Eur Respir J 2009; 34: 310–315
DOI: 10.1183/09031936.00067008

PERSPECTIVE

A hypothesis for the initiation of COPD

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ABSTRACT: Chronic obstructive pulmonary disease (COPD) is generally thought to depend on an aberrant immune response to a noxious or infectious agent, which may cause chronic inflammation. However, the initiation of this abnormal response is not fully understood. Here, we propose a new hypothesis for the beginning of COPD, that the immune response to inhaled agents, mainly cigarette smoke, is directed toward the airway epithelium, due to oxidative DNA damage of the lung epithelial barrier cells (LEBCs). The steps of this model are as follows. 1) Cigarette smoke induces oxidative DNA damage of LEBCs. 2) The acquired mutations are expressed at the microsatellite DNA level of LEBCs. 3) The altered LEBCs are recognised by dendritic cells (DCs) as ''nonself''. DCs travel, with the new information, to the lymph nodes, presenting it to the naïve T-lymphocytes. 4) A predominant CD8+ cytotoxic T-lymphocyte proliferation occurs. The CD8+ cells, by releasing perforin and granzymes, attack the altered LEBCs activating cell death cascades.

Obviously, the above scenario needs further experimental exploration. However, it is an attractive model for the initiation of the abnormal inflammation in COPD, comprising oxidative DNA damage of LEBCs and host immune response.

KEYWORDS: CD8+ T-lymphocyte, dendritic cell, lung epithelial barrier cells, microsatellite instability, oxidative DNA damage, perforin

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Received:
April 30 2008
Accepted after revision:
March 05 2009

Chronic obstructive pulmonary disease (COPD) is a major health problem worldwide, with increasing prevalence, morbidity and mortality [1–3]. Recent guidelines emphasise that COPD is characterised by progressive, not fully reversible airflow limitation, as a result of an abnormal inflammatory reaction of the lungs to inhaled noxious gases and particles [2, 3]. The vast majority of COPD patients share chronic cigarette smoke exposure as a common and primary environmental aetiological factor [1–5]. Cigarette smoke is a major source of particles, free radicals and reactive chemicals, all of which can produce an overwhelming oxidant burden on the lungs [1–3, 6]. The molecular effect of these highly reactive molecules can explain a number of pathologies observed in COPD patients: inflammation, lung destruction and emphysema, airway fibrosis, mucus hypersecretion and skeletal muscle wasting [5]. However, only a minority of smokers are diagnosed with clinically relevant COPD [1–4, 7], suggesting that COPD is the result of host–environmental interaction, most probably genetically predetermined [8]. Among the candidate genes that have been studied in COPD are those affecting the production of proteases and antiproteases, the ones that modulate the metabolism of toxic substances of smoke, those involved in mucociliary clearance and genes that influence inflammatory mediators [8–10]. However, it seems most unlikely that a single molecular pathway will account for the pathogenesis of COPD.

Lately, epigenetic mechanisms such as acquired somatic mutations may represent another fundamental contributor to the molecular pathogenesis of COPD [11]. Somatic mutations do not affect the germ line; they are not heritable, although the susceptibility to acquiring such mutations might be controlled by inherited genes [11]. During a lifetime, genomic integrity is subject to various types of acquired DNA damage, by exogenous (*e.g.* ultraviolet light, ionising radiation, toxic chemicals and cigarette smoke) or endogenous agents (*e.g.* by-products of normal metabolism, such as reactive oxygen species (ROS) and free radicals, stalled replication forks, meiotic recombination and immune system maturation) [12, 13]. These critical DNA lesions can result in cell death or a wide variety of genetic alterations, including deletions, translocations, loss of heterozygosity and microsatellite DNA instability [12, 13]. In normal conditions, cells are equipped with a number of repair pathways that remove

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

the damage and restore the intact DNA [13]. However, increased and persistent oxidative stress may inactivate the human DNA mismatch repair (MMR) system leading to acquired mutations. These mutations permanently alter DNA autorepair ability, resulting in genomic instability [14]. Smoking-induced somatic mutations persist for years or decades once they are acquired [15]. In agreement with the above hypothesis, somatic genetic alterations have been detected at the microsatellite DNA in COPD patients [16–18].

