Few smokers develop COPD. Why?

N. M. SIAFAKAS AND E. G. TZORTZAKI

Department of Thoracic Medicine, University of Crete, Medical School, Heraklion, Crete, Greece

Abstract COPD is a common disease and its major risk factor, cigarette smoking, has been identified. However, only a minority of smokers develop clinically relevant disease. Although, the current understanding of the pathogenesis includes an 'abnormal inflammation' as a response to various noxious agents, its various pathways are not clear. Oxidative stress, inflammation, tissue damage and tissue repair (remodeling) are parts of the complex procedure leading to COPD. This is a review of the available literature concerning the “susceptible” smoker. An epidemiological model is discussed, putting emphasis on the timing of the exposure to cigarette smoke. There are evidences that respiratory adenoviral infection in early life could be also an important factor. Differences in nutrition could also play a role in protecting against the oxidative stress. Airway hyperresponsiveness failed to clarify the whole picture and is still open for debate. Genetic differences are the most likely explanations to describe the ‘susceptible’ smoker. However, the only well-established genetic risk factor is the alpha-1-antitrypsin. Other candidate genes were reviewed, alpha-1-antichymotrypsin, blood group antigens, vitamin-D binding protein, a2-macroglobulin, immunoglobulin deficiency, extracellular superoxide dismutase, secretory leukocyte proteinase inhibitor, cathepsin G, tumor necrosis factor-a gene and others. Microsatellite DNA instability in COPD could be a useful tool to identify the locus of genetic alterations leading to COPD. Thus, in addition to exposure to exogenous factors, host factors, most likely several genes, are involved and affect various pathways of the pathogenesis of COPD.

INTRODUCTION

It is unclear why only some smokers develop a clinically significant chronic obstructive pulmonary disease (COPD) (1–3). From a number of epidemiological studies, it has become apparent that there are “susceptible” smokers that will develop COPD (1,3). Early pioneering and influential findings by Fletcher showed that lung function falls gradually over a lifetime, but in most non-smokers and many smokers clinically significant airflow obstruction never develops. In contrast, in “susceptible” people, smoking causes irreversible obstructive changes (1).

However, the characteristics of such a susceptible individual are not known (4). The questions “is there a distinct group of susceptible smokers?” and “what is the distribution (bimodal or unimodal) of susceptible” are extremely difficult to be answered on the basis of the current scientific knowledge.

According to a new definition developed by the GOLD expert panel (5), COPD is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and results from an abnormal inflammatory response of the lungs to noxious particles and gases.

It is likely that the answer to why only few smokers develop COPD is within the phrase “abnormal inflammatory response to noxious agents” of the definition. COPD is the result of an environmental insult and the response of the host that is primarily genetically predetermined.

Another mystery concerning the pathogenesis of COPD is why some patients develop predominantly parenchymal (emphysema) and other predominantly airways disease (chronic bronchitis). This brings up the possibility of sub-groups of “susceptible” individuals, some with primarily defects at the level of the major airways and others at the level of parenchyma. Are these defects genetically determined? (4).

In an effort to review the current literature, a brief summary of the pathogenesis of the disease will be presented first. Then, the environmental insults (noxious agents) will be discussed followed by the genetic risk factors.

PATHOGENESIS OF COPD: A SYNOPSIS

Figure 1 is a schematic summary of the pathogenesis of COPD. It is obvious that this scheme has several
limitations. A number of information come from experimental animal studies and those from humans are limited by the number of subjects, the selection of the patients, the tissue and the method studied. Thus, there are a number of missing links in the pathogenesis of COPD as presented in Fig. 1.

**Oxidative stress**

Cigarette smoke, the major environmental noxious agent, contains abundant amounts of oxygen-based free radicals, peroxides and peroxynitrite which results in severe oxidative stress in the lungs (6–9). The above substances by oxidizing cellular proteins, lipids, DNA bases, enzymes and extracellular components as matrix collagen and hyaluronic acid cause airway and parenchymal injury (10,11).

One of the consequences of the oxidative stress is chemotaxis, potent leukocyte adhesion and thus, initiation of inflammation. The recruitment of inflammatory cells such as activated macrophages and neutrophils may also contribute to the oxidization by releasing specific enzymes (10–12).

Thus, cigarette smoke and local release of oxidants initiate a vicious cycle that may promote an “abnormal” inflammatory response. For example, oxidants activate the transcription of the nuclear factor-kB (NF-kB), which promote genes of key inflammatory players, such as IL-8 and TNF-a (13,14). In addition, oxidants could oxidize antiproteinases resulting in a reduction of the antiproteinases shield and by activating matrix metalloproteinases to cause proteolysis (11,15). Oxidative injury causes impairment in the barrier function of endothelial and epithelial cells (16,17). Finally, if the oxidative stress is significant and prolonged, cells may undergo apoptosis or direct necrosis (18,19).

