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REVIEW

Towards a vaccine for chronic obstructive pulmonary disease

R. L. Clancy

Department of Immunology, Hunter Area Pathology Service and Hunter Immunology Limited, Newcastle, New South Wales, Australia

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Correspondence
Robert L. Clancy, Hunter Immunology Ltd., Level 4, DMCSB (David Maddison Building), University of Newcastle, Callaghan, NSW 2308, Australia.
Email: robert.clancy@newcastle.edu.au

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Abstract

This review discusses chronic obstructive pulmonary disease as an outcome of two pathogenic pathways: the first resulting from inhalation of toxins and the second a consequence of bacterial colonisation of damaged airways. Earlier assessment of the role played by bacteria in acute exacerbations was compromised by a deficiency of quality data and the use of parameters more relevant to invasive infection. Data are reviewed to support a hypothesis that states intrabronchial inflammation reflects an excessive and inappropriate host response (largely mediated by Th17 cells derived from gut-associated lymphoid tissues) to colonising bacteria acting as an 'antigen sump' (in essence, a hypersensitivity reaction). It is proposed that both viral and bacterial infections exacerbate inflammation through a common pathway that involves colonising bacteria. An oral vaccine containing inactivated non-typeable *Haemophilus influenzae* augments a protective loop that involves the aspiration of bronchus content into the gut and reduces the severity of acute exacerbations including the need for hospital admission by reducing the 'load' of bacteria comprising this final common path. The positive clinical results from trials using oral NTHi support both the concept that bacterial colonisation of damaged airways is a potent second pathogenic pathway and that oral immunotherapy provides a significant therapeutic advance in limiting damage in chronic obstructive pulmonary disease.

Introduction and background to chronic obstructive pulmonary disease

A second pathogenic pathway in chronic obstructive pulmonary disease (COPD) dependent on bacterial colonisation of damaged airways, in particular non-typeable *Haemophilus influenzae* (NTHi), is not a new idea. However, interest waned due to a lack of data of therapeutic value. This review focuses on NTHi as a keystone in understanding the importance of bacterial colonisation in provoking exacerbations of COPD, in part as a result of studies that suggest benefit from an oral mucosal vaccine that reduces the load of bacteria in damaged airways.

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In this review, Robert Clancy summarises 30 years of work in mucosal immunology. The review does not include any original data that is not in the public record.

Twenty-five years ago, an oral inactivated NTHi vaccine was shown to reduce the frequency and severity of exacerbations in smoking-related airways disease (SRAD).[1] This was followed by three further vaccine studies[2–4] and a Cochrane Report[5] supporting benefit, with no reported significant adverse events. These studies recorded a reduction of all pathogens in sputum but did not detect an increase in secretary immunoglobulin A (IgA) antibody in saliva. In these studies, several unselected organisms were used possibly accounting for a variation in results with only five of six studies showing protection.[1–4] At that time, there was little interest in the idea of a vaccine for a disease where inhalation of toxins was widely considered to be the sole cause of damage and IgA antibody was the required marker of mucosal immunity. This was consistent with a longstanding negativity and neglect with respect to SRAD, which was regarded as a self-induced disease of the elderly, for which nothing could be done; however, these circumstances have changed. Recognition of the potential of a vaccine to limit damage in COPD can best be understood in the context of change in acceptance of the importance of bacterial colonisation of damaged airways as a second major cause of airways damage in COPD. Four stages in this process will

be discussed: evolution of ideas on the nature of SRAD, the importance of acute exacerbations, the role of bacteria and then the host response in determining acute episodes, and the evidence that an oral vaccine can downregulate inflammation.

In assessing the value of any 'oral vaccine', it is important to consider a number of often opposing receptor-response pathways that contribute to the 'net response' involving both innate and adaptive immune systems. Most microbes present both specific (antigenic) and 'pattern' recognition units to mucosal antigen-presenting (or dendritic) cells (APCs), to trigger cytokine-mediated upregulation – and downregulation – of the inflammatory response. Any particular 'net outcome' depends on characteristics of the stimulus and the manner in which it is presented, and to the 'state' of the mucosa. For example, constant, low-dose, soluble antigen presentation leads to a non-responsive or tolerant state, while an intermittent bolus of particulate antigen stimulates net immunity throughout the mucosal system. With respect to the 'state' of mucosa, variations include the level of inflammation present and whether or not the subject is atopic. In the presence of pathological inflammation, the IgA antibody response is suppressed[6] and replaced by a Th17 response – mucosal colonisation is then controlled by the neutrophil flux.[7] Atopic subjects express IgEFcR on the surface of mucosal APCs that can trigger a Th2 or Treg cell response depending on circumstances of allergen presentation.

