

Novel aspects of pathogenesis and regeneration mechanisms in COPD

Edvardas Bagdonas
Jovile Raudoniute
Ieva Bruzauskaite
Ruta Aldonyte

State Research Institute Center for
Innovative Medicine, Vilnius, Lithuania

Abstract: Chronic obstructive pulmonary disease (COPD), a major cause of death and morbidity worldwide, is characterized by expiratory airflow limitation that is not fully reversible, deregulated chronic inflammation, and emphysematous destruction of the lungs. Despite the fact that COPD is a steadily growing global healthcare problem, the conventional therapies remain palliative, and regenerative approaches for disease management are not available yet. We aim to provide an overview of key reviews, experimental, and clinical studies addressing lung emphysema development and repair mechanisms published in the past decade. Novel aspects discussed herein include integral revision of the literature focused on lung microflora changes in COPD, autoimmune component of the disease, and environmental risk factors other than cigarette smoke. The time span of studies on COPD, including emphysema, chronic bronchitis, and asthmatic bronchitis, covers almost 200 years, and several crucial mechanisms of COPD pathogenesis are described and studied. However, we still lack the holistic understanding of COPD development and the exact picture of the time-course and interplay of the events during stable, exacerbated, corticosteroid-treated COPD states, and transitions in-between. Several generally recognized mechanisms will be discussed shortly herein, ie, unregulated inflammation, proteolysis/antiproteolysis imbalance, and destroyed repair mechanisms, while novel topics such as deviated microbiota, air pollutants-related damage, and autoimmune process within the lung tissue will be discussed more extensively. Considerable influx of new data from the clinic, in vivo and in vitro studies stimulate to search for novel concise explanation and holistic understanding of COPD nowadays.

Keywords: dysbiosis in COPD, autoimmune COPD, occupational COPD, chronic obstructive pulmonary disease

Pathogenesis mechanisms in COPD

Several clinical features comprise COPDs such as chronic bronchitis, destruction of small airways, and enlargement/disorganization of alveoli, ie, loss of alveolar tissue. Our review will focus on the changes taking place in alveolar space, ie, emphysema component of COPD. We have illustrated emphysematous vs healthy alveoli with several well-known pathogenesis processes in Figure 1. Some novel aspects are also added therein, eg, microbiota-produced inflammatory influences. The schematic overview of the complex interplay among structural, inflammatory, and progenitor cells; mediators' milieu; and simplified structural shifts within the lung alveoli and its surroundings represent the challenges, both inner and exogenous, that lung tissue components are exposed to.

In general, COPD remains unappreciated, and COPD prevalence surveys are remarkably lower in numbers in comparison with prevalence surveys available in asthma, cancer, cardiovascular, or other major diseases. This may be among the reasons

Correspondence: Ruta Aldonyte
State Research Institute Center for
Innovative Medicine, Zygimantu 9,
Vilnius LT 01102, Lithuania
Tel +370 5 262 8636
Fax +370 5 212 3073
Email r.aldonyte@imcentras.lt

