Review Article

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Immunological Features of Chronic Obstructive Pulmonary Disease (COPD) Induced by Indoor Pollution and Cigarette Smoke

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Etiology, prevalence and types

COPD is a major cause of mortality and morbidity worldwide and poses an increasing global healthcare problem (1). The definition of COPD recognises the "abnormal", exaggerated or amplified inflammatory response in the lung and systemically to cigarette smoking and noxious pollutions (2). The pattern of inflammation involves recruitment of lymphocytes, macrophages and neutrophils, as well as activation and damage to structural cells following the release of inflammatory chemokines and cytokines (2–5). In the Western world, the major driver of disease is cigarette smoke (CS) which is a complex mixture of organic chemicals, heavy metals and reactive oxygen species (ROS) (6–11). Importantly, Sopori (12) highlighted that chronic inhalation of cigarette smoke can modulate both innate and adaptive immune responses. Moreover, it has been speculated that many of the health consequences of chronic cigarette smoking might be due to its adverse effects on the immune system (13). Many inflammatory cells and their mediators, both of the innate and adaptive immune system, participate in the inflammatory processing of COPD. Macrophages, neutrophils and CD8+ T cells are the cells usually considered the prime effector cells in pathogenesis of COPD (14), but recently DCs have been suggested to be a potentially important new player/orchestrator of the pattern of inflammation that characterizes COPD (15, 16).

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) and American Thoracic Society (ATS)/European Respiratory Society (ERS) COPD guidelines have defined COPD as a preventable and treatable disease characterized by airflow limitation that is partially reversible (17, 18). It is likely that CS-induced inflammation is responsible, at least in part, for this airflow limitation. Multiple intracellular signaling events occur by CS, which ultimately leads to the synthesis and release of

pro-inflammatory mediators, such as interleukin-8 (IL-8)/CXCL8, IL-1β, and tumor necrosis factor-α TNF-α) (19, 20). CXCL8 levels, for example, are markedly increased in induced sputum of patients with COPD and this increase correlates with the increased proportion of neutrophils (22-26).

Besides CXCL8 and other inflammatory cytokines and chemokines, there is evidence for enhanced presence of markers of oxidative stress in COPD including nitric oxide (NO) (27), hydrogen peroxide (27) and lipid peroxidation products (28, 29) in COPD patients. NO is generated in COPD from the enzyme inducible NO synthase (NOS2), which is expressed in macrophages and lung parenchyma of patients with COPD, particularly in patients with severe disease (30). In addition, there is increased expression of neuronal NOS (NOS1) in these patients (31). NO is markedly increased in exhaled breath of patients with mild asthma, reflecting the inflammatory process in the airways (31) but in patients with COPD exhaled NO levels are little raised above normal (32, 33) but are more clearly increased during exacerbations (32). This may reflect formation of nitrotyrosine adducts which are markedly increased in COPD (14).

COPD in Never Smokers

Since the 1950s tobacco smoking has been linked to COPD (17) and smoking has long been widely considered as the single most important risk factor for COPD. A great percentage of COPD mortality and morbidity in both genders can be attributed to cigarette smoking (14). Because of such well-association, a number of studies have concentrated on the role of smoking in COPD, focusing only on smokers, in particular, those with at least 20 pack-years of cigarette smoking exposure (34).

However, published data in recent years demonstrate a significant prevalence of COPD among never smokers. Increasing evidence suggests that non-smokers may account for between one fourth and one third of all COPD cases in contrast to the 50-70% whose COPD is smoking-related (37-40). This prevalence varies across nations in both developed and developing regions and mainly relates to exposure to indoor pollution (35). However, little is known about the pathological features and molecular mechanisms underlying this type of COPD in non-smokers (36).

Recognizing the etiology of generalized bronchopulmonary lesions in nonsmokers with an unknown history of previous exposure is a challenge to physicians and researchers. Indoor air pollution includes coal and biomass fuel combustion, as well as ETS, which is one of the major etiologies for non-cigarette smoking-induced COPD (41-44). Biomass fuels such as wood, charcoal, crops, twigs, dry grass and dung are widely used for cooking or heating in low-income countries (43). According to the World Health Organization WHO) estimation, approximately 50% of all households and 90% of rural households utilize biomass or coal fuels for cooking and heating worldwide (43). This suggests that about three billion people worldwide are exposed to smoke produced from biomass or coal fuel burning (43).