In summary, mucosa-associated bacteria can either trigger a low level of inflammatory response when conserved 'patterns' ligate surface Toll-like receptors (commensals), or by also binding intracellular receptors (such as NOD), they induce an excessive inflammatory response and tissue damage (pathogens). In addition, bacteria-associated antigens are processed to induce uncommitted CD4 T cells in mucosa-associated lymphoid tissue to differentiate into Th1, Th2, Th17 and Treg cells depending on the characteristics of APCs and cytokine milieu. The outcome of net immunity, hypersensitivity (inappropriate Th2-IgE secretion, inappropriate Th17-neutrophil recruitment) or tolerance (Treg) reflects the balance between these T-cell subsets. These concepts underpin innovative approaches to immunotherapy: sublingual desensitisation for allergy;[8] helminth-induced suppression of chronic inflammation, including Crohn's disease and multiple sclerosis;[9] downregulation of infection-related bronchospasm in children following low-dose polybacterial lysate mixes;[10] and by regulating Th1/Th2 balance to optimise mucosal protection using probiotics.[11] It is within this framework that durable immunity within the airways (seen as a reduction in bacterial antigen load) following cyclic ingestion of high-dose particulate bacteria can be both understood and differentiated from separate net outcomes that follow these other immunotherapeutic products.

The term chronic obstructive pulmonary disease (COPD) was introduced to unify SRAD through the common denominator of progressive airways obstruction.[12] This allowed a simple and objective diagnosis to be made, enabling the development of international consortia such as GOLD that could then promote optimal management strategies and develop diagnostic criteria for multinational clinical trials.[13] There had been division across the Atlantic as to the nature of SRAD: in America, the focus was on changes in structure and function in emphysema thought to be exclusively caused by inhaled toxins,[14] while in the United Kingdom, the focus was the influence of smog-initiated epidemiological studies of 'chronic cough and sputum' (or 'simple bronchitis'), and its complications of 'infection episodes' and 'chronic obstruction'. These clinical disorders may or may not be associated with the tissue changes of emphysema.[15] Initially, the 'infection episodes' were considered to be caused by bacteria, especially NTHi and *Streptococcus pneumoniae*. However, a 'poor fit' with parameters derived from study of invasive infection including sputum bacteriology, serology and response to antibiotics,[16] together with a lack of epidemiological support for the idea that acute episodes worsened airways obstruction,[15] lessened interest in bacterial infection as a significant pathogenic factor in COPD. Recent intense interest in COPD derives from a recognition of the sheer magnitude of its human and economic costs,[17] the realisation that intrabronchial inflammation provides new opportunities for therapeutic intervention and likely relates to new ideas on the lung microbiome[18] and a re-evaluation of the importance of exacerbations in acute and chronic phases of COPD.[19] Thus, COPD is the only major cause of death that is increasing in frequency with about 20% over 60 worldwide having COPD;[17] there are immense economic costs, and in many countries, exacerbations of COPD are the major medical cause for hospital admission.[20] Inflammation in COPD has been contrasted with that in asthma, substituting neutrophils for eosinophils but linking inflammation exclusively to inhaled toxins.[21] The use of asthma therapy has been trialled (inhaled corticosteroid/bronchodilator). The most substantial database is with fluticosone/salmeterol from two multicentre studies designed to detect retention of airflow[22] or reduction in mortality.[23] There was a significant reduction of about 40% in exacerbations requiring corticosteroid therapy, but reduction in hospital admissions was less clear, being recorded at 0% and 17%, respectively.[22,23] In both of these studies, the incidence of antibiotic-treated episodes increased in the treated groups,[24] consistent with the

proneness to infection recorded with long-term inhalation of corticosteroids.[25] Any conclusion that inhalation of toxins is the sole cause of inflammation is conflicted by progression of disease in severe COPD, where most have long ceased smoking.[19] Alternative 'drivers' of inflammation such as colonising bacteria, feedback loops based on enzyme-release from damaged tissue and autoimmunity must be considered.