In the present review, we propose a novel hypothesis for the initiation of COPD, that oxidative stress associated with cigarette smoking might damage DNA of lung epithelial barrier cells (LEBCs), which are further misinterpreted by the host immune system as ''nonself''. An atypical adaptive immune response is generated, with the predominance of CD8+ cytotoxic cells, leading to cell death cascades (fig. 1).

HYPOTHESIS

The links between cigarette smoke, oxidative stress, lung inflammation and COPD have been extensively studied, and several theories have been proposed to explain the pathogenesis of COPD [19–26]. In this article, we advance a new hypothesis for the initiation of COPD (fig. 1).

In detail, in the beginning of COPD, cigarette smoking affects the air–lung barrier system and, in particular, its cellular component *via* repeated oxidative stress leading to oxidative DNA damage [27–30]. Oxidative stress damages the DNA of LEBCs, leading to acquired somatic mutations [11, 14, 15], and further suppresses key genes of the DNA MMR system, affecting the DNA autorepair ability [11, 14].

Somatic mutations of the LEBCs are an indirect way of activating and polarising the dendritic cell (DC) network, by danger/alarm signals expressed by the altered cells (fig. 2) [31, 32]. In the ''danger model'', antigen-presenting cells, such as DCs, can be activated by danger/alarm signals produced by self-injured cells, after exposure to pathogens, toxins, mechanical damage and cigarette smoke. This model has been supported by the discovery of endogenous, nonforeign alarm signals [32], including altered mammalian DNA, RNA,

