

Nontypeable *Haemophilus influenzae* in chronic obstructive pulmonary disease and lung cancer

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Abstract: Chronic obstructive pulmonary disease (COPD) is predicted to become the third leading cause of death in the world by 2020. It is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases, most commonly cigarette smoke. Among smokers with COPD, even following withdrawal of cigarette smoke, inflammation persists and lung function continues to deteriorate. One possible explanation is that bacterial colonization of smoke-damaged airways, most commonly with nontypeable *Haemophilus influenzae* (NTHi), perpetuates airway injury and inflammation. Furthermore, COPD has also been identified as an independent risk factor for lung cancer irrespective of concomitant cigarette smoke exposure. In this article, we review the role of NTHi in airway inflammation that may lead to COPD progression and lung cancer promotion.

Keywords: COPD, NTHi, inflammation

Introduction

The pooled global prevalence of chronic obstructive pulmonary disease (COPD) in adults aged 40 years or older is ~10%, and it is a leading cause of morbidity and mortality in the US.¹⁻⁵ COPD is believed to be caused by inflammation induced by inhaled smoke and particulates, and possibly by infecting pathogens as well, leading to the structural changes in airways and alveoli that result in irreversible airflow limitation (Figure 1). Despite the fact that smoking causes most cases of COPD, only 25% of smokers develop COPD.^{6,7} Conversely, epidemiologic data indicate that approximately 1 of 6 patients with COPD has never smoked.⁸ This variable susceptibility to COPD most likely reflects genetic variations in the inflammatory and structural responses to inhaled smoke and to microorganisms colonizing the airways of smokers.⁹⁻¹¹ The most common colonizing bacterium is nontypeable (ie, unencapsulated) *Haemophilus influenzae* (NTHi).^{12,13} This organism is found in the lower respiratory tract of ~30% of individuals with COPD at any time.^{12,14-17} In addition to colonization during clinically stable periods, acquisition of new strains of NTHi is an important cause of lower respiratory tract infection, resulting in exacerbations of COPD.^{13,18-20} Together, these findings suggest that persistent or repetitive exposure of the airway to NTHi products may contribute to airway inflammation in COPD.¹⁸ Furthermore, several studies have found that smokers with COPD have an increased risk of lung cancer (3- to 10-fold) compared with smokers with comparable cigarette exposure but without COPD.^{21,22} In this review, we will discuss the existing data regarding the roles that NTHi plays in COPD development and lung cancer promotion.

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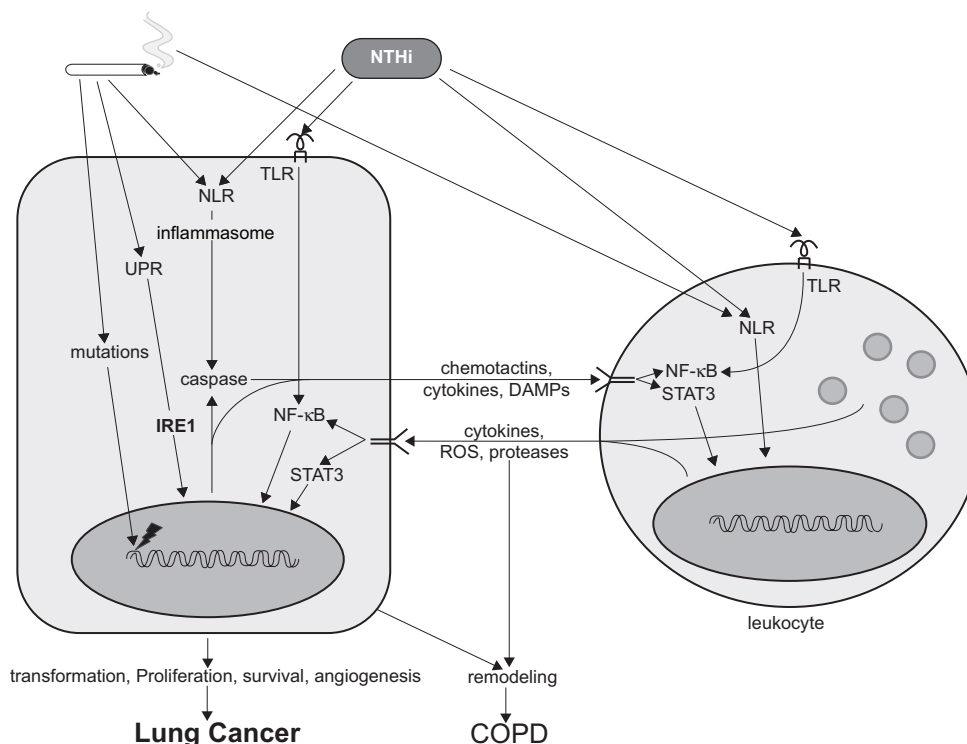