**Inflammation**

Many cells have been reported to be involved in the pathogenesis of COPD. However, their presence or activation in the affected tissues or into fluids, such as sputum or BAL, does not necessarily confirm their role in the process of the development of the disease. The number of macrophages is increased in COPD (20). Furthermore, cigarette smoke activates macrophages to release mediators, including IL-8, LTB4 and TNF-a (21). Thus, macrophages may orchestrate the inflammation in COPD.

Neutrophils are the most studied cells in COPD; however, their role is not yet clear (22–24). Neutrophils cause elastolysis by secreting neutrophil elastase, cathepsin G and proteinase 3 (25). In addition, neutrophil proteinases are mucus stimulants. Recruitment of neutrophils is the result of potent chemotaxis by IL-8, LTB4, and increased adherence (Mac-1, E-selectin) (26). Neutrophilic survival in the respiratory tract is increased in COPD by the increase of cytokines, such as GM-CSF. Although neutrophils are increased and/or activated in other diseases, such as cystic fibrosis, their elastolytic effect is not as prominent as in COPD. Thus, other factors may be involved in promoting the elastolytic activity of neutrophils in COPD (27).

T-lymphocytes are increased in lung parenchyma, and in both peripheral and central airways in COPD (28,30). Particularly, CD8⁺ cells are increased and may cause cytolysis and apoptosis of alveolar epithelial cells (29,30). Although there is an association of T-lymphocytes and

**Fig. 1.** Schematic representation of the pathogenesis of COPD as result of host exposure to environmental risk factors. Abnormal inflammation may play significant role in the pathogenesis (for more details, see text).
the amount of alveolar destruction and airflow limitation (28, 31) the role of T-cells in the pathogenesis of COPD is not yet certain.

The role of eosinophils in COPD is obscure. There are conflicting reports as far as their numbers in stable disease are concerned, but most reports have shown an increase during exacerbations (32–34). Furthermore, their interaction with neutrophils and their degranulation is under investigation. Recently, it has been shown that airway epithelial cells are important cells secreting inflammatory mediators. Cigarette smoke activates epithelial cells to produce TNF-α and IL-8, thus may initiate the abnormal inflammatory response (35). Many inflammatory mediators could be involved in the pathogenesis of COPD. Among them the best known are the lipid mediator LTB₄ (21, 33, 35–37), the chemokine IL-8 and the cytokine tumor necrosis factor-α (TNF-α). Other mediators that have been reported in COPD are IL-5, GM-CSF, TGF-β, EGF, the endothelin-1 (LT-1) and others (33, 38–40). The inflammatory response in COPD may be up-regulated by the above mediators.

Tissue damage

The best-studied mechanism of tissue damage in COPD is that of proteinase-antiproteinase (elastase/antielastase) imbalance. Proteinases are enzymes that degrade matrix proteins. Elastin is an important target, but collagen, proteoglycans, laminin and fibronectin are also degraded (41–43).

The most potent proteinases are the neutrophil elastase, cathepsin G and proteinase 3 and matrix metalloproteinases (44, 45). Neutrophils are the major provider of the above proteinases but other cells such as macrophages and airway epithelial cells could also contribute.

The elastolytic activity of proteinases is balanced by the antiproteinases, such as α1-antitrypsin. α1-antitrypsin is the major endogenous tissue antiproteinase (plasma/lung parenchyma) and SLPI is the major antiproteinase in the airways (41, 46). A logical follow-on from the imbalance in the proteinase/antiproteinase system in favor of proteinases (Proteinolysis) (47) is emphysema.

Tissue repair and “abnormal” remodeling

A tissue damage incidence is followed by an epithelial and parenchymal repair process. This repair procedure is extremely complex and so far not fully understood. In the airways, the repair (remodeling) process includes repair of the tight junctions, cell migration, cell differentiation and metaplasia, mitosis and hyperplasia of basal cells and mitotic redifferentiation among others (48). It has been shown that smoke impairs lung repair mechanisms (49, 50) and disrupts procedures that are able to restore tissue structure. This may lead to bronchial fibrosis and narrowing, particularly at the site of small airways. Fibronectin and TGF-β produced by the epithelial cells are involved in the normal repair processes but may be an excess of those factors that cause fibrosis and abnormal remodeling (39, 51).

