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REVIEW

Chronic Obstructive Pulmonary Disease (COPD): Evaluation From Clinical, Immunological and Bacterial Pathogenesis Perspectives

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Chronic obstructive pulmonary disease (COPD), a disease manifested by significantly impaired airflow, afflicts ~14.2 million cases in the United States alone with an estimated 63 million people world-wide. Although there are a number of causes, the predominant cause is excessive tobacco smoke. In fact, in China, there have been estimates of 315,000,000 people that smoke. Other less frequent causes are associated with indirect cigarette smoke, air pollutants, biomass fuels, and genetic mutations. COPD is often associated with heart disease, lung cancer, osteoporosis and conditions can worsen in patients with sudden falls. COPD also affects both innate and adaptive immune processes. Cigarette smoke increases the expression of matrix metalloproteases and proinflammatory chemokines and increases lung titers of natural killer cells and neutrophils. Yet, neutrophil reactive oxygen species (ROS) mediated by the phagocytic respiratory burst and phagocytosis is impaired by nicotine. In contrast to innate immunity in COPD, dendritic cells represent leukocytes recruited to the lung that link the innate immune responses to adaptive immune responses by activating naïve T cells through antigen presentation. The autoimmune process that is also a significant part of inflammation associated with COPD. Moreover, coupled with restricted FEV1 values, are the prevalence of patients with single or multiple infections by bacteria, viruses and fungi. Finally, we focus on one of the more problematic infectious agents, the Gram-negative opportunistic pathogenic bacterium, *Pseudomonas aeruginosa*. Specifically, we delve into the development of highly problematic biofilm infections that are highly refractory to conventional antibiotic therapies in COPD. We offer a non-conventional, biocidal treatment that may be effective for COPD airway infections as well as with combinations of current antibiotic regimens for more effective treatment outcomes and relief for patients with COPD.

Keywords: Chronic obstructive pulmonary disease (COPD), smoking, innate and adaptive immunity, airway infections, *Pseudomonas aeruginosa*, biofilm

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive, debilitating lung disorder characterized by non-normalizing airflow limitation. COPD is currently the third major cause of death in the United States and is projected to become the fifth most significant contributor to the worldwide burden of disease by 2020. Primary tobacco smoke exposure is the most common cause but contact with indirect smoke, air pollutants, and biomass fuels, and genetic mutations, especially alterations in alpha 1 antitrypsin, may be significant etiologic factors. Classically, COPD has been considered to be two overlapping processes, chronic bronchitis and emphysema, but more recent investigations suggest that there are multiple COPD phenotypes with varying presentations, clinical course, and response to management. The principal respiratory manifestations of COPD are cough, phlegm production, and breathlessness. Physiologically, COPD is defined by the presence of airflow limitation (obstruction), a reduction in the ability to exhale at a normal rate and volume. The clinical course of COPD is marked by an insidiously progressive decline in lung function that may be accelerated by disease exacerbations, episodes of increased respiratory symptoms usually precipitated by bacterial, viral, or mixed pulmonary infections. Although COPD is usually recognized and diagnosed by respiratory symptoms, more recent investigations show that COPD is a systemic disorder with significant cardiovascular, endocrine, musculoskeletal, and psychological comorbidities that are believed to be caused by systemic inflammation. Management of COPD utilizes pharmacologic interventions including short and long acting bronchodilators, corticosteroids, oxygen and nonpharmacologic treatments such as smoking cessation and pulmonary rehabilitation.

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Features of COPD

Epidemiology

A meta-analysis of global studies of COPD prevalence estimated the worldwide prevalence of physiologically defined COPD among adults 40 years of age or older to be 9–10% with significant regional variation (Halbert *et al.*, 2006). The Burden of Obstructive Lung Disease (BOLD) study measured lung function in 9425 subjects from 12 global sites and found the overall prevalence of COPD to be 10.1%, 11.8% in men and 8.5% in women with a wide range (Buist *et al.*, 2007). Despite these high measured prevalence rates, it is estimated that less than half of individuals with airflow limitation have been diagnosed with COPD (Mannino *et al.*, 2000). The World Health Organization estimates that by 2020, COPD will be the fifth leading process contributing to the burden of disease worldwide (WHO. World health statistics. Available at: http://www. who.int/whosis/whostat/EN_WHS08_Full.pdf. Accessed January, 2008). Currently, COPD is the third leading cause of death in America.

There are significant gender differences in death rates for COPD; in the US, the death rate for men declined from 57.0 per 100,000 in 1999 to 46.4 per 100,000 in 2006 whereas, for women there was no significant change, 35.3 per 100,000 in 1999 to 34.2 per 100,000 in 2006 (Ford *et al.*, 2012). For the past decade, more women than men have died of COPD in the US (Camp and Goring, 2007). Further, women are twice as likely to be diagnosed with chronic bronchitis compared with men: in 2011, 56.7 women per 100,000 and 29.6 men per 100,000 (http://www.lung.org/finding-cures/our-research/trend-reports/copd-trend-report.pdf. Accessed January, 2014). COPD may present differently in women from in men, have different associated disorders, and a better prognosis after COPD exacerbations (Aryal *et al.*, 2013). These differences may be related to changes in tobacco smoking rates, differences in smoking habits and behaviors, susceptibility to tobacco and environmental smoke, pulmonary anatomy and physiology, hormones, and response to respiratory medications (Aryal *et al.*, 2013).

COPD presentation

The development of a cough is often the initial respiratory symptom in patients with COPD and persistent cough with phlegm production portends increased frequency of exacerbations, hospitalizations, and overall decline in pulmonary function (Kornmann *et al.*, 2003; Miravitlles, 2011). Although cough and sputum production may be the earliest respiratory symptoms, breathlessness, usually with exertion initially and insidiously progressing to dyspnea at rest, is the most concerning and debilitating respiratory symptom (Bestall *et al.*, 1999; Witek and Mahler, 2003; Rabe *et al.*, 2007; Hill *et al.*, 2008). This shortness of breath is often attributed to other causes such as deconditioning, obesity, aging, or comorbidities such as heart disease that leads to delays in its evaluation and the diagnosis of COPD.

As the respiratory manifestations of COPD progress, patients decrease their physical activity to avoid the discomfort of breathlessness. Inactivity initiates a gradual downward spiral: activity associated breathlessness leads to avoidance of exercise that accelerates deconditioning that causes weakness and inability to perform activities which further increases inactivity. As the activity level decreases, patients' prognosis declines and the number of exacerbations and hospitalizations increases, quality of life declines, and mortality increases (Garcia-Aymerich *et al.*, 2006; Pitta *et al.*, 2006; Kocks *et al.*, 2011). In fact, COPD affects general health status more negatively than cardiovascular disease and diabetes (Janson *et al.*, 2013).

Respiratory symptoms are neither constant nor do they progress linearly; dyspnea, cough, and sputum production may vary throughout the day, week, and even over long periods of time (Espinosa de los Monteros *et al.*, 2012). In general, respiratory symptoms are worse in the morning hours and improve as the day progresses. Patients with COPD may also have significant symptoms at night that may impair the ability to fall asleep or maintain sleep. Obstructive sleep apnea may occur in patients with COPD and has been labeled the "overlap syndrome". The simultaneous occurrence of OSA and COPD may be associated with more profound sleep disturbances and oxygen desaturations at night.