Figure 1 Effects of cigarette smoke and NTHi in the pathogenesis of lung cancer and COPD. Two inputs (cigarette smoke and microbial infection, top) act on two broad cell types (epithelial, left, and leukocyte, right) within the lungs to induce two outputs (lung cancer and COPD, bottom) as follows. Hydrocarbons and ROS in cigarette smoke induce mutations in epithelial cells that initiate carcinogenesis. Hydrocarbons, ROS, and particles in smoke activate the UPR and NLRs in epithelial cells and resident leukocytes, such as macrophages, inducing inflammation through key mediators such as IRE1, the inflammasome, and NF- κ B. Microbial infection of smoke-damaged airways, particularly with NTHi, results in further inflammation by activating pattern recognition receptors such as the NLRs and TLRs. Activated epithelial cells amplify inflammation by signaling through chemotactins, cytokines, and DAMPs to resident and recruited leukocytes ($CD8^+$ T cells, $CD4^+$ Th17 cells, and B cells). In turn, activated leukocytes signal to epithelial cells through NF- κ B and STAT3 to further amplify inflammation and generate proliferative and survival signals that promote carcinogenesis. COPD results from phenotypic changes in epithelial cells, such as mucous metaplasia or cell death, along with other structural changes such as proliferation or death of mesenchymal cells and deposition or destruction of extracellular matrix that together are termed remodeling.

Abbreviations: COPD, chronic obstructive pulmonary disease; DAMPs, damage-associated molecular patterns; IRE1, inositol-requiring enzyme 1; NF- κ B, nuclear transcription factor- κ B; NLRs, Nod-like receptors; NTHi, nontypeable *Haemophilus influenzae*; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TLRs, Toll-like receptors; UPR, unfolded protein response.

Inflammation in COPD

Based on the definition of the Global Initiative for Chronic Obstructive Lung Disease, COPD is “a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases”.²³ Although COPD and asthma both involve inflammation in the respiratory tract, the inflammatory processes differ markedly.^{10,24} Histopathological studies show a predominant involvement of peripheral airways (bronchioles) and lung parenchyma in COPD, whereas asthma involves inflammation in all airways but minimal involvement of the lung parenchyma.²⁵ Both innate and adaptive immune responses are involved in lung inflammation in patients with COPD.

Inflammatory cells

In histopathologic specimens of distal lung and in bronchoalveolar lavage fluid (BALF) from COPD patients,

macrophages and neutrophils are prominent.^{26–28} There is also an increase in the total numbers of T lymphocytes in lung parenchyma and peripheral and central airways of patients with COPD, with a greater increase in $CD8^+$ than in $CD4^+$ cells.^{25,29,30} Although the absolute number of $CD8^+$ cells is more than $CD4^+$ cells, there is strong evidence to support a role for $CD4^+$ T helper 1 (Th1) cells in maintenance of chronic inflammation in COPD.^{31,32} Loss of lung function in patients with COPD is associated with a high percentage of $CD4^+$ and $CD8^+$ T lymphocytes that express chemokine receptors CCR5 and CXCR3 (markers of Th1 cells), but not CCR3 or CCR4 (markers of Th2 cells).^{33,34} $CD8^+$ cells are typically increased in airway infections, and it is possible that chronic or recurrent colonization of the lower respiratory tract of COPD patients by bacterial and viral pathogens contributes to this inflammatory response.³⁵ It has also been shown that $CD8^+$ T cells are required for inflammation and destruction in cigarette smoke-induced emphysema in mice.³⁶