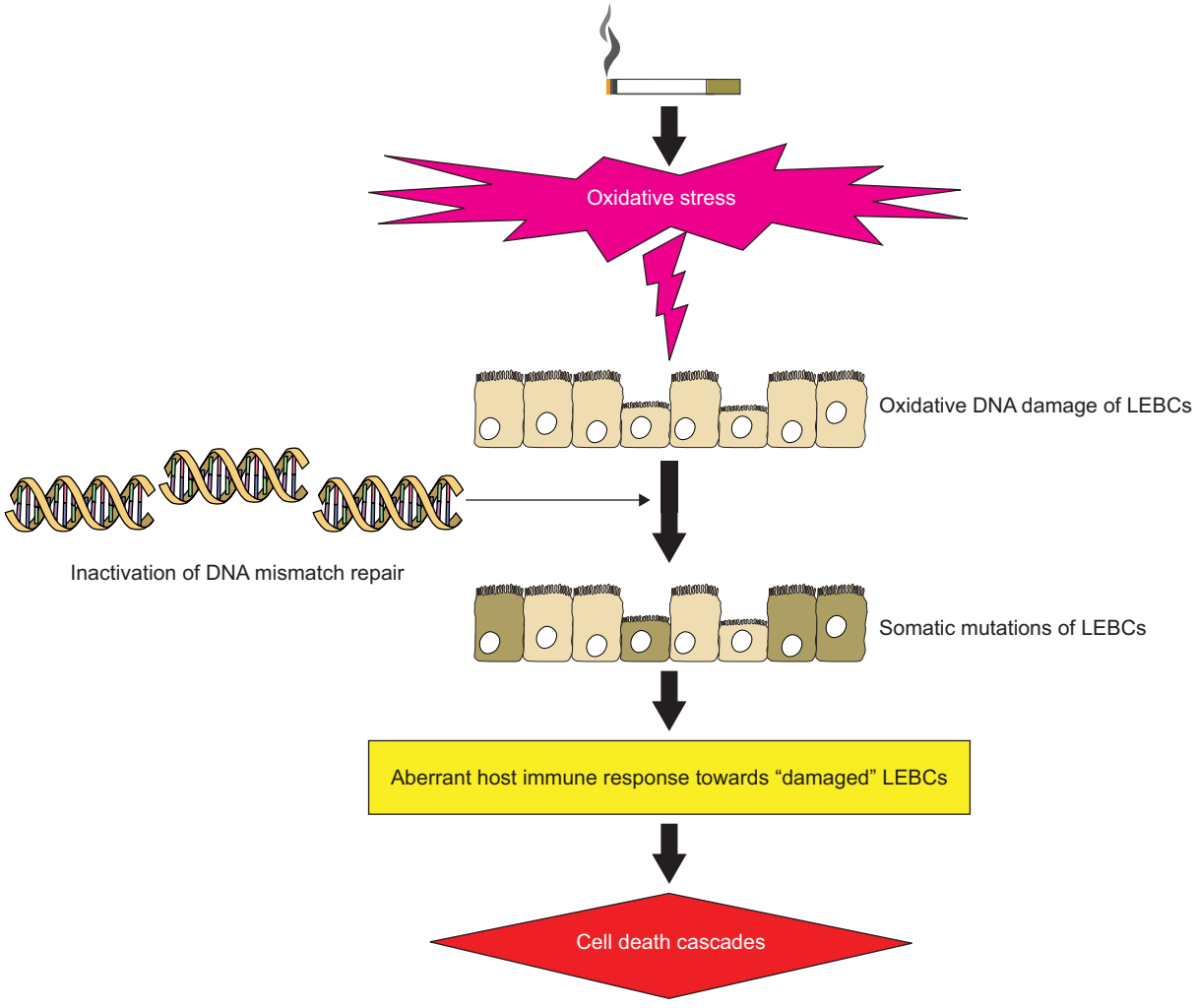

FIGURE 1. The proposed model for the beginning of chronic obstructive pulmonary disease. The oxidative burden of smoking damages the DNA of lung epithelial barrier cells (LEBCs) and inactivates the human DNA mismatch repair system, leading to acquired somatic mutations of LEBCs. Altered LEBCs are recognised as ''nonself'' by the host, inducing an abnormal immune response towards the altered LEBCs, resulting in the activation of cell death cascades.