The course of COPD is marked by exacerbations, episodes of increased pulmonary and systemic inflammation, worsened symptoms, and reduction in quality of life that often precipitate healthcare encounters, and are associated with increased healthcare costs and mortality (O'Donnell and Parker, 2006; Wedzicha and Seemungal, 2007; Wedzicha *et al.*, 2013). These episodes may be caused by infections due to bacteria, viruses, or a mixture of organisms, or exposure to pulmonary irritants (e.g., air pollution). Recent evidence suggests that some patients with COPD have frequent exacerbations whereas others have few, if any exacerbations (Hurst *et al.*, 2010). The best predictor of future exacerbations is a history of exacerbations in the prior year (Hurst *et al.*, 2010) (Table 1).

Although respiratory symptoms are the hallmarks of COPD, nonpulmonary manifestations occur frequently. COPD is associated with an increased risk of significant comorbidities and is now considered a multisystemic disorder (Barnes and Celli, 2009; Huertas and Palange, 2011; Vijayan, 2013) (refer to Table 2). These nonpulmonary manifestations of COPD are believed to be mediated by inflammatory processes that are initially triggered within the lungs and propagate systemically both causing and accentuating comor-

Table 1. Characteristics of individuals with frequent exacerbations

Higher levels of inflammation measured by CRP, fibrinogen, Interleukin 6
Enhanced bacterial colonization
Increased susceptibility to viral infections
Increased COPD morbidity and mortality:
Greater dynamic hyperinflation
Worse quality of life and faster erosion of functional status
More rapid decline in lung function
Greater healthcare utilization
Higher risk of death
Increased COPD comorbidities:
Greater anxiety and depression
Increased cardiovascular risks
More extra-pulmonary manifestations

Table 2. Systemic manifestations of COPD (adapted from Tobin *et al.*, 2009)

Vascular	
	Coronary artery disease
	Congestive heart failure
	Hypertension
	Stroke
Metabolic	
	Diabetes
	Metabolic syndrome
	Obesity
	Dysfunction of:
	Pituitary
	Thyroid
	Gonads
	Adrenals
	Pancreas
Bone disease	
	Osteoporosis
	Osteopenia
	Axial and long bone fractures
Lung cancer	
Muscle weakness	
	Fall proclivity
Psychological	
	Anxiety
	Depression
	Cognitive decline

bidities (MacNee, 2013). Circulating inflammatory biomarkers, such as C-reactive protein, fibrinogen, tumor necrosis factor alpha, interleukin (IL)-6, IL-8, leptin, and ghrelin, are potential mediators of these systemic effects (MacNee, 2013). Most COPD research has focused on pulmonary pathogenetic processes and management of the lung manifestations. New evidence shows that the extra-pulmonary manifestations of COPD are very common, have significant effects on patient health, longevity, and wellbeing, and justify systemic screening and management (Agusti and Soriano, 2008). Among the systemic manifestations of COPD are weight loss, skeletal muscle dysfunction, cardiovascular disease, osteoporosis, depression, anemia, diabetes, and malignancy (Agusti, 2007; Cazzola *et al.*, 2010; Nussbaumer-Ochsner and Rabe, 2011; Clini *et al.*, 2013; Mullerova *et al.*, 2013).

COPD and heart failure occur concomitantly in clinical practice (de Miguel Díez *et al.*, 2013) approximately one third of patients with chronic heart failure have obstructive lung disease whereas 17% of individuals with COPD have ventricular dysfunction (Macchia *et al.*, 2012). The risk of lung cancer is 3-4 fold greater in smokers with COPD compared with individuals who have equivalent smoking histories but do not have COPD (Tockman *et al.*, 1987; Wasswa-Kintu *et al.*, 2005). In individuals with COPD, the prevalence of osteoporosis and osteopenia range from 9–69% and 27–67%, respectively (Jorgensen *et al.*, 2007; Graat-Verboom *et al.*, 2009, 2011, 2012; Lehouck *et al.*, 2011; Rittayamai *et al.*, 2012). In a longitudinal cohort study of 102 patients with COPD, 16 of 48 (33%) patients with normal initial bone density developed osteoporosis over 3 years (Graat-Verboom *et al.*, 2012). In the TORCH trial, 18% of men and 30% of women had osteoporosis and 42% of men and 41% of women had osteopenia at baseline based on bone mineral density measurement (Ferguson *et al.*, 2009). Osteoporosis and falls are major risk factors for fractures and occur commonly in individuals with COPD. In an analysis of 14,828 subjects participating in the National Health and Nutrition Examination Survey (NHANES) from 1999–2008, individuals with physician-diagnosed COPD were more likely than those without physician-diagnosed COPD to have osteoporosis (16.9% vs 8.5%) and more likely to report mobility difficulty (55.6% vs 32.5%) and dizziness/balance problems (41.1% vs 23.8%) (Schnell *et al.*, 2012). The 3 year fracture rate hazard ratio ranged from 5.1–6.3 across the various treatment and placebo groups in the TORCH trial (Ferguson *et al.*, 2009). Hip fractures occurred most commonly, followed by wrist, spine, and rib fractures. The prevalence of vertebral fractures in patients with COPD ranges from 24%–63% (McEvoy *et al.*, 1998; Papaioannou *et al.*, 2003; Jorgensen *et al.*, 2007; Nuti *et al.*, 2009). Approximately 10% of patients admitted with COPD exacerbations have chest radiographs documenting vertebral compression fractures (Majumdar *et al.*, 2010).

In a study comparing 36 patients with COPD with 20 normal individuals, hypoxemia, dyspnea, and fatigue were associated with balance impairment and falls (Ozalevli *et al.*, 2011). In a retrospective study, 46% of 39 participants with COPD (mean FEV1% predicted 42%) reported at least one fall in the preceding year. Those with self reported falls scored lower on the Activity-Specific Balance Confidence (ABC) Scale (66 v 82) and the Berg Balance Scale (BBS) (45 v 49), and had prolonged times on the Time Up and Go Test (TUG) 17 v 14 s. Falls correlated with the use of supplemental oxygen and dyspnea severity (Beauchamp *et al.*, 2009). Another retrospective study found that 25% of 80 patients with COPD (mean FEV1% predicted 47.5%) reported a fall in the prior year and 29% expressed a fear of falling (Hellstrom *et al.*, 2009). In a prospective study of 101 patients with COPD (mean FEV1% predicted 46%) for 6 months, 31.7% reported at least one fall and the fall incidence rate was 0.1 (95% CI: 0.06–0.14) falls per person/month (Roig *et al.*, 2011). Factors contributing to the increased risk of falling in patients with COPD include alterations in proprioception (Janssens *et al.*, 2013), impaired postural and balance control mechanisms (Butcher *et al.*, 2004; Eisner *et al.*, 2008; Smith *et al.*, 2010; Roig *et al.*, 2011), slower reaction times, reduced physical activity levels, and skeletal muscle weakness (Beauchamp *et al.*, 2012).

