS) and proteolytic enzymes (neutrophil elastase [NE] and matrix metalloproteinases [MMPs]). More research is needed to show the importance of NOD-like receptor family, pyrin domain containing (NLRP) inflammasomes in chronic obstructive pulmonary disease. HSP=heat shock protein. HMGB1=high-mobility group box 1. HA=hyaluronic acid. NF-κB=nuclear factor κB. CXCL=chemokine (c-x-c motif) ligand. RAGE=Receptor for Advanced Glycation End products.

cytotoxicity (figure 3). Smoking of cigarettes has been associated with an expansion in the population of Langerhans-like dendritic cells in the epithelial surface of the lower respiratory tract. After acute smoke exposure, myeloid dendritic cells are immediately and selectively recruited into the bronchoalveolar lavage fluid of smokers. Moreover, expression of Langerin and CD1a (markers of Langerhans-like dendritic cells) is increased, as is that of costimulatory markers CD80 and CD86 on myeloid dendritic cells from the bronchoalveolar lavage fluid of smokers compared with never-smokers.

In COPD, several dendritic cell subsets in the lung parenchyma showed significant increases in costimulatory molecule expression, which correlated with COPD severity. By immunohistochemistry we showed selective accumulation of Langerin+ myeloid dendritic cells in the small airways of patients with COPD, which increased with disease severity and was associated with increased expression of chemokine (c-c motif) ligand (CCL) 20α (figure 4). The Langerin+ subset of myeloid dendritic cells is mainly present in the airway epithelium and is segregated from the interstitial myeloid dendritic cell subset that is positive for dendritic cell-specific intracellular adhesion molecule 3—grabbing non-integrin, which is located in the lamina propria and adventitia and does not increase in patients with COPD (figure 4). Langerin+ dendritic cells have been linked to the induction of CD8+ T-cell responses by cross-presentation.

**Adaptive immune responses in COPD**

In both the airway and alveolar compartment, the CD8+ cytotoxic T cell is the predominant T cell in patients with COPD. The number of pulmonary CD8+ T cells in COPD increases substantially with higher stages of airflow limitation and emphysema. The expression of the chemokine receptor CXCR3, preferably...
expressed on type 1 T lymphocytes, and its ligand interferon-induced protein 10 (also known as CXCL10) (table 3) is increased in peripheral airways of smokers with COPD, suggesting that the axis between CXCL10 and chemokine (c-x-c motif) receptor (CXCR) 3 might be implicated in CD8+ T-cell recruitment in COPD. On activation, CD8+ T cells release proteolytic enzymes such as perforin and granzymes, which cause cell death of structural cells by apoptosis or necrosis (figure 5).

Numbers of CD4+ T cells are raised in the airways and lungs of smokers with COPD. At least two different types of effector CD4+ Th cells accumulate in the lungs of patients with stable COPD: Th1 cells and Th17 cells. Th1 cells expressing chemokine receptors CCR5 and CXCR3 produce interferon γ and promote accumulation of inflammatory cells to the lungs. Lung lymphocytes in patients with COPD and emphysema have higher percentages of CD4+ Th1 cells and secrete more interferon γ than in control smokers. Interleukin 17A, which promotes T17 cell development, is strongly expressed in alveolar macrophages, CD8+ T cells, and both bronchial and alveolar epithelia in lungs of patients with severe COPD. Th17 cells are a distinct lineage of activated CD4+ T cells, regulating tissue inflammation by producing interleukin 17A and interleukin 17F. They mediate immunity against extracellular pathogens, but have also been implicated in autoimmunity. Th17 cytokines induce epithelial cells to produce antimicrobial peptides (such as β defensins), chemokines, and the granulocyte growth factors G-CSF and GM-CSF to promote neutrophil accumulation at the site of tissue injury. Patients with COPD have increased numbers of interleukin 23+ immunoreactive cells in the bronchial epithelium and interleukin 17A+ cells in the bronchial submucosa. However, because interleukin 17A production is linked to structural cells such as endothelial cells, sources other than T cells might be important.