In China, approximately 60% of rural households use biomass fuel for cooking and 31% use coal fuel (44). A recent investigation of 13 urban and rural areas in China demonstrated that 44.6% and 73.2% of non-smokers were exposed to biomass and coal smoke respectively, and 40% had poor ventilation in the kitchen (45). This compares with the first study detailing the bronchopulmonary characteristics of 10 Iranian women who were exposed to the indoor smoke published by Amoli in 1998 (46).

There may be some variability in the presentation of COPD in patients who are never smokers but exposed to biomass smoke. In a large epidemiological study in China the prevalence of COPD in non-smokers was 5.2%. Exposure to biomass smoke and the presence of poor ventilation in the kitchen were independently associated with a higher risk of COPD among non-smokers. Interestingly, non-smokers with COPD were less likely to present with chronic productive coughs and lower BMI, while more likely to have received a physician diagnosis of asthma and respiratory diseases in childhood, than

smokers with COPD and may therefore have a distinct profile from that of smokers with COPD (47).

Overall, females older than 55, with a previous history of a respiratory disease and without expectoration or wheezing predominate in populations characterized as having COPD despite being never smokers (48). Furthermore, charcoal workers exposed to wood smoke have increased respiratory symptoms and decreased pulmonary function (49).

A Brazilian study of 1402 subjects has reported that the amount of particulate matter less than 2.5µm in diameter (PM2.5), whether from indoor or outdoor biomass fuel, was associated with worse lung function, greater respiratory symptoms and the development of COPD. These effects were associated with the duration and magnitude of biomass exposure and were exacerbated by tobacco smoke. This was not seen in individuals from the same community exposed to liquefied petroleum gas (50).

In a similar manner, Turkish women from rural areas exposed to biomass fumes were more likely to suffer from chronic bronchitis and COPD than women from urban areas even though the incidence of cigarette smoking was much greater in the urban population (51). Furthermore, in a group of 561 females from Isfahan in Iran, age, childhood pulmonary infection, bread baking, carpet weaving and use of biomass fuels were all significant risk factors for chronic bronchitis with a reduced risk if using kerosene or gas. Only 7 women were current or ex-smokers (52). Importantly, the concentration of respirable particulate matter was up to 4-fold more concentrated indoors than outdoors. This confirmed the earlier data from Amoli in 1998 (46).

It has been suggested that the majority of serious effects on morbidity and mortality related to air pollution occur via interactions with respiratory infection (53). However, the mechanisms underlying the relationship between infection and the development of lower airway symptoms after air pollution exposure are not fully understood. Oxidant pollutant exposures have the potential to exacerbate the inflammatory effects of virus infections in the lower airway, especially in individuals with pre-existing lung disease (53).

The prevalence of respiratory illnesses and symptoms was considerably higher in mud and brick houses when compared with concrete houses, and higher in those living on hills and in rural areas when compared with flatland and urban areas. Regalado et al.(54) reported that women who used a stove burning biomass fuel in Solis, close to Mexico City, showed moderate airflow obstruction with COPD at stage GOLD II,. In addition, Orozco-Levi et al. (55) reported that most of their study population of non-smoking women with COPD in Barcelona Spain) between 2000 and 2003 were exposed to wood and charcoal smoke during their childhood and youth, but remained free of exposure for more than 25 years prior to presenting with symptoms of the disease. The risk of developing COPD was greatest if subjects were exposed to both wood and charcoal (54).

Furthermore, the incidence of non-smoking COPD (GOLD stage 2) in Northern Sweden reached 7% in almost 2000 subjects studied and was associated with increasing age but not sex or exposure to environmental tobacco smoke. Of those subjects with airway obstruction as defined by GOLD, 14% of men and 27% of women had never smoked (56). The authors did not report on biomass or wood smoke exposure in these subjects. Although use of biomass fuel for cooking is not common in Western Europe, exposure to air pollutants in workplaces such as farming, coal mining, construction, gold mining, plastic, textile, rubber, leather manufacturing, manufacture of food products and automotive repair have been shown to be important and may account for the incidence of COPD in non-smokers in the UK and other parts of Europe (57).