Skeletal muscle dysfunction in patients with COPD is characterized by reduced muscle atrophy (decreased mass and cross-sectional area), deranged distribution of muscle fiber types (fewer oxidative fibers and more glycolytic fibers), altered metabolic capacity (diminished mitochondrial enzyme activities and expression), and reduced vascular supply (loss of capillary density) that cause diminished muscle strength and endurance (Kim *et al.*, 2008; Gea *et al.*, 2013). Factors that contribute to skeletal muscle dysfunction include disuse and inactivity, systemic inflammation, malnutrition, corticosteroid use, hypoxemia, senescence, and myocyte biochemical derangements including reactive oxygen and ni-

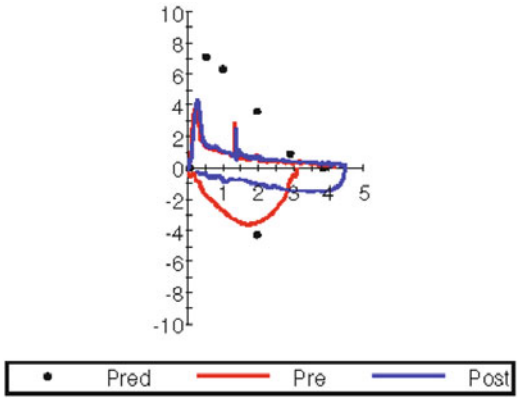

Fig. 1. Flow volume loops and volume time curves. The flow volume loops demonstrate reduced flow rates with a concave expiratory flow pattern. The volume time curves demonstrate prolonged expiratory times with persistent exhalation that never reaches a plateau. Color scheme: black, predicted; red, pre-administration of a bronchodilator; blue, post-administration of a bronchodilator.

trogen species production and muscle fiber degradation due to increased calpain and caspase activities (Kim *et al.*, 2008). Quadriceps muscle strength is 20–30% less in individuals with moderate to severe COPD compared with those who do not have COPD (Gosselink *et al.*, 1996; Bernard *et al.*, 1998; Franssen *et al.*, 2005).

COPD diagnosis

COPD is diagnosed by a constellation of respiratory symptoms (usually cough, sputum production, and/or dyspnea), the presence of airflow limitation (FEV1/FVC < lower limit of normal), radiographic features of emphysema, and the exclusion of other processes. COPD is not a disease but is a syndrome that is defined by a constellation of historic, physiologic, and radiographic features; although airflow limitation is usually considered an essential feature, individuals with radiographic emphysema and no airflow limitation are still diagnosed with COPD. Thus, COPD is not a single homogeneous disorder but is categorized by many phenotypes which define COPD subgroups that have differing presentations, courses, and responses to treatment (Friedlander *et al.*, 2007; Barker and Brightling, 2013).

The physiologic hallmark of COPD is the presence of non-normalizing airflow limitation. Spirometry is used to measure expiratory airflow when an individual exhales from maximal capacity, total lung volume, to minimal capacity, residual volume. This exhaled volume is temporally divided into the amount of air exhaled in the first second, forced expiratory volume in one second (FEV1), and the total amount of air exhaled, forced vital capacity, and the ratio, FEV1/FVC, is used to define airflow limitation. In individuals with normal lung function, approximately 80% of the FVC is exhaled in the first second; in individuals with airflow limitation, the FEV1/FVC is reduced (Table 3).

The threshold for the definition of obstruction varies from

Table 3. Pulmonary function test results from a patient with COPD. The FEV1/FVC is less than the lower limit of normal demonstrating airflow limitation. There is not a significant change in either the FEV1 or the FVC after the administration of a bronchodilator. Both the total lung capacity (TLC) and the residual volume (RV) are elevated and are greater than their respective upper limits of normal demonstrating hyperinflation and air trapping, respectively. The diffusing capacity, DLCO, is reduced demonstrating a decrease in the surface area for gas exchange.

	Baseline				Post Bronchodilator		
	Actual	Pred	%Pred	LLN	Actual	%Chng	%Pred
Spirometry							
FVC (L)	4.15	3.82	109	2.95	4.50	8	118
FEV1 (L)	1.32	2.74	48	2.00	1.38	4	50
FEV1/FVC(%)	32	72	44	62	31	-4	42
FEF Max (L/sec)	4.19	7.15	59	4.96	4.42	6	62
FEF 25-75% (L/sec)	0.44	1.94	23	0.42	0.56	27	29
Lung volumes							
TLC (Pleth)(L)	9.23	6.72	137	5.32			
SVC (L)	4.94	3.99	124				
ERV (L)	1.81	0.91	199				
IC (L)	2.99	3.08	97				
RV (Pleth) (L)	4.29	2.73	157	1.91			
RV/TLC (Pleth) (%)	46	44	106	33			
Raw ($cmH_2O/L/s$)	2.13	1.45	147	0.66			
Gaw ($L/s/cmH_2O$)	0.47	1.03	46				
Diffusion							
DLCOcor (ml/min/mmHg)	8.53	21.45	40	11.77			
DLCOunc (ml/min/mmHg)	8.69	21.45	41	13.46			
VA (L)	6.82	6.43	106	5.06			
DL/VA (ml/min/mmHg/L)	1.25	3.83	33	2.37			
IVC (L)	4.75						
Hgb (gm/dL)		12-18			15.3		

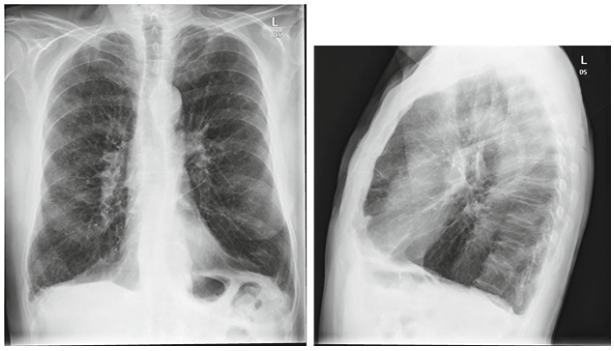

Fig. 2. Posterior-anterior and lateral chest x-rays from a patient with COPD. Hyperinflation, increased retrosternal airspace, flattened diaphragms, and equalization of the anterior-posterior and lateral dimensions, "barrel chest" are demonstrated.

a fixed threshold of 0.7 suggested by the GOLD Guidelines to the lower limit of normal which declines with increasing age. Thus, compared with the lower limit of normal, the use of a fixed threshold tends to underdiagnose airflow limitation in younger individuals and overdiagnose older individuals (Fig. 1).

The radiographic manifestations of COPD may be detected by both chest x-ray and computed tomography (CT) scan. Tram tracking and increased bronchovascular markings may occur in individuals with chronic bronchitis. Hyperinflation, diaphragmatic flattening, and equalization of anterior-posterior and lateral dimensions ("barrel chest") may be found in emphysema (Fig. 2).

CT scans provide detailed imaging of the lung parenchyma and are used to detect the various forms of emphysema, centrilobular, paraseptal, and panlobular. In addition, cysts and blebs can be identified. Quantitative CT scans may also be used to estimate lung volumes. Approximately half of individuals with moderate to severe COPD have chest CT evidence of bronchiectasis (Martínez-García *et al.*, 2013). The presence of bronchiectasis is associated with increased frequency of bacterial colonization of the lower airway, more severe airflow limitation, and previous hospitalizations for AECOPD.