Acute exacerbations

Exacerbations of COPD feared by patients, and the main precipitant of respiratory and cardiac failure, have been reassessed and are now known to promote progression of airways disease and its clinical sequelae.[19] The idea of an 'exacerbation phenotype' has recently been confirmed, with more frequent exacerbations occurring in those with most severe disease.[26,27] These clinical observations are consistent with the demonstration that there is an increase in inflammation in clinically stable COPD.[17,19] Exacerbations represent an increase in inflammation above a threshold, representing a perceived increase in volume and purulence of sputum.[19] The inflammatory process is continuous and unstable. Recognition that the 'normal' variations in level of inflammation provide a 'background noise', which complicates calculation of exacerbation frequency in clinical studies, led to exacerbations being defined as bronchitic symptoms that require a change in therapy.[22,23]

Recently, a significant role for bacteria in the pathogenesis of at least some exacerbations has been accepted but not understood largely on the basis of meta-analyses of antibiotic trials, evidence that long-term use of macrolides may reduce exacerbations, and a series of studies in COPD where novel 'exacerbation' isolates of NTHi were detected in some subjects by identifying specific serological responses.[28] These 'exacerbation isolates' caused more destruction than those isolated from sputum collected in stable disease.[29] In a study of established smokers, specific systemic antibody response over a winter period significantly correlated with exposure to NTHi.[30] NTHi was the pathogen most commonly cultured from sputum and when present was quantitatively dominant.[31,32] This is consistent with data using non-culture methods to identify the microbiome that appears restricted in COPD.[18] Recent studies indicate that most, if not all, with COPD are colonised by NTHi.[18,31] A 'Vicious-Circle' hypothesis of infection and inflammation has been generated to account for progressive disease.[28] The importance of the relationship between microbes and the host response (including the role of epithelial cells) is of current interest. A recent review has viewed direct interaction between a microbe and the epithelial cell as the role of the 'healthy soldier' and the distortion of these interactions as the response of the 'wounded soldier'.[33] The inflammatory response can thus be protective or damaging depending on the efficiency of host immunity. The concept of 'colonisation' and 'infection' differs from that associated with invasive disease – at the bronchus mucosa, these terms refer more to whether or not there is clinical disease. Yet, residual uncertainties about the role of bacteria in promoting exacerbations are compounded by finding that bacterial colonisation in COPD is polybacterial,[26] that there is a similar frequency of detection of bacteria in sputum collected in an exacerbation as in specimens taken from stable disease[26,32] and that many exacerbations appear to be triggered by a virus infection.[28] We hypothesise that the continuous presence of bacterial antigen in damaged airways stimulates a mucosal immune response that restricts expansion of colonisation. The level of inflammation within the airways is in part a dynamic reflection of the balance of a host–parasite relationship – if the inflammatory exudate exceeds a particular threshold, it is detected as an increase in volume and purulence of sputum (or an 'exacerbation' of COPD). An 'exacerbation' occurs when the mucosal immune response fails to contain colonisation, which then stimulates an inappropriate and excessive inflammatory response; in other words, purulent sputum reflects a hypersensitivity response to intrabronchial bacteria. Hypersensitivity responses with non-specific damage caused by an excessive recruitment of innate immune mechanisms in essence reflect a deficient adaptive-innate response to antigen, failing to clear that antigen, with the consequence of an excessive activation and accumulation of the innate components. These are well recognised for IgE (acute allergic reaction), IgG (arthus reaction) and Th1 (granulamatous reaction) responses. When this involves Th17 cells, the 'hypersensitivity' response is characterised by an accumulation of neutrophils (here, at a mucosal site). This hypothesis is supported by the demonstration that effector immune responses relevant to the control of bacterial colonisation are mediated by T lymphocytes generated from the gut-associated lymphoid tissue (GALT) in both rodent models[34] and humans.[30] These T cells relocate in the respiratory tract where they recruit and activate phagocytic cells, especially neutrophils.[35] Recent studies in rodent models confirm that T cells from GALT that localise in lung tissue are Th17 cells.[36] This subset of T cells is now recognised as being critical for lung protection by secreting interleukin 17 (IL-17) that acts on airways epithelium to induce neutrophil-specific chemokines and antibacterial substances.[7] Neutrophils recruited into the bronchus undergo phenotypic change secreting large amounts of IL-8, tumour necrosis factor-α and IL-1,