Regulatory T cells (Treg) are subsets of CD4+ T cells with immunoregulatory functions, which inhibit autoimmunity and suppress inflammation. The two most abundant subsets of Treg are the natural Treg and the adaptive or inducible Treg. Natural Treg leave the thymus as effector cells and are essential in maintaining self-tolerance, whereas induced Treg mature in the periphery and are activated by exogenous antigens. Treg exert their suppressive effect on other T cells or on antigen-presenting dendritic cells via contact-dependent mechanisms or the production of anti-inflammatory cytokines such as interleukin 10 and transforming growth factor β (TGFβ). They are characterised by expression of the transcription factor FOXP3 (forkhead box P3) and bright expression of the surface marker CD25, the interleukin 2 receptor α chain. Smokers with COPD and emphysema had significantly fewer Treg cells in the lungs, less messenger RNA (mRNA) for FOXP3, and less interleukin 10 secretion from the whole lung than controls. CD4+ CD25-bright cells were significantly increased in bronchoalveolar lavage fluid in smokers with normal lung function compared with people who had never smoked and patients with moderate COPD in one study, whereas bronchoalveolar lavage CD4+ CD25-bright cells were increased in smokers and patients with COPD compared with healthy people who had never smoked in another study. Because these cells encompass both FOXP3+ Treg and non-regulatory activated Th cells, additional multicellular flow cytometric and functional studies of bronchoalveolar lavage fluid and lung digestes are warranted to resolve this discrepancy. T cells that are FOXP3+ and CD4+ are raised in lymphoid follicles of patients with moderate COPD compared with control smokers, suggesting that Treg might regulate inflammatory responses to prevent autoimmunity. Treg that are CD4+, CD25+, and FOXP3+ resolve experimental lung injury in mice after intratracheal lipopolysaccharide administration and are present in the bronchoalveolar lavage fluid of patients with acute lung injury, suggesting that Treg modify innate immune responses during resolution of lung injury.

### B cells
B cells are increased in large airways of patients with COPD, as shown in central bronchial biopsy specimens.
Moreover, B cells organised into lymphoid follicles have been shown around small airways\(^1\) and in lung parenchyma\(^6\) of patients with COPD, especially in severe and very severe disease. As seen in other tissues that are affected by chronic inflammation, lymphoid follicles in patients with COPD result from lymphoid neogenesis\(^7\) and belong to the inducible Bronchus-Associated Lymphoid Tissue (iBALT), an ectopic lymphoid tissue that is formed on infection or inflammation in both mice and man.\(^8\) iBALT acquires antigens from the airways, initiates local immune responses, and maintains memory cells in the lungs.

Lymphoid follicles are anatomically and functionally well organised, consisting of a specific arrangement of memory and naive B cells, T cells, dendritic cells, and follicular dendritic cells, which allow for T-cell and B-cell priming and clonal expansion.\(^9\) Plasmacytoid dendritic cells have been recorded in peribronchiolar lymphoid follicles of patients with COPD.\(^10\) B-cell activating factor of tumour necrosis factor family (BAFF) is overexpressed in lymphoid follicles in lungs of patients with COPD, compared with controls, suggesting that the BAFF to BAFF-receptor pathway contributes to the development and maintenance of lymphoid follicles in COPD.\(^11\)

Moreover, pulmonary BAFF expression correlates to the degree of airflow limitation and hypoxia, two important markers of disease severity.