RISK FACTORS

Risk factors for COPD include both environmental exposures and host factors, and the disease usually develops from an interaction between the two (5). Figure 2 provides a schematic classification of risk factors for COPD.

**RISK FACTORS**

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Environmental exposures

Cigarette smoke (epidemiological model of COPD)

Among the risk factors that have been related to COPD, cigarette smoke is the best studied and is a consistent finding in numerous studies (1,52,53). Thus, all recent guidelines of COPD considered cigarette smoking the best-established risk factor for the development of the disease (54,55). In addition, passive cigarette smoking was related to chronic cough and sputum and is also a candidate risk factor for the development of airflow limitation (53,56,57).

However, from the above epidemiological studies, it is apparent that not all smokers develop clinically important COPD, and second that there is not a direct dose–effect relationship. A passive smoker may develop the disease, whereas a heavy smoker may not. These observations lead to the hypothesis of the “susceptible” smoker.

The epidemiological “longitudinal” studies suggested that a more important factor than the dose (pack/years) is the timing of the exposure to cigarette smoke (58,59). This was summarized in an epidemiological model of COPD risk shown in Fig. 3. Although COPD is a disease of middle/late adult life, events that occur during early life may play a significant role. For example, active or passive smoking during adulthood when the lungs are developed may position the individual smoker to the lower part of the “horse race” and thus, making him “susceptible” to develop COPD (Fig. 3). Furthermore, an additional effect in the maturation of the respiratory system could be the maternal smoking during pregnancy [Fig. 3 (a)]. Finally, the third phase of adult life where the susceptible smoker is characterized by a rapid decline in FEV1 is well known [Fig. 3 (c)]. Thus, the timing of exposure to cigarette smoke is crucial and may have different and/or addictive effects. Cigarette smoke could operate before birth (lower initial lung volume), during growth (lower maximal attained volume), plateau phase (earlier start of decline) and during the late phase with an accelerated decline (1,60,61).

Noxious agents

A combination of exogenous risk factors could be an alternative hypothesis of the “susceptible” smokers. Exposure to a mixture of known noxious agents, such as active plus passive smoking, and environmental pollution and occupational pollution could cause COPD. However, the current data do not support the hypothesis of combination of risk factor as the basis of the “susceptible” smoker (3).

Respiratory infections

There are studies that suggest a link between severe childhood respiratory infections and COPD in adult life (62). However, this association is rather weak because it is not retrospectively easy to exclude the possibility that these infections are the result of lung function impairment and not the cause. Nevertheless, viral infections may directly contribute to the development of COPD by incorporating viral DNA into the airway cells. This could alter their genetic material and thus their response

Fig. 3. Epidemiological model of COPD during the life cycles. From top: during pregnancy (gray area); during lung growth (0–20 years) (gray area); during the plateau phase (20–40 years) (gray area); during lung function decline (40 years) (gray area). FEV1: forced expiratory volume in one second. Arrow (↑): Birth, (gray area): life cycle under discussion. [Modified from: Rijcken and Britton (3) with permission].

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to subsequent exposure to cigarette smoke. In fact, increased levels of adenoviral DNA have been found in COPD patient in comparison with the control ones (63). Furthermore, incorporation of adenoviral DNA in animal epithelial cells has been shown to amplify the inflammatory response, when exposed to cigarette smoke (64,65). Therefore, a possible scenario could be that “susceptible” smokers are the ones with an early-life viral infection leading to an excessive load of adenoviral DNA in the epithelial cells. Those cells then can orchestrate an “abnormal” inflammatory response to cigarette smoke. However, other investigators had not duplicated these results in human.

The British hypothesis (chronic mucus hypersecretion) supported that goblet cell hyperplasia in peripheral airways occurs in smokers (62,66) and therefore, predisposes them to respiratory infection. Recent results from the lung health study, in 5887 smokers, conducted by Kanner showed that smoking and lower respiratory illnesses (LRI) had an interactive effect on FEV1 in people with mild COPD while in smokers frequent respiratory infections may influence the long-term prognosis of the disease (67).

**Nutrition**

Nutritional elements with antioxidant properties could play a role in the development of COPD. Such dietary factors are the antioxidant vitamins C and E, magnesium and fish oils. In addition to the endogenous enzymatic antioxidant systems, the above nutritional antioxidants (vitamins C and E) may enhance the host defense against the oxidative stress of cigarette smoke. Fish oil contains highly polyunsaturated ω-3 fatty acids that act as competitive inhibitors of arachidonic acid metabolism. Thus, fish oil could down-regulate the inflammatory potency of lipid mediators, as LTB4, and protect against COPD (68,69). It is possible that smokers who develop COPD have a deficit of the above nutritional element in their diet. However, this hypothesis is not supported by longitudinal studies (70) and could not explain the whole problem, since there are so many confounding factors between diet and cigarette smoking (alcohol intake, etc).