Management of COPD

The goals of COPD management are: 1) reduce mortality, 2) preserve lung function, 3) decrease COPD-associated complications, 4) treat COPD-related comorbidities, 5) decrease the number and severity of COPD exacerbations, 6) relieve respiratory symptoms, especially breathlessness and cough, and 7) improve overall well being.

Guidelines for the management of COPD

There are multiple guidelines for the management of COPD that have been developed by numerous national and international health organizations. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines are the most widely disseminated international strategies for the management of COPD and are continually being updated with the most recent revision published in 2014 (GOLD).

Table 4. GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV1: forced expiratory volume in one second (Adapted from GOLD Guidelines)

GOLD classification of airflow limitation severity		
GOLD 1	Mild	FEV1 ≥ 80% of predicted
GOLD 2	Moderate	50% ≤ FEV1 < 80% of predicted
GOLD 3	Severe	30% ≤ FEV1 < 50% of predicted
GOLD 4	Very Severe	FEV1 < 30% of predicted

2014 GOLD guidelines for the management of COPD

The GOLD guidelines recommend that each individual with COPD be evaluated to determine the severity of COPD, the effect of COPD on the individual's overall health and well being, the risk of future COPD-related events (healthcare encounters or death), and COPD-related comorbidities (Table 4). Management is based upon stratification of patients into one of four groups (Fig. 3). COPD severity is determined by the level of airflow limitation measured by spirometry and compares the measured FEV1 to the predicted FEV1. The effects of COPD on an individual's overall health and well being are evaluated by the COPD Assessment Test (CAT) or the modified British Medical Research Council (mMRC) breathlessness scale. The risk of future COPD exacerbations is estimated by the number exacerbations in the prior year. Patients with less symptoms, better spirometry, and fewer exacerbations are group A; patients with more symptoms, worse spirometry, and more frequent exacerbations are group D. Groups B and C are intermediate categories and have either more symptoms and fewer exacerbations or less symptoms and more exacerbations.

Pharmacologic treatment of COPD

Medications for the treatment of COPD consist of short act-

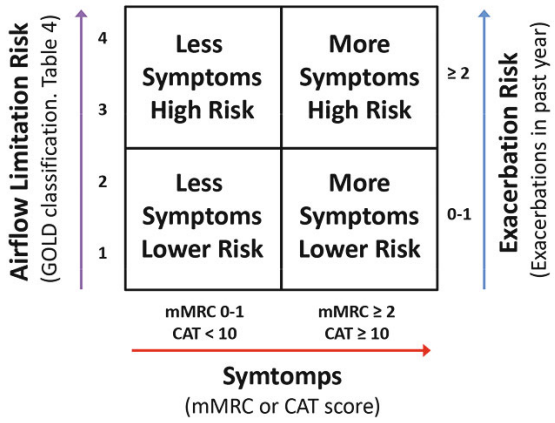

Fig. 3. Stratification of individuals with COPD based upon the impairment in spirometry, symptoms, and risk of exacerbations. GOLD, Global Initiative for Chronic Obstructive Lung Disease; CAT, COPD Assessment Test; mMRC, Modified British Medical Research Council breathlessness scale (Modified from GOLD Guidelines).

ing beta agonists (SABA), long acting beta agonists (LABA), short acting anticholinergics (SAch), long acting anticholinergics (LAch), inhaled (ICS) and enteral corticosteroids. Other medications include phosphodiesterase inhibitors, macrolide antibiotics (used for their anti-inflammatory not antibiotic effect), and mucolytics.

Beta agonists and anticholinergics: SABAs are usually the initial medication and are used as rescue treatments because their short half lives limit their utility as maintenance therapy (Cazzola *et al.*, 2013). If symptoms persist despite use of a SABA or if SABA use is excessive (greater than 3–4 times daily), an anticholinergic bronchodilator is usually added. LAch's are usually preferred because of the ease of use and theoretical advantage of improved adherence with less frequent dosing. The most frequently prescribed LAch is tiotropium that reduces airflow limitation, dynamic hyperinflation, and COPD exacerbations, and improves quality of life and breathlessness (Tashkin *et al.*, 2008; Yohannes *et al.*, 2013). A LABA may also be added. LABA's improve exercise tolerance, reduce breathlessness and dynamic hyperinflation, augment quality of life, and decrease COPD exacerbations (Cazzola *et al.*, 2011, 2012, 2013; Wang *et al.*, 2012).

Corticosteroids: ICS are recommended for patients with GOLD severe or very severe disease (FEV1<50%) and who have had two or more exacerbations in the prior year (GOLD 2012). Use of triple inhalers in COPD, tiotropium plus combination salmeterol + fluticasone, improves lung function and symptoms but does not reduce exacerbations compared with either treatment alone (Aaron *et al.*, 2007). Many patients with less severe and /or less COPD-related risk are prescribed ICS (Seaman *et al.*, 2010). It has been estimated that 70% of patients with COPD are treated with high-dose combination inhalers yet only 10% qualify under the current guidelines (Barnes, 2011).

Phosphodiesterase inhibitors: Phosphodiesterases (PDEs) are a family of at least 11 isoenzymes that hydrolyze cAMP and cGMP. Inhibition of PDEs stimulates bronchodilation and also reduces pulmonary inflammation. Methylxanthines such as aminophylline or theophylline are nonspecific PDE inhibitors that have been used for the treatment of asthma and COPD. These medications are limited by a narrow therapeutic serum drug range, frequent interaction with other medications, and poor tolerability. Most recently, specific inhibitors of PDE isoforms, especially PDE4, have been developed and shown to be effective in the management of COPD. Roflumilast was approved by the FDA in 2011 as adjuvant treatment to reduce COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations (Reid and Pham, 2012).

Macrolides: Macrolides such as erythromycin and azithromycin have anti-inflammatory properties in addition to anti-microbial effects. Recent studies have shown that either daily erythromycin or azithromycin decrease the frequency of COPD exacerbations in patients with a history of exacerbations (Martinez *et al.*, 2008; Seemungal *et al.*, 2008; Albert *et al.*, 2011). Although it remains unclear which subgroup of patients with COPD will benefit best from macrolide treatment and whether dosing should be daily or thrice weekly, current recommendations are to consider daily macrolide treatment in patients who, despite maximal standard bronchodilator therapy, have at least 2 exacerbations yearly (Mammen and Sethi, 2012).

Mucolytics: Mucolytics such as *N*-acetylcysteine and carbocysteine may reduce COPD exacerbations and improve health related quality of life in patients with COPD (Decramer and Janssens, 2010). In one prospective study, *N*-acetylcysteine reduced hyperinflation (Stav and Raz, 2009).

Supplemental oxygen

Supplemental oxygen improves survival in patients with hypoxemia at rest (PaO_2 <55 torr or SpO_2 <88%; or PaO_2 < 60 and >55 torr with evidence of cor pulmonale) (Make *et al.*, 2010; Stoller *et al.*, 2010). The mechanism(s) by which supplemental oxygen improves mortality is not known. Oxygenation should be measured on room air at rest, with exertion, and during sleep after the administration of supplemental oxygen to insure that desaturation is prevented. Although Medicare and most insurances reimburse for supplemental oxygen during exercise or at night with evidence of exercise or nocturnal desaturation, there is no evidence that supplemental oxygen during exercise or at night is beneficial in individuals with stable COPD (Panos and Eschenbacher, 2009).