The compartmentalisation of lymphoid follicles in B-cell and T-cell areas is—as in lymph nodes—regulated by homeostatic chemokines, which dictate homing and motility of lymphocytes and dendritic cells in lymphoid tissues.\(^12\) CCL19 and CCL21 attract naive T lymphocytes expressing CCR7 and mature dendritic cells into the T-cell areas, whereas the CXC chemokine CXCL13 attracts B cells expressing CXCR5 (table 3). Whereas in lymph nodes lymphotixin a induces the expression of the chemokines CCL19 and CXCL13, interleukin 17 is needed for the induction of these chemokines in iBALT.\(^13\)

Once formed, iBALT is longlasting and participates in local immune responses.\(^14\)

In these lymphoid follicles—especially in germinal centres of secondary lymphoid follicles—antigen retention, immunoglobulin class switching, and affinity maturation occur.\(^15\) The B cells are oligoclonal in nature, suggesting antigen-specific induction of the B-cell follicles.\(^16\) Which antigens might be involved is not known, but microbial antigens, cigarette smoke-derived antigens, breakdown products from extracellular matrix, and autoantigens have been suggested.\(^17\) Therefore, the pathogenic role of the follicular B-cell response is controversial; it might be beneficial, if protective against microbial colonisation and infection of the lower respiratory tract; or by contrast, it could be detrimental, if directed against lung tissue antigens, suggesting an autoimmune component in the pathogenesis of COPD and especially emphysema.\(^18\)

**Figure 3:** Dendritic cells driving CD4+ T helper cell differentiation and CD8+ T-cell cytotoxicity

MHC I-restricted dendritic cells present antigenic peptides to CD8+ T cells, whereas MHC II-restricted dendritic cells drive the differentiation of naive CD4+ T cells towards T helper (Th) 1, Th2, Th17 cells or regulatory T cells (Treg) depending on the cytokine balance in the local microenvironment. The different Th cell subsets and Treg cells are characterised by different transcription factors and membrane-bound receptors (eg, chemokine receptors) and produce different cytokines. IL=interleukin. CXCR=chemokine (c-x-c motif) receptor.

Infection in COPD

Infections of the respiratory tract contribute to the pathogenesis and course of COPD in at least two different ways.\(^19\) First, viral and bacterial infections are the most important cause of acute exacerbations of COPD. As airflow obstruction progresses in COPD, the frequency of exacerbations greatly increases.\(^20\) Second, colonisation and chronic infection of the lower airways by respiratory pathogens can amplify and perpetuate chronic inflammation in stable COPD. The frequency of chronic bacterial colonisation and infection increases progressively with disease severity.\(^21\)

Importantly, in COPD, phagocytosis of bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae* by alveolar macrophages is impaired.\(^22\) Monocyte-derived macrophages and alveolar macrophages from patients with COPD showed significantly decreased phagocytic responses to bacteria compared with non-smokers and smokers.\(^23\) The impairment of phagocytosis of bacteria by alveolar macrophages in COPD contributes to chronic bacterial colonisation and to acute infectious exacerbations.

Bacteria such as *H influenzae*, *S pneumoniae*, and *Moraxella catarrhalis* are detected in about 25% of patients with stable COPD and in more than 50% of patients during COPD exacerbations.\(^24\) Bacterial exacerbations lead to increased airway and systemic inflammation, as a
result of direct effects of bacteria and of the host response.⁶⁵ Several PAMPs of bacteria, including lipopolysaccharides, peptidoglycans, and outer-membrane proteins, are sensed by specific PRRs on epithelial cells and innate immune cells (table 4), triggering the nuclear factor-kB pathway and other signal transduction pathways such as p38 mitogen-activated protein kinase and interferon regulatory factors, resulting in the production of pro-inflammatory cytokines and chemokines (figure 2). TLR signalling in macrophages seems to link phagocytosis, a biological process to remove extracellular organisms, to autophagy, (webappendix p 3).⁶⁶ Not only an increase in the concentration of bacteria that colonise the lower airways in patients with COPD, but also the acquisition of a new bacterial strain is crucial in the pathogenesis of exacerbations.⁶⁷ In patients with severe COPD, exacerbations can be caused by *Pseudomonas aeruginosa*.