The accumulated volume of B cells in small airways (mainly seen in lymphoid aggregates)² and in bronchial biopsies of large airways³⁷ is higher in patients with COPD than in controls without airflow limitation and even higher in more severe COPD. The humoral, or antibody, immune response is essential for host defense against viral and bacterial pathogens. B cells probably contribute to the inflammatory process and/or the development and perpetuation of COPD by means of a specific antigen-driven process.³⁸ The lung has the ability to respond quickly to some pathogens through stimulation of resident antigen-specific memory B cells. Alternatively, after exposure to a new pathogen, the lung can generate de novo both a systemic and local (mucosal) antibody response. The resulting production of antigen-specific IgG and IgA acts in concert to help clear the invading pathogen and reduce subsequent colonization of respiratory epithelium.³⁹ Primary adaptive responses are triggered by immature myeloid dendritic cells (DCs), which carry antigen from the lungs to regional lymph nodes.⁴⁰ Antigen presentation by these mature DCs is required to activate naive CD4⁺ T cells, which are essential to generate polarized type 1 or type 2 responses and for robust immunologic memory. Persistent and chronic inflammation recruits natural killer (NK) cells and more DCs.⁴⁰ NK cells exposed to interleukin-12 (IL-12) favor survival of DCs that prime for Th1 responses, whereas NK cells exposed to IL-4 do not exert DC selection, leading to Th2 responses. Additionally, previous pulmonary infections or immune responses increase the number of lung DCs and populate the lungs with clones of memory B cells and T cells that are immediately available to respond to infections.⁴⁰

In addition to the traditional Th1 and Th2 subtypes, recent developments in cytokine biology imply that COPD might be better explained by the T helper 17 (Th17) phenotype.⁴¹ Effector molecules produced by CD4⁺ Th17 cells include IL-17A, IL-17F, and IL-22.⁴¹ IL-17A and IL-17F both bind to the IL-17 receptor (IL-17R).⁴² It has been shown that IL-17R signaling is required for lung CXC chemokine and granulocyte colony-stimulating factor (G-CSF) expression, neutrophil recruitment, and host defense against Gram-negative bacteria.⁴³ IL-17A, the prototype of this new cytokine family, is a 20–30-kDa glycosylated homodimeric cytokine produced predominantly by T cells.⁴⁴ Many of the inflammatory effects of Th17 cells are attributed to the expression of IL-17A. For example, IL-17A upregulates the expression of a number of CXCR2 chemokines, including CXCL1, CXCL6, and CXCL8, together with the neutrophil survival factors granulocyte-macrophage colony-stimulating factor (GM-CSF)

and G-CSF from the airway epithelium. This would suggest that Th17 cells are important in promoting and sustaining the neutrophilic inflammation observed in COPD. In addition, IL-17A can act synergistically with viral infection as well as other inflammatory mediators that include tumor necrosis factor (TNF) to potentiate these responses.^{45,46} Transgenic overexpression of IL-17A in the lung induces inflammation and mucous metaplasia.⁴⁷ Primary human tracheobronchial epithelial cells stimulated by an extensive panel of cytokines upregulated the *MUC5AC* and *MUC5B* genes in response to IL-17A.⁴⁸ Additionally, IL-17A potently induces epithelial cells and fibroblasts to secrete neutrophil attractants, notably CXCL8.^{48–51} Finally, IL-17 family members increase the sensitivity of macrophages to pathogen-associated molecular patterns and may even directly induce TNF.⁵¹

Inflammatory mediators and signaling pathways

Cellular inflammation in COPD is accompanied by increased levels of proinflammatory cytokines, which amplify inflammation via the activation of the NF- κ B pathway, leading to increased expression of multiple inflammatory genes.^{52–56} NF- κ B activation occurs primarily through I κ B kinase (IKK)-dependent phosphorylation and subsequent degradation of specific inhibitors, the I κ Bs, which retain NF- κ B in the cytoplasm. Upon activation, NF- κ B dimers enter the nucleus, where they modulate transcription of a large variety of target genes.^{52,53,57,58} These genes code for mediators of immune and inflammatory reactions, such as TNF, IL-1 β , IL-6, IFN- γ , IL-18, IL-32, and Th17 cytokines, the chemokine IL-8, and cell adhesion molecules.