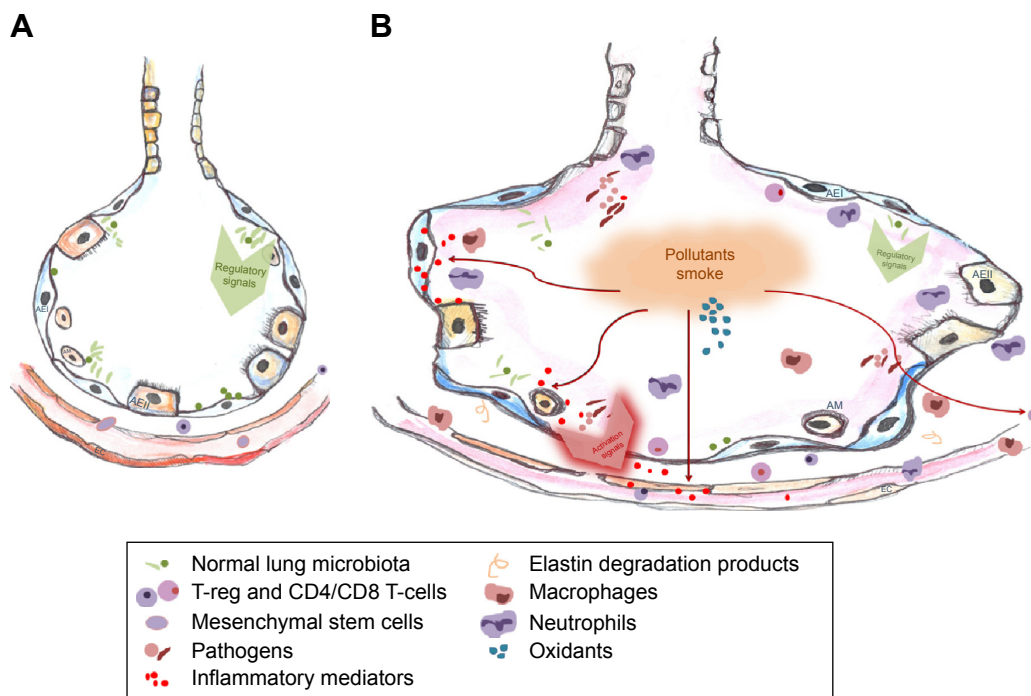


Figure 1 Mechanisms underlying COPD development.

Notes: (A) The airway epithelium consisting of several diverse cell types maintains a balanced interaction with normal lung microbiota, and only regulatory signals are induced. Progenitors (mesenchymal stem cells) are recruited from circulation when needed. (B) Pathogenic bacteria (eg, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*) activate proinflammatory signaling pathways in the epithelium and also release chemokines and cytokines themselves. In parallel to air pollutants, cigarette smoke, oxidants also induce similar changes (fine red arrows). Air pollutants, including cigarette smoke, are a rich source of oxidants capable to recruit macrophages and neutrophils. Pollution also decreases a pool of progenitor cells in the circulation and locally (fine red arrow). Inflammatory cells (eg, neutrophils, dendritic cells, monocytes/macrophages, cytotoxic T-cells) arrive at the sites of inflammation and become the additional source of cytokines, oxidants, and proteases. CD4 and CD8 T-cells (Tc1/Th1-dominant) are present and release chemokines and perforins. At the same time, representatives of normal microbiota induce relatively weak signals to prevent the exaggeration of the inflammation. These stimuli-induced processes result in alveolar wall cells and vascular endothelial cells apoptosis/loss, and extracellular matrix proteolysis. Inflammation might be further triggered by elastin fragments. Structural alveolar disintegration, loss of epithelium and endothelium, and the overall inflammatory ambience are seen.

Abbreviations: AEI and AEII, alveolar type I and II epithelial cells; AM, alveolar macrophages; EC, endothelial cells.

why COPD was accepted as exceptionally smoking-related disease for a long time. However, this is not acceptable any longer, since extensive search reveals more new risk factors. As elegantly reviewed by Salvi and Barnes, data from population-based studies show that cigarette smoking as a risk factor may only be attributed to half of COPD cases, and some unpublished data included in the review present nearly 70% nonsmokers among COPD cases collected at the city slum in India.¹ Some of the reports originating from South Africa, the People's Republic of China, and Korea state distinct proportions of nonsmoking-COPD among men and women (where nonsmoking-COPD morbidity among women exceeds 50%) suggesting that exposure to household fumes might be implicated.²⁻⁴ Tuberculosis infection, occupational exposures, and frequent childhood infections are also discussed as major risks. Thus, other than smoking factor-associated COPD, morbidity is equally huge and unrecognized. The alternative environmental risk factors implicated in the nonsmoking COPD development include indoor and outdoor air pollution, ie, biomass fuel, dust, and fumes^{5,6}

and the use of agricultural pesticides (comprising herbicides and insecticides). In the recent systematic review, hundreds of studies that were published after 1990 (final number of relevant papers included in the calculations was 23) were revised, and statement on the correlation between COPD and the use of pesticides was made.⁷ Pesticides are regarded as a very important source of reactive oxygen species (ROS) and other insults, and a direct association between the common pesticide use and COPD development has been reported a long time ago.⁸ Indeed, farming is recognized as a risk factor for COPD, and a population-based study reveals that risk for nonreversible airways obstruction attributable to farming is 7.7%.⁹ Moreover, recent study has extended the existing knowledge by following a general population cohort for 25 years and assessing the impact of pesticides on lung function. They have found that exposure to pesticides is associated with accelerated decline in lung function, as much as loss of 6.9 mL/year of forced expiratory volume in 1 second (FEV₁).¹⁰ This is also corroborated by the data from other cohorts and short term, specific pesticide-related studies.^{11,12}

Another theory relates COPD to the fetal stage (fetal origin hypothesis), ie, findings from large retrospective studies suggest that COPD might be programmed early in life. Indeed, Barker et al have found that low birth-weight and respiratory infections in infancy lead to reduced lung function in adults.¹³ Other “reprogramming” factors include use of anti-inflammatory medicines in pregnancy, vitamin D deficiency, certain diet, and vitamin C intake during pregnancy and inadequate duration of breastfeeding.^{14–17} Another important group of factors related to birth, ie, limited exposure to microbial world prior to, during, and after the delivery due to maternal use of antibiotics; increased rates of C-section; and sterile environment around the infant, will be discussed later in our paper.