Viruses can be detected in 10–15% of sputum samples in patients with stable COPD, and in 30–60% of those with COPD exacerbations, dependent on the sensitivity of the detection method.⁶⁸ Rhinoviruses and influenza virus are the respiratory viruses that are most frequently isolated from nasal and lung fluid from patients with acute exacerbations of COPD,⁶⁹ indicating that rhinoviruses infect upper and lower airway epithelial cells.

In induced rhinovirus infection in volunteers with COPD, Johnston and colleagues⁷⁰ showed a causal relation between rhinovirus infection and COPD exacerbations. After rhinovirus infection, patients with COPD developed lower respiratory symptoms, airflow obstruction, and systemic and airway inflammation that were greater and longer than in controls; virus load was higher in patients with COPD, whereas interferon production by systemic and airway inflammation in COPD. Patients with COPD develop increased concentrations of neutrophils, CXCL8, TNFα, and proteases (neutrophil elastase and MMP-9) in COPD patients and bacterial colonisation compared with those without bacterial pathogens.⁷¹ The colonisation-induced triggering of PRR by microbial PAMPs thus amplifies the chronic neutrophilic airway inflammation in COPD. Patients with COPD develop specific adaptive immune responses to colonising bacteria. These adaptive immune responses contribute locally to the development of B-cell lymphoid follicles and mucosal IgA production, and systemically to the production of IgG antibodies in serum. Strain-specific immunoglobulin responses reduce the risk of exacerbation after acquisition of a new bacterial strain and contribute to clearance of the pathogen.⁷²

![Figure 4: Dendritic cells in chronic obstructive pulmonary disease (COPD)](A and B) Accumulation of dendritic cells in small airways of patients with COPD. Langerin+ dendritic cells (brown) in human lung tissue cryosections of (A) a smoker without COPD and (B) a patient with stage II COPD according to the global initiative for chronic obstructive lung disease (GOLD) scale. (C) Immunohistochemical double staining for dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin+ (DC-SIGN+) intestinal-type dendritic cells (red) and langerin+ Langerhans type dendritic cells (blue) in small airways of human lung. Langerin+ dendritic cells are mainly present in the epithelium, whereas DC-SIGN+ cells are located in the lamina propria and adventitia. There are no double positive cells, confirming the segregation of these two dendritic cell subsets in human lung. (D) Identification of plasmacytoid dendritic cells in a lymphoid follicle of a patient with COPD GOLD stage II. Triple staining of a cryosection of human lung tissue for CD3 (blue colour indicates the T-cell zone), CD20 (red-pink colour indicates plasma cells) and BDCA-2 (brown colour indicates plasmacytoid dendritic cells). Plasmacytoid dendritic cells (arrows) are mainly found in the T-cell zone of lymphoid follicles.

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Simplified and non-limiting table of chemokines and chemokine receptors implicated in COPD. CCL=chemokine (c-c motif) ligand. CXCL=chemokine (c-x-c motif) ligand. MCP=monocyte chemotractant protein 1. MIP=macrophage inflammatory protein. RANTES=regulated on activation normal T-cell expressed and secreted. SLC=secondary lymphoid tissue chemokine. GRO-α=growth related oncogene α. Mig=monokine induced by interferon γ. IP-10=interferon-γ inducible protein of 10 kDa. I-TAC=interferon-inducible T-cell-α chemoattractant. BCA-1=B-cell attracting chemokine.

<p>| Table 3: Chemokines and chemokine receptors in chronic obstructive pulmonary disease (COPD) |
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Is COPD an autoimmune disease?