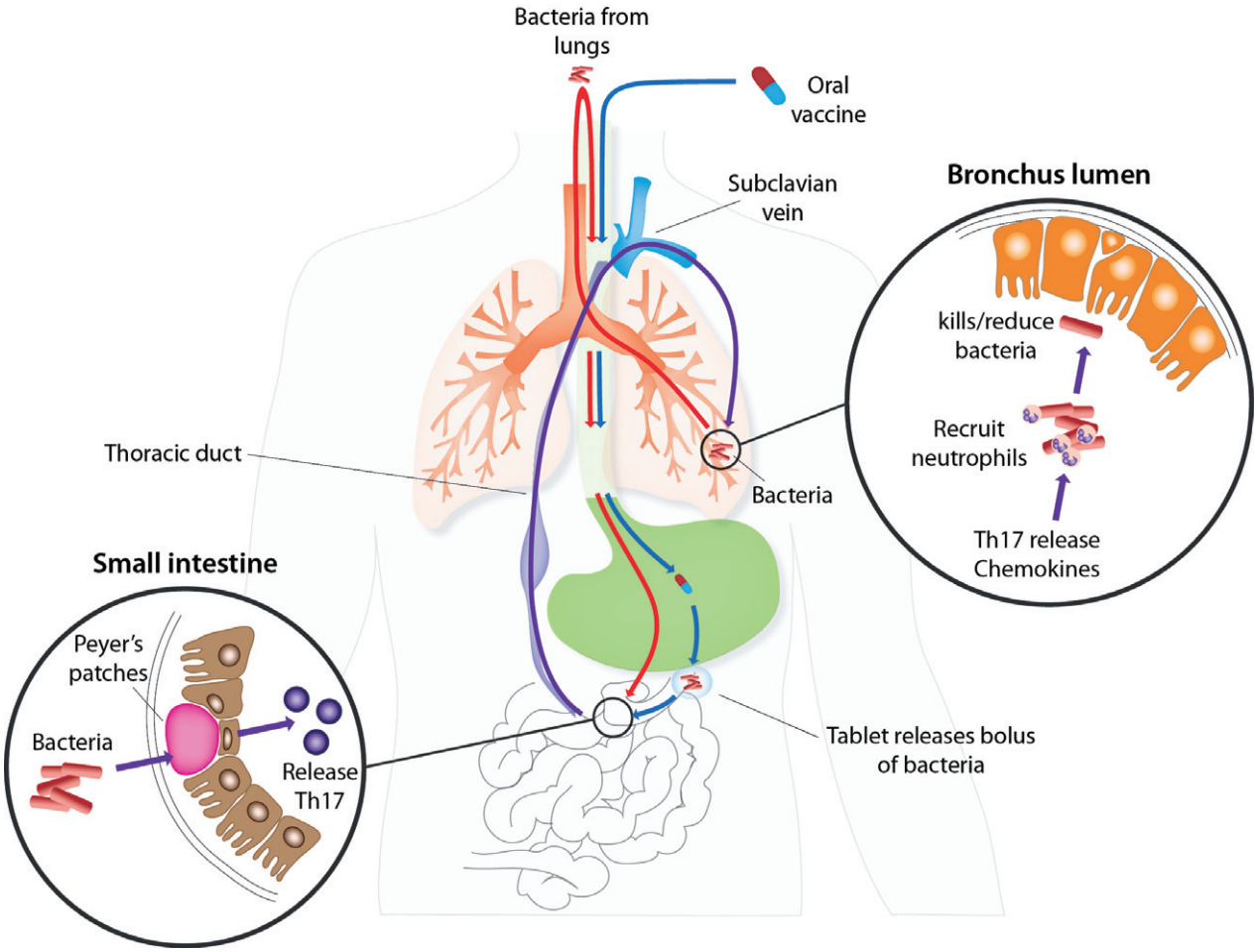

Figure 1 Schema showing aspiration of bronchus content (including non-typeable *Haemophilus influenzae* (NTHi)) into gut to activate Peyers patches from where TH17 cells traffic to airways. Oral tablets containing inactivated NTHi 'optimise' process enhancing bronchus protection.