TNF is mostly produced by macrophages, and also by many other cells, such as mast cells, epithelial cells, and B and T cells, and activates NF- κ B.⁵⁹ TNF levels are increased in the sputum of patients with COPD during exacerbations⁶⁰ and contribute importantly to cigarette smoke-induced emphysema in mice.⁵⁹

IL-1 β stimulates alveolar macrophages from patients with COPD to secrete inflammatory factors,⁶¹ and together with TNF induces ICAM-1 expression on endothelial cells.⁵⁴ IL-18, part of the IL-1 superfamily, plays an important role in Th1 polarization and various Th1-type diseases, inducing TNF, IL-1 β , GM-CSF, and chemokine production by monocytes and T lymphocytes.⁶² In mouse models, it has been shown that constitutive IL-18 overproduction in the lungs induces emphysema.⁶³

IL-6 is acutely produced by monocytes and macrophages at the site of inflammation, as well as by T cells in chronic

inflammation. IL-6 activates intracellular signaling of signal transducer and activator of transcription 3 (STAT3) in epithelial and immune cells.⁶⁴ STAT3 upregulation was observed in lung tissue from both smokers and nonsmokers with COPD, and this was associated with upregulation of its target genes.⁶⁵ Increased levels of IL-6 have been found in induced sputum, exhaled breath condensate, and BALF from COPD patients,⁶⁶ and when overexpressed in the mouse airways results in emphysema-like airspace enlargement and airway inflammation.⁶⁷

IFN- γ is produced by Th1 cells, activated macrophages, and DCs, inducing them to produce IL-12, the main Th1-inducing cytokine. When overexpressed in the murine lung it results in emphysema, and it has also been shown to be upregulated in lymphocytes isolated from emphysematous lung tissue samples, BALF, and sputum from COPD patients.^{33,68,69}

IL-32 produced in NK cells, T cells, epithelial cells, and monocytes has been recently recognized as a proinflammatory cytokine.⁷⁰ An increased expression of IL-32 in the lung tissue of patients with COPD has been demonstrated and correlates with the level of TNF and the degree of airway obstruction, suggesting that IL-32 is involved in the specific immune response observed in COPD and may have an impact on disease progression.^{71,72}

IL-8, a CXC chemokine, is a neutrophil chemoattractant and activator. It is secreted by macrophages, neutrophils, and airway epithelial cells and may contribute to COPD progression by attracting neutrophils to the lung.⁷³ Increased levels found in sputum samples from COPD patients correlate with the airway bacterial load, and its constitutive overexpression in the lungs of mice induces emphysema, furthermore suggesting its important role in the pathogenesis of COPD.^{53,63}

NTHi and immune responses

NTHi is a small ($1.0 \times 0.3 \mu\text{m}$) Gram-negative coccobacillus that lacks a polysaccharide capsule (hence its nontypeable classification). It colonizes the upper respiratory tract of up to 75% of normal adults and primarily acts as a mucosal pathogen.⁷⁴ Although it lacks a mucinous capsule, NTHi possesses characteristic coat proteins.