On the basis of the presence of B-cell lymphoid follicles in patients with advanced COPD and the detection of diverse autoantibodies in serum of a subgroup of patients with COPD,\textsuperscript{44,74} COPD has been regarded as an autoimmune disease.\textsuperscript{11} Lee and colleagues\textsuperscript{44} showed the presence of anti-elastin antibody and Th1 responses in patients with COPD, which correlated with severity of emphysema. However, several other research groups could not detect increased anti-elastin antibody titres in patients with COPD, which correlated with severity of emphysema.\textsuperscript{75} Greene and colleagues\textsuperscript{75} reported no significant differences in the levels of anti-elastin or anti-N-acetylated proline-glycine-proline (Pro-Gly-Pro) autoantibodies in three groups of patients with chronic inflammatory lung disease (cystic fibrosis, α1-antitrypsin deficiency, and COPD) compared with healthy controls. Moreover, smoke exposure but not disease state seemed to be the main determinant of anti-elastin antibody concentrations.\textsuperscript{76} Antibodies against primary pulmonary epithelial cells, including anti-Hep-2 epithelial cell IgG autoantibodies, were recorded more frequently in patients with COPD than in controls.\textsuperscript{77} Serum concentrations of antitissue antibodies, especially antismooth muscle-antigen antibodies, were present in a fifth of patients with COPD with a recent history of hospitalisation for exacerbation of COPD, and correlated with the severity of airflow limitation.\textsuperscript{78} Finally, antinuclear autoantibodies were more prevalent in patients with COPD than in healthy controls, but these molecules were not associated with smoking status or FEV\textsubscript{1}, hence the pathological importance of these observations is unclear.\textsuperscript{74,75}

Why does pulmonary inflammation persist despite smoking cessation?

Airway inflammation in patients with COPD persists after smoking cessation.\textsuperscript{79} Although autoimmunity might contribute to the perpetuation of pulmonary inflammation in a subgroup of patients with advanced COPD and
emphysema, several other mechanisms are likely to be involved in persistence of the inflammatory response despite smoking cessation. These mechanisms are not mutually exclusive and encompass self-perpetuating innate immune responses, airway wall remodelling, impaired macrophage clearance, chronic colonisation and infection of the lower airways, oxidative stress, tissue hypoxia, genetic susceptibility, and epigenetic changes.21

First, destruction of extracellular matrix (collagen, elastin, and hyaluronan), releases proinflammatory fragments that are chemotactic for neutrophils and monocytes, sustaining inflammation even when the initial stimulus is gone.27 CXC chemokines mediate neutrophil attraction to sites of tissue injury, and matrix fragments of collagen—including the tripeptide N-acetyl Pro-Gly-Pro—are important neutrophil chemoattractants.79 Furthermore, fragments of elastin have major chemotactic activity, inducing recruitment of monocytes and macrophages in experimental models of emphysema. Additionally, other constituents or fragments of the extracellular matrix such as tenascin C, biglycan, and low molecular weight hyaluronan contribute to the perpetuation of the inflammatory response, partly through activation of TLR2, or TLR4, or both (table 2).

Second, the removal of apoptotic cells (termed efferocytosis) is a highly conserved process, and is essential for the resolution of inflammation and the maintenance of lung integrity.20 In COPD, apoptotic cells are increased because of enhanced induction of apoptosis and deficient phagocytosis of apoptotic cells by alveolar macrophages, which lead to impaired resolution of inflammation. In a murine model of COPD, acute and subacute cigarette exposure suppresses efferocytosis by alveolar macrophages.78 Moreover, apoptosis of neutrophils followed by efferocytosis not only prevents damage, but also induces an anti-inflammatory macrophage phenotype (M2 or alternatively activated macrophages). By contrast, failed efferocytosis of apoptotic neutrophils leads to secondary necrosis, which results in the release of DAMPs, neutrophil elastase, and other toxic components into the extracellular space (figure 2).

Third, impaired innate lung defences predispose patients to microbial colonisation and infection of the lower respiratory tract, which causes additional airway epithelial injury. This cyclical sequence of events amplifies chronic inflammation and perpetuates microbial infections that are characteristic of severe COPD.23 Fourth, local tissue hypoxia in patients with COPD can perpetuate lung inflammation, because of an important bidirectional communication between hypoxia and inflammation.24 In hypoxic conditions, hypoxia-inducible factor 1α (HIF-1α) translocates from the cytoplasm to the nucleus, inducing transcription of multiple genes, including those of TLRs and nuclear factor-κB. HIF-1α prolongs the lifespan of neutrophils by inhibiting apoptosis. Conversely, inflated tissues often become hypoxic and activation of the nuclear factor-κB pathway leads to the transcription of inflammatory genes and HIF-1α.85 Fifth, expression of several microRNAs— which negatively regulate protein expression (webappendix p 4)—is significantly decreased in induced sputum of patients with COPD.86 Of particular interest is the decreased expression of microRNA let-7c in sputum of smoking patients with COPD, because let-7c not only regulates several inflammatory genes such as TNFR-II, but has also been implicated in oncogenesis (eg, lung cancer).87