which maintain lumenal protection through additional neutrophil recruitment, enhanced phagocytosis and prolongation of cell lifespan through autocrine mechanisms.[37] IgA antibody appears irrelevant to protection as it is stimulated only over a narrow dose range and suppressed in the presence of inflammation.[6] The relationship between bacteria and viruses in the respiratory tract is complex, but in addition to direct damage by virus,[28]mechanisms of synergy are being described. For example, co-infection of mice with NTHi and influenza virus shows an interdependence with an increase in titre of both microbes, thus influencing the bacterial load.[38] Accrediting an exacerbation to either 'bacterial' or 'viral' infection fails to recognise this synergy. Colonising bacteria – in particular NTHi – may be a final common path influencing the severity of both 'bacterial' and 'viral' infections. Studies using 'infection-prone' and 'non-infection-prone' phenotypes, as probes, have shown that those 'prone' to recurrent exacerbations have distinctive characteristics with respect to handling of colonising bacteria, which could influence clinical outcomes.[39] Recent studies in smokers have demonstrated a 'loop' of gut-driven protection of the airways by showing an increase in NTHi-specific T cells through a winter season because of aspiration of bronchus content (including bacteria) into the gut (Fig. 1), confirming a unique antigen delivery system.[30] Detection of elevated IgE antibodies against NTHi antigens in the serum of most with COPD (and many with steroid-resistant asthma) suggests that an allergic reaction to colonising bacteria may account for bronchospasm often noted in exacerbations.[40] These observations support the old idea known as the 'Dutch Hypothesis' that intrinsic asthma and COPD are forms of the same disease.[41]

A mucosal NTHi vaccine

The clinical trials described earlier used a high dose (3 × monthly cycles of six tablets, each tablet containing 10^{11} bacteria) of inactivated NTHi that did not stimulate a mucosal IgA antibody response in either normal subjects[6] or those with chronic bronchitis[1] but did stimulate a specific T-cell response.[30,42] In rodent models, oral immunisation also selectively stimulated a specific T-cell response,[6] which could transfer immunity to naive recipients.[34] In human studies, a 3-log reduction in NTHi colonisation density followed oral immunisation with three cycles of NTHi.[3] In other studies, there was a significant reduction in frequency of pathogen detection in sputum.[4] The duration of this reduction was about 6–9 months, which was similar to the period of clinical benefit,[3,5] indicating a need for annual pre-winter oral NTHi. Taken together with data from studies of recurrent acute bronchitis (earlier), these data are consistent with the idea that vaccine-induced reduction in colonisation reduces the inflammation drive, providing a buffer to limit an inflammatory response to an intercurrent infection. The clinical evidence of vaccine-induced protection is clearest in subjects with moderate-to-severe COPD – the level of protection in either mild COPD or in other forms of septic airways disease requires further study.

Early studies of an oral NTHi vaccine in COPD used different uncharacterised isolates and gave variable benefit, with five of six studies showing protection when exacerbations were defined as 'an increase in volume and purulence of sputum'.[5] The most consistent benefit was a significant reduction in antibiotic usage;[5] the only early study that included data on admission into hospital showed a significant reduction at about 90%.[1] To improve the vaccine to react more broadly with NTHi isolates and be less dependent on concomitant colonisation, rat models of intratracheal or intestinal immunisation followed by respiratory infection challenge were used to screen vaccine candidates to identify HI-164 as broadly protective against a panel of NTHi isolates (M. Dunkley *et al*., unpubl. data, 2006). The vaccine isolate HI-164 prevents penetration of NTHi into small airways and reduces parameters of inflammation in the airways of smokers.[30] In subjects with COPD, protection is greatest against the most severe episodes that were defined as requiring corticosteroid therapy and/or admission into hospital in those with the most severe disease.[42] A parallel study included subjects with less severe COPD and showed less dramatic benefit than in those with severe disease (unpublished data) possibly because a similar quantum of reduction in inflammation would have more clinical impact in those with the most compromised airways. Significant protection against recurrent exacerbations, duration of exacerbations and a reduction in antibiotic usage were recorded in those treated with the NTHi vaccine. In this trial, subjects continued on their background therapy, with about 90% on inhaled corticosteroid/bronchodilator combinations. Thus, observed benefit is additional to any current best practice therapy. There was a significant reduction in the isolation rate of all pathogens in sputum[42] supporting earlier conclusions that enhanced protection is based on specific activation of sensitised T cells, which in turn enhances phagocytosis, a non-specific effector mechanism.[6] While activation of mucosal protection is specific, the effector mechanism is non-specific, that is, phagocytes reduce all pathogens creating an 'antisepsis' environment within the bronchus lumen. In the mouse model of co-infection with NTHi and influenza virus, the synergistic increase in titre of both organisms was abrogated by pretreatment with oral NTHi.[38] By reducing the level of inflammation within the airways, there is a shift in the severity of exacerbations. The apparent protection in the majority of those immunised reflects the high frequency of T-cell sensitisation in COPD (R. Clancy *et al*., unpubl. obs., 2012) with or without restimulation of T cells relocating in the airways by colonising NTHi.[43] Protection by oral NTHi must not be confused with the oral polybacterial products used in Europe. The bacterial content of these latter products is about 1% of that in the NTHi oral vaccine. They are often lysates less geared to uptake into Peyer's patches. They are short-lived polyclonal activators acting as super antigens inducing Treg cells, with no evidence of enhancing mucosal immunity and without proven benefit in COPD.[44] In a direct comparison with oral NTHi vaccine, a polybacterial product was significantly less effective at preventing exacerbations of COPD.[45]