Nonpharmacologic treatment of COPD

Smoking cessation is the singularly the most important intervention for the prevention and treatment of COPD. Most smoking cessation programs combine counseling with pharmacologic treatments such as nicotine replacement therapy (NRT), varenicline, or bupropion (Tonnesen, 2013). Varenicline is as effective as dual NRT and more effective than single NRT or bupropion (Tonnesen, 2013).

Both influenza and pneumococcal vaccinations are recommended for individuals with COPD. Influenza vaccination reduces mortality, outpatient visits, hospitalizations, and exacerbations caused by influenza (Varkey *et al.*, 2009). In contrast, although pneumococcal vaccination reduces the incidence of invasive pneumococcal disease, it has not shown any significant effect on mortality, rates of pneumonia or exacerbations, lung function decline, or cost effectiveness (Varkey *et al.*, 2009; Walters *et al.*, 2010). Vaccination against both influenza and pneumococcus may reduce COPD exacerbations more effectively than either vaccine alone (Varkey *et al.*, 2009).

Pulmonary rehabilitation is a multidisciplinary program of education and exercise that teaches patients with COPD about their disease, its treatment, and mechanisms to cope with its consequences as well as an exercise and conditioning program. PR is most effective when it is integrated into a comprehensive COPD management program that encourages behavior change and a shift from provider initiated to patient initiated care. Patients with COPD who maintain physical activity have less breathlessness with exertion, better health related quality of life, improved long term function and independence, and better psychological and physiological function. PR also improves respiratory symptoms (Lacasse *et al.*, 2006). In addition, PR decreases health care utilization and may improve survival (Ries, 2008). Home-based PR programs have the equivalent benefit of hospital

based programs (Bourbeau, 2010).

Lung volume reduction performed either surgically or endoscopically via endobronchial valve placement may also be effective in certain patients with COPD (Sciurba *et al.*, 2010; Tidwell *et al.*, 2012). LVRS is only beneficial in patients with upper lobe emphysema and poor exercise tolerance and is detrimental in individuals with FEV1<20% of predicted, DLCO<20% of predicted, or diffusely distributed emphysema (Fishman *et al.*, 2003). Patients who are being considered for LVRS should be referred for pulmonary consultation. Endoscopic LVRS is a newer technique that may also be beneficial in select patients.

Management of COPD-related nonpulmonary comorbidities

Because COPD is a multisystemic disorder with systemic nonpulmonary manifestations, it is essential to evaluate and treat patients with COPD for conditions such as anemia, diabetes, lung cancer, osteopenia/osteoporosis, and cardiovascular disease.

Immunolgy of COPD: Relationships to Cigarette Smoking

Prevalence of cigarette smoking

Tobacco use is the leading cause of preventable deaths in the United States (Mokdad *et al.*, 2004), and cigarette smoking is by far the most common form of tobacco use in the United States (Capehart, 2006). There are an estimated 91.8 million current or former smokers in the United States (Mariolis *et al.*, 2006). The prevalence of cigarette smoking among adults in the United States has declined more than 50% between 1965 and 2006 (Giovino, 2007). However, the rate of decline in cigarette smoking among US adults since 2005 appears to be leveling off (Agaku *et al.*, 2012). In many low and middle income countries, the prevalence of cigarette smoking continues to increase significantly (Edwards, 2004). Evidence from a prospective study of the mortality associated with cigarette smoking suggests that an estimated 50% of smokers will die as a result of their tobacco use (Doll *et al.*, 1994). Projections based on current rates of tobacco use predict a continuing increase in the global mortality associated with cigarette smoke (Mathers and Loncar, 2006).

The pulmonary symptoms associated with long-term cigarette smoking include coughing, wheezing, mucous hypersecretion and dyspnea (Jansen *et al.*, 1999). Smokers also report more frequent pulmonary infections that are also more severe than illness in non-smokers (Cohen *et al.*, 1993). Lung function declines with age faster in smokers than in non-smokers and is typified by airways obstruction and increased lung tissue compliance (Tager *et al.*, 1988; Sherrill *et al.*, 1991). Pathological examination of the lungs of smokers reveals a varying spectrum of features which may include respiratory bronchiolitis (pigmented macrophage accumulated in the bronchioles and distal airways), mucous cell metaplasia, squamous metaplasia, fibrosis, coalescing of alveoli, smooth muscle hypertrophy, and inflammation of the alveoli or insterstitium. Genetic and other environmental factors influence whether these pulmonary effects lead to the development of lung cancer, interstitial lung disease, COPD or chronic pulmonary infection.

Smoking and the immune system

The ideal immune response quickly identify damage or infection, effectively deals with pathogens, clears damaged tissue and cellular debris, and promotes wound healing while causing minimal collateral damage. An exaggerated immune response can cause excessive collateral damage to affected tissues in the process of battling the infection. A weakened immune response may fail to eradicate an infectious agent in a timely manner. Effective clearance of infection involves the coordination of two intertwined branches of the immune system: the innate immune system and the adaptive immune system, both of which are discussed in the following paragraphs. The innate immune system consists of myeloid cells such as granulocytes, monocytes and macrophages and innate lymphocytes such as natural killer cells (NK cells). The innate immune response is dependent upon invariant receptors that recognize conserved patterns found on molecules associated with pathogens or endogenous compounds indicative of tissue or cellular damage (Barton, 2008). The cells of the innate immune system provide the initial response to infection or tissue damage. The adaptive immune system includes lymphocytes that develop in the thymus known as T cells or develop in the bone marrow termed B cells. These adaptive lymphocytes express cell surface receptors encoded by DNA loci that undergo somatic recombination to during cellular development to create a vast diversity of antigen specificities (Davodeau *et al.*, 1994). The adaptive immune system is responsible for immunological memory and focuses the immune response to specific pathogens (Grossman, 1984). Together the adaptive and innate arms of the immune system cooperate to mount a balanced response to infectious challenges and clear damaged tissue for subsequent repair.

Innate immune system

The traditional view stood that cigarette smoke is an inhibitor of the immune system (Stampfli and Anderson, 2009). Alveolar macrophages are resident cells of the lung that are important in the surveillance of the lung and orchestration of proper pulmonary immune responses. Cigarette smoke increases the number of alveolar macrophage in the lungs (Wallace *et al.*, 1992). Alveolar macrophages exhibit defective phagocytosis of bacteria and apoptotic cells after exposure to cigarette smoke. However, cigarette smoke also increases the expression of matrix metalloproteases and proinflammatory chemokines, suggesting that cigarette smoke exposure skews the functional responses of alveolar macrophages (Russell *et al.*, 2002; Woodruff *et al.*, 2005). Neutrophil reactive oxygen species (ROS) mediated by the phagocytic respiratory burst and phagocytosis is impaired by nicotine exposure during differentiation providing an attractive mechanism for the impaired clearance of bacterial infections observed in smokers (Xu *et al.*, 2008). Total neutrophil titers in the COPD lung is substantially increased, however, and their subsequent clearance after apoptosis is impaired. This increases the possibility that neutrophil elastase and other

lytic granules are released into the extracellular space, further contributing to cigarette smoke-associated pulmonary damage (Vandivier *et al.*, 2006).