Importantly, in patients with COPD oxidative stress persists after smoking cessation, and has a crucial role in perpetuation of pulmonary inflammation (webappendix p 2). The reduced histone deacetylase 2 activity in COPD has been linked to increased acetylation of the central antioxidant transcription factor Nrf 2, impairing Nrf 2 activity and the upregulation of antioxidant enzymes in response to oxidative stress.88 Impairment of Nrf2-mediated antioxidant defences and epigenetic changes induced by reactive oxygen species (eg, histone modifications) contribute to the persistence of oxidative stress in COPD.89,90

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>PAMP</th>
<th>PRR (cellular [soluble])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Lipoteichoic acid, pneumolysin</td>
<td>TLR2, TLR4, PAFR</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Peptidoglycan</td>
<td>TLR2, TLR4, CD14, [LBP]</td>
</tr>
<tr>
<td>CpG dinucleotides</td>
<td>Phosphorylcholine</td>
<td>TLR9</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>TLR4</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>LPS</td>
<td>TLR4 and CD14, [LBP]</td>
</tr>
<tr>
<td>OMP P6, OMP P2</td>
<td>Peptidoglycan</td>
<td>TLR2</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>LPS</td>
<td>TLR4 and CD14, [LBP]</td>
</tr>
<tr>
<td>Usp A1</td>
<td>Usp A2</td>
<td>NODD, NOD2, CAECAM1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAFR</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>LPS</td>
<td>TLR4 and CD14, [LBP]</td>
</tr>
<tr>
<td>Flagellin</td>
<td></td>
<td>TLR5</td>
</tr>
</tbody>
</table>

Table 4: Microbial pathogens, pathogen associated molecular patterns (PAMP), and pattern recognition receptors (PRR) in chronic obstructive pulmonary disease

Therapeutic implications

Smoking cessation attenuates the accelerated decrease in lung function in patients with COPD.31,32 A pooled analysis of three bronchial biopsy studies showed that, in established COPD, the numbers of inflammatory cells in the bronchial mucosa are much the same as in former smokers and in persistent smokers.26 However, analysis of bronchoalveolar lavage fluid of COPD patients suggests that smoking cessation partly changes the macrophage polarisation from a
proinflammatory M1 towards an anti-inflammatory M2 macrophage phenotype.86

Regular treatment with long-acting bronchodilators is the mainstay of therapy in symptomatic patients with moderate to severe COPD, but only a few randomised controlled trials have investigated the effects of pharmacological treatment on the underlying bronchial and pulmonary inflammation.90–92 3-month treatment with the inhaled corticosteroid fluticasone-propionate did not reduce numbers of CD8+ T cells, macrophages, or neutrophils in bronchial biopsy samples,93 whereas combination therapy of salmeterol xinafoate and fluticasone was associated with a reduction in CD8+ cells on biopsy samples.94 Whereas—especially mild to moderate—asthma is usually highly responsive to inhaled corticosteroid therapy, COPD patients respond poorly to such treatment. The differences in corticosteroid responsiveness between asthma and COPD are due to several molecular mechanisms, including oxidative stress.95 Cigarette smoke and stress from reactive oxygen species can prevent nuclear translocation of the glucocorticoid receptor or decrease the activity of the co-repressor protein histone deacetylase 2, reducing the ability of corticosteroids to switch off inflammatory gene expression.96,97 Additionally, differences in the type of the underlying immune responses between asthma and COPD, might contribute to differential corticosteroid responsiveness.98,99 The Th2-driven eosinophilic airway inflammation in mild allergic asthma is highly responsive to inhaled corticosteroids, implicating this drug class as the mainstay treatment in persistent asthma. By contrast, the chronic neutrophilic airway inflammation in COPD is resistant to steroids.95 Short-term courses of oral corticosteroids are effective for acute exacerbations of COPD. However, chronic treatment with systemic corticosteroids should be avoided in patients with stable COPD.95