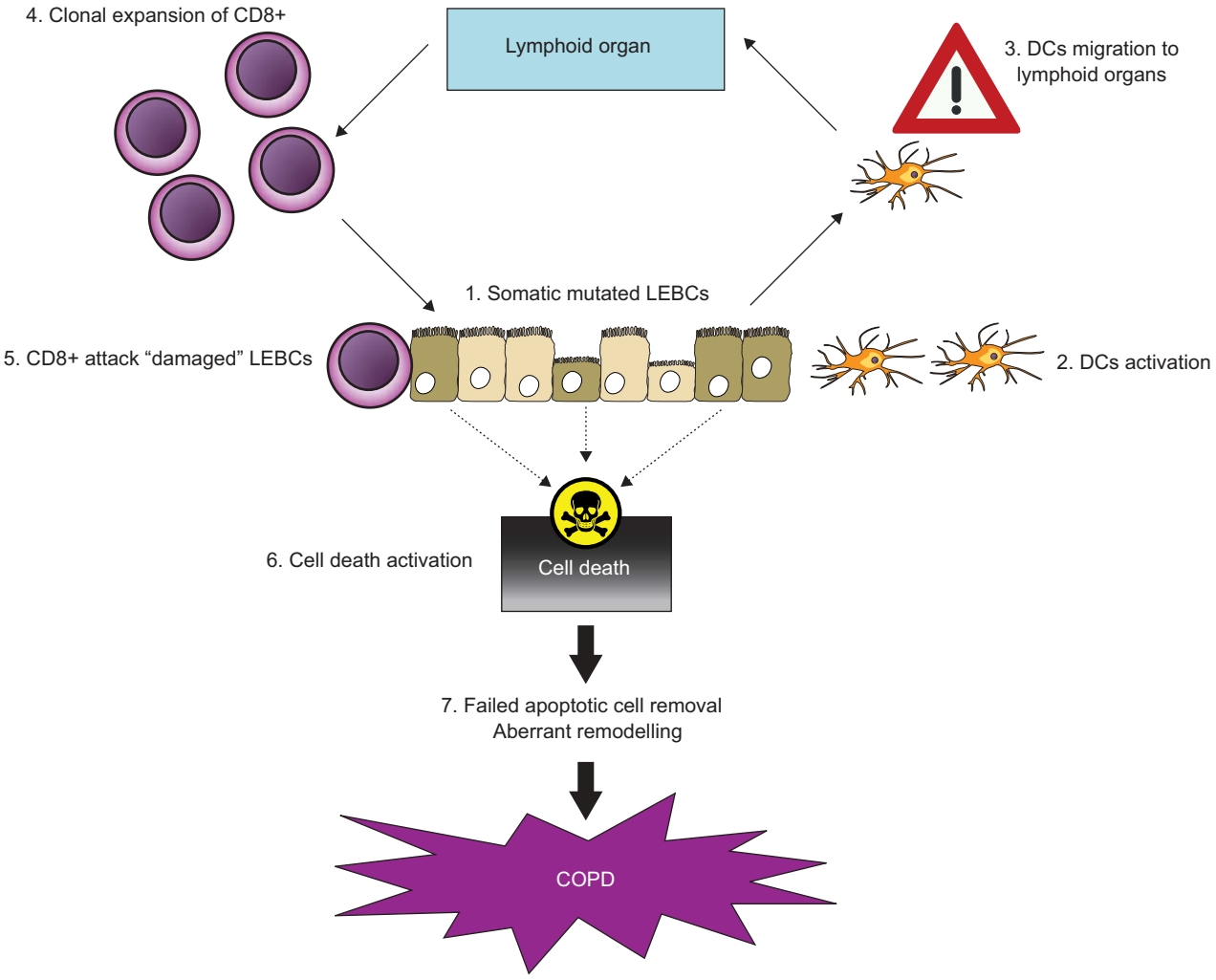

FIGURE 2. Acquired somatic mutations of lung epithelial barrier cells (LEBCs) in chronic obstructive pulmonary disease (COPD). 1) Somatic mutated LEBCs are recognised as nonself by the host. 2) Dendritic cells (DCs) are activated by the ''danger/alarm'' signals emitted from the altered LEBCs, then 3) DCs travel to the draining lymph nodes to present the new signal. 4) A proliferation of CD8+ cytotoxic T-lymphocytes occurs, then 5) the CD8+ T-cells migrate to the sites of the initial insult and, by releasing perforin and granzymes, attack altered LEBCs, 6) activating cell death. 7) Failed apoptotic cell removal may contribute to COPD pathogenesis by impeding both the resolution of inflammation and the maintenance of alveolar integrity.

heat-shock proteins, interferon (IFN)-a, interleukin-1b, CD40-L and breakdown products of hyaluron [32, 33].

Thereafter, the DCs travel to the draining lymph nodes with this endogenous alarm signal, produced by the damaged LEBCs, and they present it to the naïve T-lymphocytes, inducing a proliferation of CD8+ cytotoxic T-lymphocytes [34–36]. The CD8+ T-cells migrate to the sites of the initial insult and, by releasing perforin and granzymes, attack the altered LEBCs. Perforin forms pores in the target cells' membranes, while granzymes, as serine proteases, enter the cytoplasm of the target cells, altering their function and/or activating cell death (fig. 2) [37–39].

STUDIES SUPPORTING THIS HYPOTHESIS

Oxidative DNA damage of LEBCs in COPD

The defence against noxious or infectious agents in the respiratory tract is provided by a number of different mechanisms. In the airways, the LEBCs constitute the first line of defence and have been shown to differ in number and function in smoke-related diseases [40, 41], such as COPD [1–3]. Smokers are exposed to thousands of radicals and reactive chemicals with every cigarette, thus oxidative stress is believed to be a central component in COPD pathogenesis [5]. ROS, reactive nitrogen species (RNS) and carbon-centred radicals are constituents in both the tar and gas phases of smoke [5]. In addition, there are also endogenous sources of ROS, such as those produced by activated alveolar macrophages and neutrophils in response to smoke, which further promote epithelial cell damage [5]. Increased ROS production has been directly linked to oxidation of proteins, DNA and lipids, which may cause direct lung injury or induce a variety of cellular responses, through the generation of secondary metabolic reactive species and ROS-mediated molecular mechanisms [12–15, 27]. ROS can attack nucleic acids, resulting in base modifications, double base lesions, strand breaks,

mismatches and cross-links, leading to increased levels of somatic mutation [11, 14, 27]. In the majority of cases, these mutations can be corrected by endogenous repair processes, such as the human DNA MMR system. However, post-translational inactivation of repair enzymes by free radicals may enhance the accumulation of DNA damage [14]. Interestingly, female smokers have accelerated metabolism of cigarette smoke leading to increased burden of DNA adducts and mutations, but reduced DNA repair capacity [42, 43] Thus, they are more susceptible to somatic mutations, which may predispose to COPD [42, 43].