Natural killer (NK) cells are innate lymphocytes important in the response to viral infections and in controlling cancer. Our laboratory has identified hyper-responsiveness in NK cells following chronic *in vivo* exposure to cigarette smoke through several studies. Cigarette smoke exposure causes the surface expression of ligands for the NK cell activating receptor NKG2D (Borchers *et al.*, 2009) on the surface of epithelial cells. When NK cells encounter cells expressing ligands for NKG2D, the NK cell can respond by killing the cell. This cytotoxicty may contribute to the apoptosis and lung destruction resulting from cigarette smoke exposure, and chronic activation of this pathway is sufficient to cause emphysema in mice (Borchers *et al.*, 2009). Chronic stimulation through NKG2D primes NK cells to produce more IFN-γ in response to IL-12 and IL-18 (Motz *et al.*, 2010; Wortham *et al.*, 2012). The consequences of this NK cell priming in the context of influenza infection are increased pulmonary damage, but no difference in the viral titers in the lung (Wortham *et al.*, 2012). These results suggest that cigarette smoke exposure can lead to an exaggerated NK cell response that results in excessive tissue destruction.

Adaptive immune system

Dendritic cells are a key population of leukocytes that link the innate immune responses to adaptive immune responses by activating naïve T cells through antigen presentation (Geppert and Lipsky, 1989). Cigarette smoke has been demonstrated to increase the recruitment of dendritic cells into the airways (Casolaro *et al.*, 1988; Soler *et al.*, 1989). The surface expression of costimulatory molecules necessary for the activation of naïve T-lymphocytes is increased by cigarette smoke exposure, suggesting an enhanced ability to initiate T cell responses (Bratke *et al.*, 2008). Bronchial biopsies from smokers reveal infiltration of $CD8^+$ T cells into the epithelial space (Saetta *et al.*, 1993). Th17 $CD4^+$ T cells expressing the cytokine IL-17A are increased in response to cigarette smoke exposure (Harrison *et al.*, 2008; Shan *et al.*, 2009). Mice deficient in IL-17A are protected from cigarette smoke-induced emphysema, suggesting a role for the Th17 $CD4^+$ T cells in progression of COPD (Shan *et al.*, 2012). Increased B-lymphocytes are found in the lungs of smokers, especially in patients with COPD, where they are typically found in lymphoid follicles (Bosken *et al.*, 1992). Cigarette smoke exposure initiates and modulates a complex, chronic inflammatory response, and affects the homeostasis of every major inflammatory cell type.

More recent studies have looked for inflammatory involvement from T cells and B cells that is antigen dependent in the context of COPD. Oligoclonal expansions of the T cell receptor repertoire among $CD4^+$ T cells have been described in the lungs of patients with COPD (Sullivan *et al.*, 2005). Reactivity to elastin fragments resulting in proliferation and cytokine production among peripheral blood $CD4^+$ T cells has been demonstrated in COPD patients (Lee *et al.*, 2007). Since 2007, several studies have identified circulating autoantibodies in patients with COPD that bind to antigens derived from epithelial, endothelial, smooth muscle, extracellular matrix, or carbonyl-modified proteins (Lee *et al.*, 2007; Feghali-Bostwick *et al.*, 2008; Karayama *et al.*, 2010; Kirkham *et al.*, 2011; Nunez *et al.*, 2011). These studies provide evidence for an autoimmune process that is a part of the inflammation associated with COPD.

However, it remains unclear what role autoimmunity plays in the pathogenesis and progression of COPD. COPD patients are more likely to have cancer and bacterial colonization in the airways, and these confounders make interpretation of studies suggesting an autoimmune response in COPD more difficult. In our laboratory, we have employed a mouse model of chronic cigarette smoke exposure that does not perfectly replicate the response to smoke found in people, but does allow us to avoid some of the potential confounders present in the patient population. Previous studies from our laboratory have shown that chronic exposure to cigarette smoking leads to oligoclonal expansions among both $CD4^+$ and $CD8^+$ T cells in the lung, suggestive of an antigen-specific response to cigarette smoking, that persist after smoking cessation (Motz *et al.*, 2008). Additionally, T cells from cigarette smoke-exposed animals are sufficient to cause destruction of lung tissue and inflammation when transferred to immunodeficient recipient mice that have never been exposed to cigarette smoke (Motz *et al.*, 2010). These studies present strong evidence that chronic cigarette smoke exposure is sufficient to initiate an autoimmune response.

Microbiology of COPD: Relationships to Exacerbations

Smoking adults infected with varicella are more likely to develop pneumonitis (Mohsen *et al.*, 2001). Complications of respiratory syncytial virus are more common among young children exposed passively to cigarette smoke (Bradley *et al.*, 2005). Community acquired pneumonia is more frequent among smokers and an increased risk persisted for more than 5 years following cessation of smoking (Almirall *et al.*, 1999). Pneumococcal pneumonia is uncommon among immunocompetent adults under the age of 65, but active and passive cigarette smoke significantly increases the risk of invasive pneumococcal pneumonia for this group (Pastor *et al.*, 1998; Nuorti *et al.*, 2000). Epidemiological studies have also demonstrated an increased risk of tuberculosis infection among smokers (Buskin *et al.*, 1994). Given the complexity of the defenses against infection and the immune responses to control and resolve infection, the mechanisms responsible for the effects of cigarette smoke on the susceptibility and course of pulmonary infections remain unclear.

Problematic bacteria in COPD with a focus on *Pseudomonas aeruginosa*

Bacteria are typically considered the main cause of highly problematic exacerbations. The majority of exacerbations are mediated by persistent or acute bacterial infections and also viral infections. Of the most common pathogenic bacteria associated with COPD include *Haemophilus influenza*, *Streptococcus pneumonia*, and *Moraxella catarrhalis* and *Haemophilus parainfluenzae* and *Staphylococcus aureus*, albeit are far lower frequencies. COPD bacterial flora can be