The P2 protein is the major outer membrane protein (OMP) of NTHi, constituting ~50% of the protein content of the outer membrane. P2 is a target of human serum bactericidal antibody, indicating that the protein is important in the human immune response to NTHi.⁷⁵ P6 is a 16-kDa peptidoglycan-associated lipoprotein that is present in the

outer membrane of all strains of NTHi⁷⁶ and shows a high degree of sequence conservation among strains.⁷⁷ Several lines of evidence suggest that P6 elicits bactericidal antibody responses.^{76,78} Berenson et al have shown that P6 is a specific trigger of bacteria-induced human macrophage inflammatory events, with IL-8 and TNF as key effectors of P6-induced macrophage responses.⁷⁹ Another study has shown that although the migration of mature DCs into the pulmonary lymph nodes is attenuated after repeated airway challenge of mice with OMPs of NTHi, the in vitro P6-specific T cell proliferation in cultured pulmonary lymph node cells was enhanced and subsequently linked to the development of P6-specific IgA production and the development of protective immunity in the airway of mice.⁸⁰

NTHi lipooligosaccharide (LOS) is a major virulence determinant and may play a role in colonization and invasion of mucosal surfaces in the respiratory tract.⁸¹ NTHi LOS is analogous to the lipopolysaccharide (LPS) of enteric Gram-negative bacteria in that it contains lipid A linked by 3-deoxy-D-manno-octulosonic acid to a heterogeneous sugar polymer.^{82,83} NTHi LOS, however, differs from classic enterobacterial LPS in that it does not contain repeating O-antigen units and is therefore more similar to that derived from *Neisseria* and *Bordetella* species.^{82,83}

Like most other bacterial infections, NTHi infections induce inflammation with prominent release of cytokines and chemokines. Extensive in vitro studies have shown that NTHi lysate activates the NF- κ B pathway in airway epithelial cells and markedly increases expression and release of proinflammatory mediators, including IL-6, IL-8, and TNF.^{18,84–86} Airway epithelial cells are capable of sensing and responding to inflammatory stimuli through innate immune mechanisms that result in lung inflammation.⁸⁷ These mechanisms mostly function through Toll-like receptors (TLRs), a family of evolutionarily conserved transmembrane receptors that serve as pattern recognition receptors. They recognize conserved microbial motifs in molecules such as bacterial LPS, peptidoglycan, flagellin, unmethylated CpG DNA, and double- and single-stranded RNA. Activation of the corresponding TLR by any of these molecules results in the activation of antimicrobial responses⁸⁸ and the initiation of an inflammatory response, alerting the host to the presence of microbial invasion and initiating an immune response.^{89,90}

Because the TLRs share sequence similarity with the IL-1 receptor (IL-1R) family in their cytoplasmic regions, it is not unexpected that downstream events are mediated by common components, such as myeloid differentiation primary response gene (88) (MyD88). MyD88 is an adaptor protein

that links the IL-1R to IL-1R-associated protein kinase (IRAK), a serine–threonine kinase that is related to the pelle kinase of *Drosophila*. Upon binding of ligand to IL-1R, IRAK phosphorylates, subsequently dissociates from the receptor complex and associates with TNF-receptor-activated factor 6 (TRAF6). This process results in the activation of two different pathways that involve the c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase (MAPK) family and the Rel family transcription factor NF- κ B.^{91,92}

TLR-4 is the signaling receptor for LPS, the major proinflammatory component of the Gram-negative cell wall, in concert with CD14, which serves as the ligand-binding part of the LPS receptor complex. Triggering of TLR-4 results in the activation of two distinct intracellular pathways, one that relies on the common TLR adaptor MyD88 and one that is mediated by Toll/IL-1R domain-containing adaptor-inducing IFN- β (TRIF).⁹³ Wieland et al have shown that alveolar macrophages from CD14 and TLR-4 knockout (KO) mice are virtually unresponsive to NTHi in vitro.⁹³ Intranasal administration of NTHi in the mouse results in a brisk elaboration of IL-1 β , IL-6, TNF, macrophage-inflammatory protein (MIP)-1 α , and MIP-2 in BALF and a corresponding mobilization of intrapulmonary neutrophils. After intranasal infection with NTHi, CD14 and TLR-4 KO mice showed an attenuated early inflammatory response in their lungs with diminished IL-1 β , IL-6, TNF, MIP-1 α , and MIP-2 in BALF and a notable absence of intrapulmonary neutrophils, which was associated with a strongly reduced clearance of NTHi from the respiratory tract.^{93,94} Additionally, MyD88 KO, but not TRIF mutant mice, showed an increased bacterial load in their lungs upon infection with NTHi.⁹³ These data demonstrate that the MyD88-dependent pathway of TLR-4 is important for an effective innate immune response to respiratory tract infection caused by NTHi. This suggests that the airway epithelia might contribute to sensing NTHi infection and signaling the innate immune response.⁹⁴