Recent studies have reported increased levels of oxidative stress and DNA damage in peripheral blood of patients with COPD [29, 30]. Furthermore, microsatellite DNA instability has been reported in sputum cells [16–18] and tissues [15, 44] from COPD patients and smokers, probably reflecting a defective DNA MMR system [14].

Acquired somatic mutations of LEBCs

Molecular damage in the lung epithelium of current and former smokers has been demonstrated, and seems to persist even after smoking cessation [11, 15]. Microsatellite DNA instability has been found in sputum cells of COPD patients [16–18, 45]. Although sputum is a relatively heterogeneous sample to consider, studies have shown microsatellite instability exclusively in the sputum epithelial cell subpopulation [45, 46], isolated by immunomagnetic separation and further processed for genomic analysis [46].

Microsatellite alterations involve changes in the size of the simple nucleotide repeats of polymorphic microsatellite DNA sequences, resulting in altered electrophoretic mobility of one or both alleles [47]. Insertion or deletion of these DNA sequences has been correlated with a high somatic mutational rate and is associated with a defective DNA MMR system [48]. The MMR system is a DNA repair mechanism known to be very effective at identifying and subsequently rectifying errors in DNA replication that escape DNA polymerase proofreading during the replication process [49]. The MMR corrects base substitutions and frameshift errors, as well as expansions and contractions within microsatellite sequences. In particular, the MMR system is actively involved in the repair of endogenously generated DNA lesions and in the DNA damage signalling response [49]. It has been demonstrated that oxidative stress associated with cigarette smoke and chronic inflammation might damage protein components of the MMR system, leading to its functional inactivation [14, 48, 50, 51]. CHANG *et al.* [14] reported that noncytotoxic levels of H_2O_2 inactivate both single-base mismatch and loop repair activities of the MMR system in a dose-dependent fashion, and that this inactivation is most likely due to oxidative damage to hMutSa, hMutSβ and hMutLα protein complexes. Oxidative DNA damage can lead to microsatellite slippage mutations in several model systems [50, 51]. In human cell lines, an increase in microsatellite mutations of up to 27-fold after oxidative DNA damage has been reported [51]. Moreover, MAKRIS *et al.* [18] showed a significant association between microsatellite DNA instability and COPD exacerbations, indicating a link between altered DNA MMR system and oxidative DNA damage due to frequent COPD exacerbations or *vice versa* [18].

Host immune response to the altered LEBCs

Various forms of cell damage lead to DNA fragmentation and destruction of the repair potential that allow its recognition as ''foreign'' [52, 53]. This hypothesis considers that once the LEBCs suffer oxidative DNA damage, they are detected as ''nonself'' by the primary sensors of the immune system, the DCs [54]. DCs, as the main antigen-presenting cells of the lung, populate the whole respiratory tree and can be subdivided into two types: myeloid DCs and plasmacytoid DCs (PDCs) [54].

On the basis of the recognition of self or nonself signals in the presence or absence of danger signals, the interplay of DC antigen uptake and presentation leads to immunosilencing or immunoactivating properties, which designate the outcome of tolerance or defensive immunity within lung [54].

After antigen sampling in the periphery, DCs migrate in a CC chemokine receptor (CCR)7-dependent fashion to regional lymph nodes, where they activate naïve T-cells to proliferate [55]. DCs and natural killer (NK) cells appear to regulate one another's maturation reciprocally [56]. The reciprocal interaction of NK cells with PDCs or myeloid DCs profoundly affects innate resistance functions [56]. In lung homeostatic conditions, a balance exists between PDCs and myeloid DCs necessary for maintaining tolerance to antigens and avoiding overt inflammation [56]. In adaptive immunity, DCs are recruited from the circulation and migrate towards epithelial surfaces, where they capture antigens and recognise ''danger signals''. Subsequently, they migrate to regional draining lymph nodes and present sequestered antigens to naïve lymphocytes to induce either a primary immune response or tolerance, dependent on co-stimulatory molecule expression, cytokine release and their state of maturity [57–59].