**Host factors**

**Airway hyperresponsiveness (Dutch hypothesis)**

The relationship among increased airway reactivity, atopy and the development of COPD was first proposed by Orie et al. in 1961 (71). In other words, smokers with hyperreactive airways could be the “susceptible” ones that will develop COPD. This hypothesis is still open for debate, because it is not clear, if hyperresponsiveness is the cause or the effect of the decrease in FEV1 in smokers. Airways reactivity and atopy are complex disorders related to a number of genetic and environmental factors leading to allergic inflammation (asthma). This inflammation, however, has been shown recently to be different from that caused by cigarette smoke (72). In addition, other investigators suggested that hyperresponsiveness seen in smokers is the result of abnormal geometry of the airways (73,74). In addition, the majority of the studies investigating FEV1 decline have tested airways reactivity at the end of the study (after the initiation of smoking) (73,74) and only few at the beginning (75,76). Recently, two studies that reported association of hyperresponsiveness in sub-groups of smokers lacking statistical testing of the smoking strata (77,78). When the study was performed (79,80), the association of reactivity and smoking was not found significant.

Finally, another argument against the Dutch hypothesis is the observation that only approximately 12% of the patients with COPD showed increased responsiveness. If the Dutch hypothesis was completed, then hyperresponsiveness would be the feature of most of the COPD. In conclusion, the Dutch hypothesis failed to clarify the issue of ‘susceptible’ smoker and is still open for debate.

**Genes**

It is most likely that the answer to the mystery why only few smokers develop COPD is found in the ocean of genetics. Familiar aggregation has been reported in COPD (81,82) but other confounding factors are difficult to exclude. In addition, COPD is a disease of middle age and by that time parents or grandparents are rarely alive in order to perform a classical hereditary study. Furthermore, it is most likely that many genetic factors interact to increase or decrease the risk to develop COPD. Thus, Mendel’s laws of inheritance of “susceptibility” to cigarette smoke could be ruled out (83).

**Alpha-1-antitrypsin:** Until now, the only established genetic risk factor for COPD is homozygosity of al-antitrypsin (al-At) gene. The first study was performed by Laurell and Eriksson in the 1960s. Al-antitrypsin is a potent antiprotease produced by the liver. Homozygous Z patients have a very low al-AT and show rapid decline in FEV1, even without smoking (84,85). In smokers with al-AT homozygous, COPD is developed at a younger age (86,87). However, this homozygous state is rare in the general population (1 in 5000 live-births) (88) and therefore, genetic risk factor can explain less than 1% of the COPD.

Furthermore, numerous investigations have assessed the risk of heterozygous genotypes. The common gene variants are M, S and Z and their frequency is reported 0.93, 0.05, and 0.02, respectively. MM genotype is considered normal. MS show mild reduction in al-AT (~ 80% normal), MZ has lower al-AT (~ 60% normal), SZ even lower (~ 40% normal) and ZZ (< 15% normal). A large
number of studies have compared subjects with the MZ genotype to those with MM and found no significant difference in pulmonary function or symptoms in non-smokers (83). In smokers there are conflicting results. It was shown that MZ smokers had greater loss of elastic recoil than MM smokers (89) or rapid decline in FEV1 (90). Furthermore, in addition to the mutation that affect the level of serum a1-AT, other mutations have been described that affect its function (91). One of those is the mutation in the 3′ region of the a1-AT gene (92,93). Nevertheless, this mutation is not specific for COPD since it has been found in bronchiectasis as well (92,93). Other investigations had proposed that the 3′ mutation allele could be in disequilibrium with an al-antichymotrypsin (a1-ACT) deficiency allele (94). Other investigations suggested that the 3′ mutation could affect the acute-phase response leading to an inadequate up-regulation of a1-AT during acute inflammation (95). This could be also true during the acute oxidative stress of cigarette smoking. Thus, not only the level but also the structure and function of a1-AT is genetically predetermined and could predispose to COPD in smokers.

Candidate genes

Alpha-1-antichymotrypsin (a1-ACT) is acute-phase reactant with antiprotease properties produced by the liver. a1-ACT deficiency is present only in 1% of Swedish general population transmitted by the autosomal dominant inheritance pattern (96). Two mutations in the a1-ACT gene have been associated with decreased a1-ACT serum level (97), but the relationship of low a1-ACT or its defective function with COPD is not clear.