TLR-2 also plays a critical role in mediating inflammatory responses against bacterial pathogens in the lung.⁹⁵ NTHi strongly activates NF- κ B in human epithelial cells via two distinct signaling pathways: NF- κ B translocation-dependent and NF- κ B translocation-independent pathways.⁸⁵ The NF- κ B translocation-dependent pathway involves activation of the NF- κ B-inducing kinase (NIK)–IKK α / β complex, leading to I κ B α phosphorylation and degradation, whereas the NF- κ B translocation-independent pathway involves activation of MKK3/6–MAPK. TLR-2 is required for NTHi-induced NF- κ B activation through both pathways, and OMP P6 appears to also activate NF- κ B via similar signaling pathways.⁸⁵ NTHi also

induces COX-2 and PGE2 expression in a p38 MAPK and NF- κ B-dependent manner through TLR2 in lung epithelial cells in vitro and lung tissues in vivo.⁹⁶

NTHi in COPD

NTHi is frequently present in the airways of adults with COPD.^{14–17} This Gram-negative bacterium is found in the lower respiratory tract of ~30% of individuals with COPD at any time, and with serial sampling more than 60% of COPD patients show colonization.⁹⁷ Several studies have shown higher inflammatory responses in the presence of NTHi colonization in stable COPD patients and smokers without COPD.^{98–100} For example, colonized smokers, with or without COPD, had more bronchial neutrophilia compared with noncolonized ones.¹⁵ Bacterial colonization has also been associated with increased frequency of exacerbations and an accelerated decline in lung function.⁹⁷ Furthermore, the acquisition of new NTHi serotypes is associated with exacerbations of COPD.^{12,13,18–20} In one study, NTHi was found in the bronchial tissues of 87% of patients with exacerbations compared with 33% of stable COPD patients and 0% of healthy controls.¹⁴ The immune response that develops after an exacerbation seems to be strain specific and protects against recurrent exacerbation due to the homologous strain but leaves the patient susceptible to exacerbations caused by antigenically unrelated heterologous strains, and this likely represents a mechanism for recurrent infections.¹⁰¹

Studies using molecular techniques indicate that some patients with COPD are persistently colonized with NTHi even after antimicrobial therapy and despite a negative sputum culture. This suggests that bacteria may cause a greater proportion of exacerbations than is revealed by sputum culture alone.^{102–104} Defective immune responsiveness and impaired phagocytosis by alveolar macrophages might provide an immunologic basis for persistence of NTHi in the airways of adults with COPD.¹⁰⁵ Cigarette smoke induces mucus dysfunction by several mechanisms^{106–110} and ultimately increases mucin production, reduces mucus hydration, and decreases mucus clearance, which might also contribute to airway colonization in COPD patients.¹¹¹ Other possible mechanisms of colonization include airway epithelial cell invasion,^{20,103} antigenic alteration,^{112–114} and biofilm formation.¹¹⁵

NTHi could contribute to COPD progression by inducing neutrophilic influx into the airways, neutrophil necrosis with release of neutrophil elastase and other matrix metalloproteinases, and production of oxygen radicals.^{79,86} These mediators can overwhelm the antiproteinase barrier of