Is the immunological profile of COPD due to indoor pollution the same as that due to cigarette smoke?

The immune cells involved in COPD development and progression have been summarized in several excellent reviews (58, 59). Innate immune cells such as epithelial

cells and macrophages are activated by cigarette smoke, either directly or indirectly through pathogen-associated molecular patterns (PAMPs), following binding to pattern recognition receptors such as Toll-like receptors. The adaptive immune system is also activated in response to cigarette smoke and involves stimulation of specific T helper subsets such as Th1 and Th17 CD4+ T cells, cytotoxic CD8+ cells and enhanced B-cell responses. The persistent inflammatory insult from continued smoking leads to the development of lymphoid follicles. More recently, the role of activated dendritic cells in this process has become clear (59).

Cosio and colleagues have defined a 3 step process by which the cigarette smoke-activated immune system produces the classic pathological symptoms of COPD (58). In the initial innate immune response, epithelial cells which are damaged by cigarette smoke release a number of danger signals (PAMPs) that can result in the enhanced expression of chemokines and cytokines including CXCL8, IL-1β, TNFα, CXCL10 and GM-CSF. Damaged epithelial cells can also release proteases such as elastin and MMPs, growth factors and other matrix modifying enzymes that can further the release of TLR ligands providing a feed-forward inflammatory drive and enhanced tissue injury and small airway remodelling. During this process, dendritic cells that have processes that interdigitate throughout the epithelial barrier, are activated and they migrate to local lymph nodes where they are able to activate T cell proliferation. Production of TLR ligands within the airways also leads to direct stimulation of dendritic cells resulting in enhanced expression of the cell surface markers CD80 and CD86 and a local inflammatory environment conducive to T cell antigen presentation and proliferation of Th1, Th17 and cytolytic CD8+ T cells. The role of Treg cells and γδ CD8+ T cells in limiting this progression can be overcome by the presence of IL-6 which is secreted from activated dendritic cells.

In more severe disease, tolerance is lost and an adaptive immune response develops in the lung. This is linked to the additional activation of IgG-producing B cells and the presence of increased oxidative and nitrosative stress and proteinases leading to the classical pathological features of COPD namely cell necrosis and apoptosis, immune and complement deposition, tissue injury with airway remodeling and emphysema.

To date, there is little available data on the immunologic response to indoor smoke in the literature and thus investigation on this field could help to understand the pathogenesis of COPD with various etiologies. One study has reported a pathological examination of wood smoke-associated lung disease WSLD) and compared this to pathological features of smokers with COPD (60). In Mexico, patients with WSLD were non-smoking women who used wood for cooking for a median of 45 years. Dyspnea, airway obstruction, air trapping, increased airway resistance, pathological evidence of anthracosis, chronic bronchitis, centrilobular emphysema and pulmonary hypertension were present in most patients with WSLD. Importantly, there were no significant differences in the histopathological findings for emphysema, goblet cell hyperplasia, bronchial wall inflammation, airway smooth muscle hyperplasia, bronchiolitis or aspects of remodelling between patients with WSLD and smokers with COPD.

It is evident that the effect of wood smoke exposure on lung health requires long-term exposure. Acute exposure to wood smoke at a concentration normally found in a residential area with a high density of burning wood stoves causes only a mild transitory inflammatory response as determined by fractional exhaled nitric oxide (FENO), exhaled breath condensate (EBC) and nasal lavage (61).

The innate immune response of cigarette smokers and subjects exposed to other environmental pollutants such as organic matter may also be similar. Although not studied in relation to indoor biomass fuels, the response of subjects to inhaled LPS and of cells to in vitro challenge to LPS is similar between pig farmers who are constantly exposed to high levels of pathogen-associated molecular patterns (PAMPs) and cigarette smokers (62) LPS challenge in vivo

had no effect on markers of systemic inflammation including the expression of Th2 cytokines and Toll-like receptors TLR) in peripheral blood cells in smokers or farmers compared with the marked effects seen in control subjects.