Vitamin-D-binding protein (VDBP) is a protein serrated by the liver that is able to vitamin D, endotoxin and to act as macrophage-activating factor or chemotactomer enhancer of C5a (98,99). Thus, it can regulate the inflammatory response or diminish antioxidative capacity of the host. A decrease frequency of 2-2 genotype of VDBP was reported in COPD patients (100) but this was not replicated (101).

Alpha2-macroglobulin (a2-macroglobulin) is a protease inhibitor and its serum deficiency is rare. A2-macroglobulin gene is located on chromosome 12 and its sequence has been identified. However, there are only case reports of patients with COPD and a2-macroglobulin polymorphism (102).

Immunoglobulin deficiency for selective IgA has been found to segregate with COPD in a three-generation pedigree (103). Other investigators studied the role of IgA or IgG deficiency as a etiologies of COPD in relation with recurrent infections (104,105).

Extracellular superoxide dismutase (EC-SOD) is an important extracellular antioxidant enzyme in the lung and attenuates tissue damage produced by cigarette smoke’s oxygen radicals. A polymorphism in the EC-SOD gene has been reported in 2% of general population (106). If this variant of the gene plays a role in the pathogenesis of COPD is not known.

Secretory leukocyte proteinase inhibitor (SLPI) is produced by the airway epithelial cells and is able to inhibit neutrophil elastase (107) and considered the potent anti-protease of the airways. Polymorphism of the SLPI gene has been detected but no mutations (108). This suggests that structural alterations in SLPI may not be involved in the pathogenesis of COPD.

Other candidate genes

A mutation in the cathepsin G gene, a serine protease, was found but it was not associated with COPD (109). In addition, a relationship was reported between polymorphism in the gene for microsomal epoxide hydrolase and susceptibility to emphysema (110). Recently, polymorphism of tumor necrosis factor-a gene was investigated in chronic bronchitis (111).

“Genetic markers”

Blood group antigens. An association between the ABO locus and COPD was reported and the type A blood group was associated with impaired lung function (112). Others failed to confirm any relationship between ABO alleles and pulmonary function (113,114).

Although the ABO antigens in the respiratory tract secretions may have a protective role (101,115) and could be the defect in susceptible smoker, these observations have not been duplicated in airflow obstruction (113,114,116). Similarly, the Lewis blood system had been investigated in airflow limitation and it was shown that Lewis-negative subjects were at greater risk (117). Blood group antigens have been associated with recurrent infections that may lead to COPD. However, the role of ABO, Lewis and secretor genes remain unclear in the pathogenesis of COPD.

Human leukocyte antigen locus (HLA). A significant decrease of HLA-Bw6 allele was found in COPD patient with a low FEV1 value and an increase in HLA-B7 antigen (101). However, it is not clear whether these associations due to variations in HLA genes themselves or if they express susceptibility to COPD.

Cytochrome P450IA1. A recent study reported that the high-activity allele of CYP1A1 was associated with susceptibility to centrilobular emphysema and lung cancer but this was not linked to cancer alone in the absence of emphysema (118).

Cystic fibrosis transmembrane regulator (CFTR). The above mutation has been examined in chronic bronchitis and found that none of the known mutation of CFTR is associated with COPD (119).

Microsatellite DNA instability (MSI) in COPD. Microsatellites of DNA are short tandem nucleotide repeats
commonly found throughout the genome. Microsatellite DNA instability has been correlated with high mutation rates (I20). Thus, MSI could be a useful technique to identify locus of potential altered genes. This method had been applied in sputum cells of COPD patients and it was shown that this defect is a detectable phenomenon (I21).

Recently, sputum cells from groups of smokers with and without COPD were tested for MSI (I22). Both groups had similar smoking history. MSI was detected in 24% of COPD patients but in none of the non-COPD smokers. These results indicated that MSI could be part of the complex genetic basis of COPD and could be a marker of genetic alteration caused by smoking leading to COPD. Thus, MSI may be an index of the “susceptible” smoker (I22). However, more studies are needed to verify these results.

CONCLUSIONS
In conclusion, there must be a number of host factor genetically predetermined that characterize the “susceptible” smoker. It is apparent that several genes are involved and this area of the pathogenesis (I23) of the disease urgently needs more exploration. Excellent reviews on the genetic risk factors for COPD are provided by Sandford et al. (83) and Barnes (I24).

FUTURE PERSPECTIVES
It would be revolutionary if new genes could be discovered that explain the predisposition of “susceptible” smoker to develop COPD. Linkage analysis in families of COPD patients, using polymorphic markers could identify chromosomal locations harboring genes involved in the pathogenesis of the disease.

REFERENCES