the lung and damage airway and alveolar structures, thereby amplifying smoking-induced lung damage.¹¹⁶ We have shown that weekly exposure of mice to an aerosolized NTHi lysate induces lung inflammation with a profile of mediators and inflammatory cells similar to that observed in COPD patients.¹¹⁷ Repetitive exposure to NTHi lysate results in a 10-fold increase in total inflammatory cell numbers in BALF, dominated by neutrophils (81%) with fewer macrophages and lymphocytes. This was accompanied by marked increases in inflammatory cytokines (TNF and IL-6), Th1 cytokines (IFN- γ and IL-12), and the neutrophil chemoattractant KC. Consistent with the rapid rise in the levels of inflammatory cytokines, NF- κ B was rapidly activated after each exposure. In the weeks following chronic activation of this innate immune response, a marked COPD-like inflammatory process ensues. Peribronchiolar and perivascular inflammatory cell infiltration and lymphoid aggregates (after 8 weeks), similar to those described in COPD patients, were seen. The chronic inflammatory response was characterized by infiltration of the airway wall by macrophages, CD20⁺ B cells, CD8⁺ T cells, CD4⁺ T cells, and neutrophils. Structural changes in the lung parenchyma were closely associated with the chronic inflammatory response. There was increased fibrous tissue around airways, similar to that described in COPD patients, after 25 weekly NTHi exposures, and this further increased after 50 weeks of exposure.¹¹⁷ Flow cytometric analysis of BALF and lung homogenates after eight NTHi exposures has also shown an increased number of IL-17-producing CD4⁺ T cells (Th17 cells), associated with high expression of IL-17 in inflamed lung tissue and in the BALF (unpublished data). Furthermore, exposure of mice genetically deficient in IL-17R to the NTHi lysate resulted in a lower level of neutrophilic influx into the BALF and less inflammatory cell infiltration in lung tissue, indicating a major role for Th17 cells in NTHi-induced COPD-like airway inflammation (unpublished data). As transgenic overexpression of TNF in the airway epithelium caused BALF neutrophilia and inflammatory cell infiltration around airways, but not fibrosis,¹¹⁷ this suggests that innate immune inflammation alone is insufficient to induce airway fibrosis.

Studies have shown that both human and mouse alveolar epithelial type II (AT II) cells express MHC II molecules and that AT II cells are capable of activating T cells *in vitro*.^{118–120} A recent study shows that naive CD4⁺ T cells are specifically activated against a lung-specific antigen by AT II cells independently of lymphoid tissue and DCs *in vivo*.¹²¹ These data offer the strongest evidence to date that the lung itself, independent of the classical antigen-presenting cells of the

immune system, can present foreign and self-peptides and differentiate CD4⁺ T cells into regulatory T cell subsets.¹²² We also demonstrated that NTHi lysate could induce innate resistance in the lungs, resulting in broad protection against a wide range of pathogens,⁸⁷ which occurs independent of alveolar macrophages and recruited neutrophils. This protection derives from stimulation of local innate immune mechanisms, and activated lung epithelium is the most likely cellular effector of this response. Furthermore, gene expression analysis of lungs from NTHi-exposed mice demonstrated NF- κ B signaling to be the most enriched process, followed closely by interferons, IL-6/STAT3, and TLR signaling, including MyD88, TLR-2, and TLR-4.^{123,124} To further dissect the importance of the NF- κ B pathway in this phenomenon, we have targeted NF- κ B in the airway epithelium using a genetic strategy and have demonstrated a lower level of neutrophilic influx into BALF after a single NTHi exposure (unpublished data). Together, these findings suggest that exposure of the airway to NTHi products contributes to lung inflammation and airway fibrosis in COPD, which is mediated by innate immune activation of epithelial cells that signal to adaptive immune cells.

NTHi, COPD, and lung cancer

Worldwide, lung cancer is the leading cause of cancer mortality and is expected to account for 30% of all male and 26% of all female cancer deaths in 2009.¹²⁵ Cigarette smoking causes 90% of all lung cancers and is believed to do so primarily by inducing DNA mutations.¹²⁶ In addition, epidemiologic data indicate that chronic inflammation also plays a role in lung epithelial carcinogenesis.¹²⁷ Genetic factors have also been shown to play a role in determining susceptibility to lung cancer. These genetic factors are believed to confer an inherent susceptibility (exaggerated or maladaptive response) to chronic inflammation from cigarette smoking. Consistent with many cancer models, this inflammatory stimulus in the lungs results in tissue remodeling, DNA damage, and impaired cell cycle control.¹²⁸