Animal models of wood smoke exposure also support a similar pathology to cigarette smoke-induced emphysema (63). Long term exposure (up to 7 months) of guinea pigs to pine wood smoke showed alveolar mononuclear phagocyte and lymphocytic peribronchiolar inflammation, epithelial and smooth muscle hyperplasia, and pulmonary arterial hypertension. Mild to moderate emphysematous lesions were observed in wood smoke-exposed animals after 4 months. A higher percentage of whole blood carboxyhemoglobin (COHb) and elastolytic activity in bronchoalveolar lavage macrophages and lung tissue homogenates was also observed. Increased collagen breakdown coincided with emphysematous changes as a result of enhanced MMP-2 and MMP-9 activity. Emphysema also correlated with enhanced apoptosis supporting a role for MMPs and apoptosis in emphysema secondary to wood smoke exposure. Wood smoke extract can also directly induce apoptosis of human lung endothelial cells through an oxidative stress-mediated mechanism (64).

Exposure of rats to wood smoke caused bronchiolitis, hyperplasia and hypertrophy of bronchiolar epithelial lining cells, edema, hyperplasia of lymphoid follicles, peribronchiolar and perivascular infiltration of polymorphonuclear cells, and mild emphysema after 15 days. All signs apart from emphysema got progressively worse with continued exposure (65). In addition, exposure of allergic rats to 2.5 months of low-levels of wood smoke exacerbated the inflammatory responses to ovalbumin in allergic rats (66). However, not all animal models of wood smoke exposure were able to demonstrate COPD-like responses (67).

There may also be a link between indoor pollution and COPD due to cigarette smoking in that the fine particles produced by biomass fuels may exacerbate or enhance the immune response to cigarette smoking (68). Indeed, exposure to biomass fuel smoke in a controlled indoor setting led to an altered response in COPD patients compared to control subjects. At baseline, COPD patients had more CD14+ monocytes and neutrophils than healthy controls, but fewer CD3+ T cells and an altered gene expression profile in PBMCs (57/186 genes) with the majority being down-regulated in COPD. In contrast, genes such as NF-κB1, TIMP-1, TIMP-2 and Duffy were up-regulated in COPD subjects. After 4 hours exposure to biomass fuel smoke, monocyte levels decreased in the healthy subjects but not in the COPD subjects. In addition, genes relating to immune and inflammatory responses and cell-cell signalling were differentially affected in the PBMCs of COPD subjects.

Autoimmunity and emphysema

The description of increased B-cell follicles in more advanced COPD (69) emphasized the possible importance of an autoimmune component in COPD pathology (27,59). Autoimmune diseases are characterised by circulating antinuclear antibodies (ANA) and these are found at greater levels in the serum of 25-30% of COPD patients (70, 71). Initial reports also described that auto-antibodies against elastin (72) have not been consistently reproduced (73) although autoantibodies against other matrix components of the airway such as collagen V have been reported (74, 75).

Serum autoantibodies against bronchial epithelial cells along with corresponding IgG and complement (C3) deposition (76) have also been observed in COPD lung. In addition, smoking is associated with high levels of class-switched memory B-cells IgG versus IgA in healthy controls), blood and IgG memory B-cells in the lung. There was also a greater number of anti-decorin antibody-producing cells in COPD patients compared with healthy controls (77). More recently, Packard and colleagues using an autoantigen array demonstrated that COPD patients express autoantibodies against a wide variety of self-antigens which correlate with disease phenotype and

particularly with emphysema (78). Importantly, emphysematous patients produced autoantibodies of both higher titre and reactivity than those of control subjects.

One possibility to account for these differences is that the autoantibodies are mainly directed against oxidative stress-modified self-proteins and that epitope spreading occurs to allow variable detection of autoantibodies against unmodified protein (27). This study also reported autoantibodies against endothelial cells and deposition of activated complement in the vessels of COPD lung (27). This report confirmed earlier evidence for the presence of anti-endothelial autoantibodies in patients with COPD (79). An alternative explanation for the failure to consistently show elevated serum levels of anti-eleastin antibodies may be due to the fact that these antibodies are more easily detected as elevated in bronchoalveolar lavage fluid than in plasma (80).