We consider it highly likely that DCs are implicated in the pathogenesis of COPD, not only in the initiation but also in the perpetuation of its characteristic pattern of chronic airway inflammation [55, 60, 61]. Once activated, DCs are encouraged to mature and migrate to draining lymph nodes by chemoattractants, including macrophage inflammatopry protein (MIP)-3β acting on cell surface receptors such as CCR7 [55]. Depending on the nature of the initial stimulus, DCs will cooperate with naïve lymphocytes to induce one of three predominant responses: T-helper cell (Th) type 1, Th2 or T-regulatory [62].

Induction of a predominantly CD8+ cytotoxic type I T-lymphocyte proliferation

Studies have showed that, in COPD, DCs contribute to the induction of a predominantly CD8+ cytotoxic type I T-lymphocyte response [23, 34, 54, 63]. The shift to a predominant type I immune response induces chronic airway inflammation with increased numbers of CD8+ cells and release of IFN inducible protein 10, IFN-γ and tumour necrosis factor (TNF)-α, as well as perforin and granzymes [22, 34, 35]. Moreover, B-cells and CD8+ T-cells increase in both the extent of their distribution and in volume, with organisation into lymphoid follicles [22, 24, 64]. Similar lymphoid follicles were detected in mice that had developed pulmonary inflammation and progressive alveolar airspace enlargement after smoking [65].

Cross-presentation of self antigens (*e.g.* injured LEBCs) by DCs to T-cells could lead to the development of auto-immunity

[57–59, 66]. Moreover, cigarette smoking-induced impairment in DC maturation may enhance CD8+ T-cell proliferation *via* increased cross-presentation of foreign and/or self antigens [67]. In addition, decreased DC maturation may shift the CD4+/CD8+ T-cell ratio in the lungs towards CD8+ cells, as the time required for DCs to launch the proliferative programme for CD8+ is less than that required for CD4+ cells [68]. As the proliferative capacity of CD8+ cells is greater than that of CD4+ cells, the latter are more susceptible to the effects of reduced DC maturation [69]. CD8+ T-cell differentiation into memory cells is facilitated by CD4+ T-cells. Smoking severity, degree of airway obstruction and emphysema are all related to increased CD8+ cells and/or CD8+/CD4+ ratio [67–69].

In resemblance to host immune response to a viral infection, CD8+ cells attack the altered LEBCs and perform cytotoxic functions, activating cell death cascades [20, 26, 37]. In agreement with this, CHRYSOFAKIS *et al.* [37] showed that CD8+ T-cells from patients with COPD produced more perforin and were extremely cytotoxic. Recently, VERNOOY *et al.* [39] investigated protein and mRNA expression of granzyme (Gr)A and B in lung tissue of patients with COPD and found GrA and GrB in CD8+ T-cells, CD57+ NK cells, type II pneumocytes and alveolar macrophages. Both GrA and GrB are involved in perforin-dependent processes leading to DNA fragmentation and initiation of perforin-dependent events that lead to apoptosis [39]. Apoptosis, although anti-inflammatory in nature, can lead to persistent inflammation if apoptotic bodies are not removed in time, possibly initiating autoimmune processes [70].

However, we should point out that most resident cells (LEBCs, macrophages, endothelial and extracellular matrix components), when damaged, are capable of inducing an immune response and/or contribute to the oxidative burden [71].

SUMMARY

Cigarette smoke and airway inflammation may cause acquisition of somatic mutations in LEBCs due to oxidative DNA damage. This may primarily affect the DNA MMR system, resulting in failure to preserve genome integrity of LEBCs. Damaged LEBCs are misinterpreted by the host immune system as ''nonself'', leading to the clonal expansion of the cytotoxic CD8+ cells, resulting in cell apoptosis and/or necrosis. However, we do acknowledge that a longitudinal study could answer the question of whether increased oxidative inflammatory burden in susceptible smokers is the link between somatic mutations of LEBCs and the host immune system.

STATEMENT OF INTEREST

None declared.

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