Autoimmunity also plays a role in COPD as it becomes increasingly accepted. Autoimmune mechanisms can explain the unregulated inflammatory process perpetuation that continues after smoking cessation, since the initial inflammation and environmental insults in lungs expose certain epitopes for the autoimmune attack. Naming COPD an autoimmune disease opens new horizons for the treatment strategies and for the research lines to develop. Identifying, blocking, or masking auto-epitopes; preserving and enriching lung microbiota; cleaning the breathable air; and staying physically active may become major COPD management directions as opposed to conventionally used anti-inflammatory treatment, corticosteroids, and antibiotics.

In addition to factors mentioned earlier, accelerated cell senescence has been implicated in the pathogenesis of COPD. It is clearly shown that senescence affects lung structural and inflammatory cells, fibroblasts, and progenitors, rendering repair and regeneration insufficient.^{18–22} In corroboration, autophagy was found significantly deregulated in the cells from patients with COPD.^{23,24} Insufficient autophagy contributes to the senescence by increasing accumulation of damaged cellular content. While in normal lung, autophagy mechanism maintains tissue homeostasis, ie, balance among production, degradation, and recycling of organelles and proteins, and helps tissue in adaptation, in COPD lung, chronic deficiency in autophagy leads to increased tissue senescence and insufficient repair.

COPD pathogenesis evolves with the disease exacerbations that are main drivers of health deterioration in patients with COPD. Exacerbations of COPD are defined as acute episodes of symptom worsening beyond day-to-day variations requiring change in regular medication. There have been an improvement in uniformity of the definition for the exacerbations during recent years, although current treatment

guidelines for the exacerbations often remain unrelated to clinical data and contribute to inappropriate use of corticosteroids and antibiotics.²⁵ A distinct patients’ group, “frequent exacerbation type”, is recognized and reflects a heterogeneity and multiplicity of exacerbations-related pathogenesis’ factors and mechanisms. Susceptibility to exacerbations is defined by background inflammation in the lung tissue, microbiome shifts and patterns, status of the immune system, comorbidities, and medications’ background. Major mechanisms described in COPD are further upregulated during exacerbations, including inflammatory process exaggeration²⁶ and obstruction in the airways with a subsequent hyperinflation.²⁷ Infectious pathogens, ie, viruses and bacteria, are responsible for driving the exacerbation process in more than half of all cases as reviewed by Miravittles and Anzueto.²⁸ Markers of infectious process are well documented,^{29,30} and controversial continuous prophylactic macrolide therapy for COPD is proposed.³¹ Authors claim that continuous therapy reduces COPD exacerbation frequency, although their conclusion is based on only three clinical trials, and the target group remains unclear. Among other triggers for COPD exacerbations, the indoor and outdoor air pollution^{5,32,33} and second-hand smoke³⁴ should be mentioned.

Inflammatory process: participating cells, proteolysis, and oxidative injury

It is well proven that lung tissue in every smoker is inflamed. Proofs come from the studies on biopsy material,³⁵ sputum studies,³⁶ and experimental in vitro data³⁷ that show inflammatory cell infiltration and presence of various proinflammatory molecules at the increased levels in smoker’s lungs. At certain smoking/exposure level and other predisposing situations, yet enigmatic to us, the destructive process overcomes the local protective mechanisms and lung tissue damage and loss manifests. In COPD, inflammatory tissue damage is endless, ie, inflammation perpetuates further on long after patients have quit smoking.³⁸ Inflammation-related contributors to lung tissue injury include cells and mediators of both initiative and adaptive immunity, ROS, imbalance of local proteolysis/antiproteolysis state, and other insults.