Ozone-exposed mice also exhibited increased antibody titres to carbonyl-modified protein, as well as activated antigen-presenting cells in lung tissue and splenocytes sensitized to activation by carbonyl-modified protein lung (27). Similarly, cigarette smoke exposure in mice gives rise to the production of a humoral response against elastin, collagen and decorin proteins (81). This autoimmune response was also accompanied by macrophage influx into the airway. To date there are no studies that examine the expression of autoantibodies in either non-smoking COPD patients or in response to wood smoke exposure. This is an area that needs additional research.

Can a similar response to bronchodilators be expected in COPD patients whether due to cigarette smoke or indoor pollution?

Patients with COPD are still commonly thought to show diminished acute bronchodilator responsiveness compared to asthmatics, and reversibility testing is sometimes proposed as a method of discriminating between asthma and COPD, despite previous evidence to the contrary (82).

Therapeutic agents prevent and control symptoms, reduce exacerbations, increase exercise tolerance, and improve health status (83, 84). Long-acting β2-adrenergic agonists (LABAs, such as salmeterol) combine symptom control with improvement in lung function and provide clinically relevant improvements in health status. Inhaled corticosteroids (ICS) are recommended for the treatment of patients with a more severe disease and frequent exacerbations, and inhalation of the combination of LABAs and ICS is more effective in improving lung function and symptoms and reducing exacerbations than either drug alone (85, 86). Moreover, recently, it has been demonstrated that LABAs can enhance the anti-inflammatory action of GCs.

The acute bronchodilator responsiveness in patients with COPD has not been characterized rigorously in large cohorts. This is because determination of the response to a bronchodilator is influenced by physiological and methodological factors, including differences in baseline degree of airflow obstruction, diurnal and day-to-day variability in bronchomotor tone, dose and class of inhaled bronchodilator therapy, method of bronchodilator administration e.g. metered-dose inhaler with or without a spacer or solution nebuliser), dose of bronchodilator, and timing of post-bronchodilator spirometry. These responses may also be confounded by the presence of specific mast cell subtypes which may alter the response to bronchodilators (94).

We and others have previously described the potential involvement of mast cells in the pathogenesis of lung diseases including COPD (87-89). Mast cells are thought to contribute to bronchoconstriction, mucus secretion, mucosal edema, bronchial hyperreactivity (BHR), inflammation, angiogenesis and airway remodelling in asthma (90-93). In particular, an increase in the number of airway smooth muscle (ASM) layer mast cells has been suggested to be related to asthmatic BHR (94). Bronchodilator responsiveness (BDR) has been shown to be related to BHR (95) and is probably based on similar underlying mechanisms. In addition, we demonstrated in

an in vitro study that cigarette smoke medium CSM) stimulated the release of chemokines from mast cells in a noncytotoxic manner but did not induce mast cell degranulation (88).

It is generally held that, by definition, airway obstruction in COPD is irreversible. However, significant BDR is in fact present in a large subgroup of patients with COPD, although they are mainly screened out from therapeutic studies (96, 97,). Some investigators have suggested that this BDR feature in COPD is related to "asthma-like" pathology, **i.e.** an overlap syndrome (98, 99)). A substantial number of COPD subjects have been shown to have BHR (100, 101) with a significant correlation between BDR and BHR (102). Recently, it has been shown that in COPD subjects without BDR, there was a positive relationship between mast cell density and better airway function (89). In this regard we were not able to rule out the role of eosinophils in BDR reaction since it has been shown that high sputum eosinophil count did identify a subgroup of patients with COPD who respond to inhaled corticosteroids in terms of lung function.

In summary, despite the heterogeneity across the selected studies, exposure to solid fuel smoke is consistently associated with COPD and chronic bronchitis. Women using biomass fuel for cooking typically spend about 40,000 hours and inhale a total volume of 25 million litres of polluted air during their lifetime (57). A recent meta-analysis has reported that the odds of COPD in biomass smoke exposed individuals is of a similar magnitude to that reported between tobacco smoking and COPD (57). The limited evidence available to date suggests that the pathology of biomass-induced and cigarette smoke-induced COPD are the same. This suggests that the prognosis and response to inhaled β_2-agonists and combination therapy should be similar in these groups of patients. We await the development of better anti-inflammatory treatments for COPD in order to prevent the inexorable progression of disease seen in these patients.

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