extremely variable. However, one of the most harmful bacteria detected in patients with excessively severe airflow obstruction in stable COPD and during exacerbations in *Pseudomonas aeruginosa* (PA). In patients with COPD, PA infection often precipitates hospitalization, is associated with increased mortality, and appears to occurr more frequently (Rosell *et al.*, 2005; Almagro *et al.*, 2012). Culture of respiratory secretions yield PA in 8.5–16.8% of patients with COPD exacerbations caused by identifiable bacterial infections and may reach 18% in patients requiring mechanical ventilation (Roche, 2007). At the Cincinnati VAMC, approximately 10% of respiratory cultures grew PA in 2008 (Donna Oblack, Ph.D., VAMC, personal communication). Patients receiving oral corticosteroid therapy, with more severe COPD, lower BODE score, or prior hospitalizations for COPD exacerbations are more likely to be infected by PA (Larsen *et al.*, 2009). PA isolation during hospitalization for COPD exacerbation is associated with a 2.2 fold increased risk of mortality (Almagro *et al.*, 2012). PA causes two distinct patterns of carriage within the airway: (1) short-term colonization with exacerbation followed by clearance and (2) long-term persistence that is associated with mucoid strains that the corresponding author has been studying for ~22 years (Hassett *et al.*, 1993). In longitudinal studies of 126 patients with COPD, PA was isolated one or more times in 39 patients (30%), with 57 episodes of new strain acquisition (Murphy *et al.*, 2008). Acquisition of a new PA strain increased the relative risk for COPD exacerbation by 3.36 fold and 43% of new strain acquisitions occurred contemporaneously with COPD exacerbations (Murphy *et al.*, 2008). Approximately 2/3 of new PA acquisitions are cleared, usually within 1 month, and 1/3 persist for months to years. The PA and host characteristics that lead to clearance or colonization are not known but may involve strain factors including mucoidy, biofilm formation, and other virulence factors and host characteristics including physiologic impairment and innate and adaptive immune responses (Parameswaran and Sethi, 2012). Persistent infections are usually caused by a single clone that diversifies and evolves with an increased mutation rate of the *mucA* gene (Martin *et al.*, 1993; DeVries and Ohman, 1994), increased antibiotic resistance, reduced protease production, less motile and greater biofilm production, more likely mucoid, microevolution of accessory genome, and less cytotoxin (ExoU) production. Even beyond COPD infections, PA is also a significant ventilator-associated pathogen and its infectivity may be increased in patients with bronchiectasis (Gursel and Demirtas, 2006). PA infection is hypothesized to be a causal factor for the development of bronchiectasis in patients with COPD (Parameswaran and Sethi, 2012). PA causes at least 20% of community-acquired pneumonias in veterans with spinal cord injury and disorders (Chang *et al.*, 2005). Respiratory infection with multi-drug resistant PA (MDRPA) is associated with a 6.2-fold increased mortality (Montero *et al.*, 2009). Among the risk factors for therapeutic failure in MDRPA respiratory infection are smoking (Table 3), prior PA infection, and above all, COPD (Montero *et al.*, 2009). The attributable mortality due to PA ventilator-associated pneumonia approaches 40%. By current estimates, there are 914 times more COPD patients (~64 million) vs. 70 thousand CF patients world-wide. Far more is known about the genes/gene products of PA that are retained or mutated throughout the various stages of CF lung disease (Ciofu *et al.*, 2010). In fact, when we PUBMED-searched the words "aeruginosa", "biofilm" and "COPD", we found only 10 hits, while when we substituted "cystic fibrosis" for COPD, there are 415 hits. Further, COPD (from 2006 data) was only 0.6% of the NIH budget, ranking 26th amongst diseases. Furthermore, Martinez-Solano *et al.* (2008) has shown that the overall "pattern of infection" is similar evolutionary during both COPD and CF and that treatment outcomes from CF patient data may help better understand and implement novel treatment strategies for COPD as well. Understanding the fundamental microbiologic mechanisms of PA infectivity and colonization are essential for the development of effective therapies (such as acidified NO_2^-, that have already undergone animal and human Phase I studies) to reduce the deleterious complications and mortality associated with PA respiratory infections.

There are other organisms detected less frequently yet their significance in overall COPD severity is debated (Table 5).

What are biofilms and are there PA biofilms in COPD patients?

The classical definition of one form of biofilm (e.g., Type I) is microbial growth on any substratum. In the case of the, VA patients infected with PA, these bacteria can attach to and form robust biofilms on substrata including bones, organs, arteries/veins, skin, catheters, prostheses and ventilators (e.g., in VAP). A model of biofilm formation on biotic/

Table 5. The respiratory microbiome in health, in stable COPD and exacerbated COPD (from Beasley *et al.* (2012))

Healthy individuals	Stable COPD (mild to moderate)	Stable COPD (moderate to severe)	Exacerbated COPD
Staphylococcus epidermidis	*Haemophilus influenza*	*Haemophilus influenza*	*Moraxella catarrhalis*
Cornyebacteria	*Streptococcus pneumonia*	*Streptococcus pneumonia*	*Streptococcus pneumonia*
Staphylococcus aureus	*Moraxella catarrhalis*	*Moraxella catarrhalis*	*Haemophilus influenza*
Non-hemolytic streptococci	*Haemophilus parainfluenzae*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Neisseria spp.	*Staphylococcus aureus*	*Haemophilus parainfluenzae*	*Haemophilus parainfluenzae*
Streptococcus pneumonia (occasional)		*Staphylococcus aureus*	
Haemophilus influenza (occasional)			
Prevotella spp.			
Fusobacteria			
Veillonella			

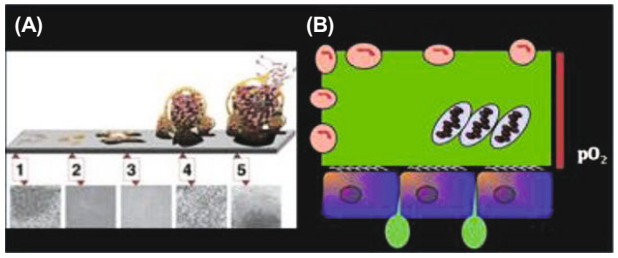

Fig. 4. PA biofilms. (A) Model of the steps in the formation of a PA Type I biofilm on abiotic surfaces (from Wikipedia). 1, Free-swimming (planktonic bacteria attachment to surface); 2, Cell growth and development or a preliminary matrix; 3, Cell division and accumulation of EPS, leading to the development of microcolonies; 4, Macrocolony development and the characteristic mushroom-shaped biofilm structures; 5, Dispersion, where bacteria synthesizes a new flagellum and migrates to new colonization sites. Please refer to several recent reviews on this subject (Hassett *et al.*, 2010; Su and Hassett, 2012). (B) Cartoon of CF (or Type II) biofilm where the bacterial (as elongated "soccerball"-shaped) structures are enmeshed within the thick CF airway mucus.

abiotic surfaces using either static or flow-through *in vitro* systems. Current dogma indicates that there are 5 developmental stages of Type I biofilms (Fig. 4A), a process requiring an ever-burgeoning number of genes (Hassett *et al.*, 2010). First, planktonic bacteria must attach (step 1). Cell growth ensues in step 2 where bacteria remain surface-bound. With cell division and exopolysaccharide (EPS) biosynthesis (step 3), leading to formation of a matrix between cells, the bacteria can form what are commonly termed "microcolonies." As the microcolonies mature (often into "macrocolonies") into a thickening biofilm (step 4), an oxygen gradient forms, where oxygen levels are highest at the top of the biofilm and lowest at the substratum. As the biofilm develops and/or senses gradients of either attractants or repellants, single cells or groups of cells can undergo a poorly understood process know as *dispersion* (step 5), and proceed to form new biofilms at destinations propelled by bacterial chemotaxis sensing systems. This appears to be an active process that involves sensing of some form of gradient (e.g., oxygen, nutrient, redox, c-di-GMP levels) where organisms activate flagellar gene transcription to propel themselves to seemingly new and inviting confines. Type II biofilms, where bacteria are enmeshed in soccerball shaped encasements are known to exist in CF, and likely COPD. However, we could find no with electron micrographs of COPD thin sections describing Type II biofilms in COPD. We have ~100 ml COPD sputum donated blindly from a PA-infected patient that has had COPD for >15 years. Shown in Fig. 5A is mucoid PA from his/her sputum and in Fig. 5B is a clear example of the soccer ball or macrocolony shaped biofilms, similar to CF biofilm, in the COPD sputum. Thus, the nature and behavior of PA in biofilms in COPD is remarkably underappreciated at present, and we intend on illuminating information gathered in this study of PA biofilm behavior in COPD sputum.