In patients with COPD, the specific phosphodiesterase-4 inhibitor roflumilast significantly reduced the absolute number of neutrophils in sputum and the concentrations of CXCL8 and neutrophil elastase in sputum supernatants. This anti-inflammatory effect of roflumilast might correlate with its reduction of exacerbations in severe COPD patients with chronic bronchitic symptoms and a history of COPD exacerbations. In this COPD subphenotype, long-term macrolide therapy was associated with significant reductions in the rate of exacerbations compared with placebo.97 Low dose macrolide treatment shows much the same benefits in other chronic neutrophilic airway diseases, including diffuse panbronchiolitis, bronchiolitis obliterans syndrome after lung transplantation, cystic fibrosis, and non-cystic fibrosis bronchiectasis. The anti-inflammatory mechanisms of macrolides need to be fully elucidated, but probably encompass pleiotropic inhibitory effects on neutrophil elastase, interleukin 17 production by T lymphocytes and CXCL8 and GM-CSF release from epithelial cells, and upregulation of the expression of the mannose receptor on alveolar macrophages, which improves the phagocytosis of apoptotic cells.98

Several molecular and cellular mediators of the innate and adaptive immune responses, are regarded as new therapeutic targets. Possible new drugs for COPD encompass modulators of TLRs, chemokine receptor antagonists, protease inhibitors (eg, small-molecule neutrophil elastase inhibitors and inhibitors of MMP-9 and MMP-12) and anti-interleukin-17 targeted therapies (including anti-interleukin-17 monoclonal antibodies and tocilizumab, which targets the interleukin-6 receptor and thereby favours the differentiation towards Treg instead of Th17 cells). However, specific targeted anti-inflammatory therapy with the anti-TNFα monoclonal antibody infliximab has failed in COPD.99 An alternative approach is to try to restore glucocorticoid sensitivity in patients with COPD.100 Dependent on the underlying molecular mechanism of glucocorticoid resistance, patient-specific therapies need to be investigated, encompassing anti-oxidants, theophylline, or kinase inhibitors such as mitogen-activated protein kinase inhibitors or phosphatidylinositol-3 kinase inhibitors.100,102,103

Contributors
GGB contributed to the literature search, writing and revision of the report. GFJ co-supervised the preclinical and clinical work that is the basis of this report and reviewed and edited the report. KRB contributed to the literature search, created the tables and figures, and reviewed and edited the report.

Conflicts of interest
GGB is a member of advisory boards for AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, and Novartis; has been a consultant for Amgen; and has received speaker’s fees from AstraZeneca, Boehringer-Ingelheim, Chiesi, GlaxoSmithKline, Merck, Novartis, Pfizer, and UCB. GFJ is a member of advisory boards for AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Merck, Novartis, and Nycomed; has received grants or has grants pending from AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Novartis, and UCB; and has received speaker’s fees from Almirall, AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Merk Novartis, Pfizer, and UCB. KRB declares that he has no conflicts of interest.

Acknowledgments
We thank all collaborators at the Laboratory for Translational Research of Obstructive Pulmonary Disease, Department of Respiratory Medicine, Ghent University Hospital and Ghent University, Ghent, Belgium; Professor Patrick Venables for helpful insights into autoantibodies; and the Interuniversity Attraction Poles Program (Belgian State, Belgian Science Policy, Project P6/35), the Fund for Scientific Research Flanders and the Concerted Research Action of Ghent University for funding.

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