Multiple studies have found that smokers with COPD have an increased risk of lung cancer compared with smokers without COPD.^{21,22,127,129,130} There is strong evidence that a patient's forced expiratory volume in the first second of expiration is an independent prognostic marker for developing lung cancer in both smokers and exsmokers,^{129,130} and the incidence of lung cancer seems to be higher for those with more severe airflow obstruction. The presence of mild emphysema, even without demonstrable airflow obstruction, confers a substantial risk of developing lung cancer.¹³¹ The

likelihood of developing lung cancer within 10 years is three-fold greater in patients with mild-to-moderate COPD and 10-fold greater in patients with severe COPD compared with smokers with normal lung function.²² It has also been shown that increased lung cancer mortality is associated with a history of COPD, even among people who have never been active smokers.¹³²

Several possible mechanisms may link lung cancer to COPD, and both genetic and environmental factors may play a role. This link could be related to inflammation and the body's attempt to repair emphysematous airspaces.¹³³ On the basis of existing in vitro studies showing that NTHi activates proliferative and antiapoptotic signaling pathways,^{84,85,134} colonization with this bacterium may also promote carcinogenesis by stimulating growth and inhibiting apoptosis. We have shown that NTHi-induced COPD-like airway inflammation promotes lung cancer in a Clara cell-targeted K-ras mutant mouse model (CC-LR) of lung cancer.¹³⁵ In contrast, existing epidemiologic data do not suggest any definite association between allergic airway inflammation (including asthma) and lung cancer, and some even suggest a protective role.^{136–140} To test this, CC-LR mice were sensitized intraperitoneally to ovalbumin (OVA) and then repetitively challenged with an OVA aerosol weekly for 8 weeks. This resulted in eosinophilic lung inflammation associated with increased levels of Th2 cytokines and mucous metaplasia of airway epithelium, similar to what is seen in asthma patients. However, this type of allergic inflammation did not result in a significant difference in lung surface tumor number.¹²⁴

IL-6 has been implicated in inflammatory responses in human COPD,^{6,26} and the IL-6 pathway has been found to be one of the mechanisms linking inflammation to cancer.⁶⁴ Therefore, to determine the causal role of cytokines that mediate COPD-like inflammation in lung carcinogenesis, we genetically ablated IL-6 in CC-LR mice. This not only inhibited intrinsic lung cancer development (1.7-fold) but also inhibited the promoting effect of extrinsic COPD-like airway inflammation (2.6-fold).¹²⁴ Taken together, we found that there is a clear specificity for the nature of inflammation in lung cancer promotion (Th17 type), and IL-6 has an essential role in lung cancer promotion. We suggest that COPD-like airway inflammation induced by NTHi provides a tumor microenvironment that favors lung tumor promotion and progression. This occurs via release of inflammatory mediators (IL-6 and TNF) from innate immune cells (neutrophils and macrophages) secondary to activation of epithelial innate immune signaling pathways (MyD88/

NF- κ B). This in turn activates more intracellular signaling pathways (NF- κ B and STAT3) in the airway epithelium and immune cells, resulting in activation of prosurvival, antiapoptotic, and proangiogenic signals accompanied by skewing toward a protumoral adaptive immune response (Th17 response) (Figure 1).

Conclusion

It appears that surface components of NTHi induce lung inflammation by innate immune signaling mechanisms and that this progresses to airway fibrosis by recruitment of the host adaptive immune system. A straightforward strategy to reduce the incidence of COPD is the prevention of tobacco consumption. However, because of the persistent risk among former smokers, additional strategies that stop the progression to advanced COPD are highly attractive. Based on the demonstrated ability of NTHi to induce lung inflammation and airway fibrosis and its persistent colonization of the airways of COPD patients, dissecting underlying mechanisms will help us to develop alternative preventive and therapeutic strategies in COPD patients. Furthermore, this would have a major impact on preventing the leading cause of cancer death by providing the basis for rationally directed therapy in patients at high risk for lung cancer development.

Disclosure

The authors report no conflicts of interest in this work.

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