PA can grow both aerobically and anaerobically

We have been researching the anaerobic biofilm mode of growth in PA (Su and Hassett, 2012). PA is capable of robust growth under both aerobic and, to a lesser extent, anaerobic conditions. Aerobic respiration obviously results in oxygen consumption and water production with resultant ATP production. Anaerobic growth is either via respiration, using an inorganic nitrogen oxide terminal electron acceptor, or exceedingly slowly by substrate level phosphorylation using arginine. Anaerobic respiration, which represents ~2/3 of the ATP production using NO_3^- as terminal electron acceptor. However, NO_2^-, or nitrous oxide (N_2O) have also been used as alternative terminal electron acceptors. The reduction of NO_3^- occurs either through an assimilatory pathway, where NO_3^- is reduced to ammonia, or by a dissimilatory pathway, where NO_3^- is reduced to N_2. Assimilation occurs under both aerobic and anaerobic conditions while dissimilatory NO_3^- reduction occurs only under anaerobic conditions and involves the sequential reduction of NO_3^- to NO_2^- to nitric oxide (NO) to N_2O to N_2. Operonic loci important in the first step involving reduction of NO_3^- to NO_2^- are termed the *nar* (nitrate reduction) genes. Those involved in reduction of NO_2^- to NO are termed *nir* (nitrite reductase). Those involved in removal of NO, *nor* (nitric oxide reductase), and finally those involved in removal of N_2O, *nos* (nitrous oxide reductase). Because of the importance of the *nar*, *nir*, *nor*, and *nos* loci in anaerobic survival during the denitrification process, it is not surprising that such genes are localized in tightly regulated operons peppered throughout the genome (www.pseudomonas.com).

Finally, a simplified model of how dissimilatory NO_3^- reduction works and the localization of enzymes required for this process is described in the following sentences. Once the multi-protein, membrane-bound NO_3^- reductase (NAR) reduces NO_3^- to NO_2^-, NO_2^- is quickly extruded from the cell by the $NarK_2$ channel, due to the inherent toxicity of NO_2^-. Within the periplasm, NO_2^- reductase (NIR) reduces NO_2^- to NO. A membrane-bound nitric oxide reductase (NOR) reduces NO to N_2O and a periplasmic N_2O reductase (N_2OR) reduces N_2O to N_2. In addition, although there is also a periplasmic NO_2^- reductase, NAP, it is involved in assimilatory nitrate reduction and not essential for anaerobic growth. The clinical relevance to COPD is the fact that many strict anaerobic bacteria have been isolated from patients with chronic COPD exacerbations (Brook and Frazier, 2003).

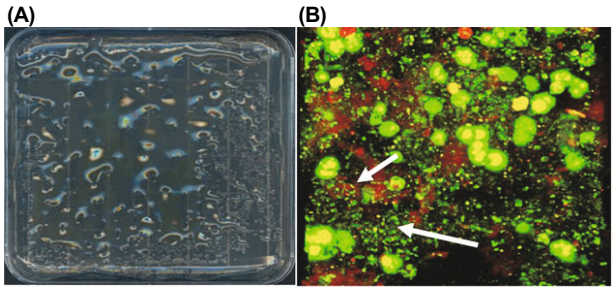

Fig. 5. Mucoid PA on LB plate (A) and macrocolony biofilms derived from COPD sputum (B). White arrows indicate PA macrocolonies and the large green cells are human cells.

Correlation between mucoid subpopulations and microaerobic or anaerobic biofilm modes of growth

A unique feature of PA strains infecting COPD, CF, chronic bronchiectasis (CB), and ventilator-associated pneumonia patients is their mucoid colony morphology, a result of alginate production. The role of alginate in pathogenesis is still unclear but it mainly appears to be a "hiding" process. Mucoidy does offer PA several advantages *in vivo* (i) antibiotic resistance, (ii) resistance to opsonic phagocytosis, (iii) ability to inhibit PMN chemotaxis, (iv) degeneration of pulmonary function, (v) mechanism of adherence and (vi) an inability to activate IL-8 because mucoid bacteria do not possess flagella because the *fliC* gene is repressed by AlgT(U) (Garrett *et al.*, 1999). Other studies have shown that alginate is capable of scavenging macrophage- and enzymatically-generated oxygen free radicals (Simpson *et al.*, 1989) and metals including iron, zinc and manganese (Gacesa, 1988). Mucoid PA is isolated more commonly from chronically colonized COPD patients than from those who are transiently infected (Murphy *et al.*, 2008). Thus, with chronic PA colonization and progression of COPD airway disease, patients may develop (i) persistent airway mucoid PA populations and (ii) respiratory failure that is characterized by hypoxemia and/or hypercarbia. We postulated that alginate production is a biomarker for the microaerobic/anaerobic biofilm mode of growth by PA in the thick COPD airway mucus and there is excellent evidence for such a hypothesis (Worlitzsch *et al.*, 2002; Yoon *et al.*, 2002). Nearly 17 years ago, the corresponding author observed some fascinating connections between *in vitro* grown organisms and the maintenance of mucoidy and these likely play a significant role in how the organism behaves *in vivo* and why mucoidy persists. Highly oxygenated bacteria in shake flasks maintain 100% mucoid. Yet, those that are placed under static conditions acquired spontaneous *algT(U)* mutations (DeVries and Ohman, 1994) and nearly 100% reverted to their non-mucoid, antibiotic-susceptible counterparts within 4–5 days. This observation was later confirmed by Dan Wozniak's group (Ohio State Univ.) in collaboration with our group in 2002 (Wyckoff *et al.*, 2002). Strikingly, anaerobically grown organisms, whether by NO_3^- or NO_2^- respiration or arginine fermentation maintain >95% mucoidy (Hassett, 1996). Do anaerobic growth conditions favor maintenance of the mucoid phenotype, a phenotype that often reverts to the non-mucoid, antibiotic-susceptible form during semi-aerobic *in vitro* culture? This is an area of study that begs revisiting and a thorough understanding of the reversion process is highly warranted and could help find additional clues as to how to reverse mucoidy in COPD patients.

Use of acidified sodium nitrite (A-NO_2^-) for treatment of *mucA22* mutant mucoid *P. aeruginosa*: a novel means by which to kill several airway pathogens

Yoon *et al.* (2006) demonstrated that the mucoid *mucA22* clinicial isolate, FRD1, was exquisitely sensitive to A-NO_2^- at pH 5.5 and 6.5, but not 4.5 (killed mice). We also showed that the reduction of A-NO_2^- to nitric oxide was essential for the killing process. When we discovered this event, we found out that at least one mechanism underlying such sensitivity is a significant decrease in protective NIR and NOR activity in FRD1 bacteria. Yet, provision of the *mucA* gene *in trans* restored A-NO_2^- resistance in planktonic and biofilm bacteria. Animal toxicology (rat/dog) and human Phase I trials are very encouraging and a cohort of patient for Phase II trials is planned in the immediate future. Interestingly, NO significantly enhanced the overall efficacy of the anti-pseudomonas aminoglycoside tobramycin (Barraud *et al.*, 2006). In another study, by our group, Major *et al.* (2010) demonstrated that A-NO_2^- killed not only PA but the *Staphylococcus aureus* and *Burkholderia cepacia*.

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