Conclusion and future studies

This review discusses two major mechanisms that contribute to airways damage in COPD: one consequent on the other. The second involves bacterial colonisation of toxin-damaged airways, and the hypothesis presented that acute exacerbations in COPD reflect a particular outcome of the host–bacteria relationship. A critical part of the argument is that an oral NTHi vaccine downregulates intrabronchial inflammation protecting the patient from acute exacerbations. A central role for NTHi is postulated with these bacteria providing a final common pathway for many, if not most, exacerbations irrespective of cause suggested by the size of the shift away from severe exacerbations following oral NTHi immunotherapy. A major task is to confirm these clinical observations in large clinical trials – such a study is current across 21 sites in Australia with 320 subjects with severe COPD. Any downside of oral immunotherapy remains to be

identified – to date, there is no evidence of bacterial replacement, immune tolerance or hypersensitivity reactions, but these must be carefully screened as more subjects are exposed to these new therapies. Of similar interest is testing the idea that oral NTHi alleviates symptoms in 'treatment-resistant' asthma. The basis of this postulate is reduction in recurrent episodes of recurrent wheezy bronchitis following oral NTHi,[2] the presence of IgE anti-NTHi antibody in subjects with 'treatment-resistant' asthma (P. Howarth *et al*., unpubl. obs., 2010), a documented role for NTHi in promoting asthma in a rodent model[46] and the reduction of NTHi 'allergen' in the lower airways following oral NTHi.[30] Any effect of oral NTHi on progressive airways disease requires long-term study and the development of surrogate parameters. The use of culture-independent methods of quantitating the lung microbiome in such studies will be invaluable. The use of imaging technology may assist evaluating oral therapy on local disease because of microanatomic changes in bacterial communities.[18] Technology is now available to study mechanisms of action as a molecular level for T-cell subsets, cytokine patterns, markers of inflammation and changes in the microbiome. NTHi is particularly able to form biofilms of structured extracellular DNA that may have particular relevance to hypersensitivity reactions,[7] making assessment of the effect of enhanced immunity an important goal. In addition, the sequence of activation of tissue repair genes related to collagen deposition around small bronchi and bronchioles, and destruction of support elastic tissue can be studied following oral NTHi.[47] Thus, an oral vaccine that induces quantitative change in the pulmonary microbiome can be used not only to analyse changes in the microbiology but also as a probe on mechanisms of damage and repair in COPD and perhaps other lung diseases. As similar characteristics of inflammation occur in other diseases of both lower airways (e.g. bronchiectasis and cystic fibrosis) and upper airways (e.g. otitis media and recurrent sinusitis), as seen in COPD, these diseases may benefit from oral vaccines that reduce bacterial load, and these ideas need to be tested.

The most important management of COPD remains the cessation of smoking. Now, it may become possible to direct more effective therapy for those many with moderate-to-severe COPD who have long ceased smoking but continue with severe recurrent exacerbations and progressive airways disease. Indeed, as smoking can on occasion be perceived to reduce acute episodes, an oral vaccine may aid the process of giving up smoking. The disappointing history of identifying useful surrogate markers may improve as we gain a better understanding of relationship of the microbiome to the host response and the benefits of intervention strategies that modulate these relationships.

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