Participating cells: neutrophils

Circulating and lung-resident inflammatory cells, including neutrophils, for a long time are recognized participants of smoking-induced tissue injury.³⁹ In corroboration with many previous reports, a recent study finds that neutrophils are increased in sputum of patients with COPD along with

increased interleukin (IL)-6 signaling.³⁶ The same tendency was detected in bronchoalveolar lavage fluid (BALF) long time ago.^{40,41} Neutrophils are known as the major destructors of the elastic matrix of the alveoli. They act via proteases and small cationic peptides and are able to attack invading bacteria, viruses, pollutants, and, under certain autoimmune circumstances, own tissue, like in Crohn's disease and psoriasis.^{42,43} Under the influence of environmental insults, including cigarette smoke, neutrophil-released enzymes and peptides are able to cleave collagen into fragments that may activate inflammatory cells and drive chronic inflammation further.⁴⁴ Moreover, study shows that neutrophils from patients with COPD are loaded with intracellular proteolytic enzymes by 25 folds more than the cells from healthy donors. Authors also report that immunohistologically proteolytic enzymes are distributed along with neutrophils, macrophages, and epithelial cells.

Signals that drive neutrophils to infiltrate the damaged lung parenchyma are partially identified. It is known that neutrophils rapidly emerge at the sites of inflammation in response to interleukin (IL)-8, a chemoattractant produced by multiple cell types, mainly, exposed and damaged epithelium and endothelium.^{45,46} A variety of other attractants have been shown to induce neutrophil migration, including chemokine CXC motif ligands 2 (CXCL2), leukotriene B₄ (LTB₄), and formyl-met-leu-phe (fMLP).⁴⁷⁻⁵¹ The latter is a host-microorganism interaction-associated chemoattractant, while others are generated by organism own immune cells and injured tissues at the site of disease. A modified alpha-1-antitrypsin (AAT) needs to be mentioned as a candidate attractant for neutrophils.⁵²⁻⁵⁴ Various modified, ie, oxidized, polymerized, cleaved, nitrosylated, and citrullinated, AAT forms are especially relevant on the inflammatory lung destruction stage, where proteolysis and ROS attack are predominant processes and AAT is a major antiprotease.⁵⁵ There is also a comparative data presented on chemotaxis induction where fMLP is shown as a most powerful attractant *in vitro*.⁵⁶ Overall, it is accepted that cellular migration behavior (orientation and motility) is defined by integrate multiple signals, both dominant and secondary. Interestingly, stem cells also home via similar vectors, including our own data on AAT/mesenchymal stem cells (MSC) interactions *in vitro*,⁵⁷ neural stem cells and LTB₄ partnership reported,⁵⁸ bone progenitors and CXC motif ligands interplay,⁵⁹ and so on.

Earlier findings that neutrophils and neutrophil-produced proteinases are major contributors to tissue breakdown in COPD lungs were challenged by the observation that macrophage-released metalloproteinases (MMP) might be

critical mediators in COPD.⁶⁰ However, neutrophils are still regarded as initiating cells and also producers of attractants and epitopes for the further perpetuation of the inflammation. It is proven that neutrophils appear in the airways immediately following the insult in animal models and remain abundantly distributed.^{61,62} Moreover, the numbers of submucosal neutrophils correlate significantly with the intensity of the cigarette smoking.⁶² Some studies, however, reveal that neutrophilic infiltration in the smokers airways does not reflect the disease state.⁶³ The scenario of neutrophils participation in the lung tissue breakdown is still being under evaluation, while new data emerge and certain issues are being revised, eg, the distinct features of neutrophils from smokers with COPD vs smokers without COPD in a case of infection.⁶⁴

Participating cells: macrophages

When parenchyma infiltrating inflammatory cells, ie, T and B cells, macrophages and neutrophils were counted *in situ* (per cubic millimeter of lung tissue), the numbers of neutrophils were found to inversely correlate with the degree of emphysema.⁶⁵ This surprising correlation was accompanied by the finding that the count of T lymphocytes and macrophages closely correlated with the degree of lung destruction. And furthermore, the numbers of T-cells correlated with the numbers of macrophages leading to the conclusions that these cells may act in a team.

Macrophages play a crucial role in COPD and are regarded as major producers of MMPs in the COPD lung.⁶⁶ They also participate in the propagation of the inflammation by releasing chemoattractant molecules. Moreover, we and others have shown that circulating progenitors of lung macrophages, ie, peripheral blood-derived monocytes, differ if compared between patients with COPD and healthy controls.⁶⁷ Such early, systemic proinflammatory commitment of monocytes may explain why COPD affects the whole organism. Lung macrophages colocalize with emphysematous zones and are found in increased numbers in patients' airways, parenchyma, BALF, and sputum.^{65,68} Moreover, macrophages' numbers in the airways correspond to the severity of COPD.⁶⁹ In COPD, macrophages are recruited to the lungs and enhances the release of tumor necrosis factor (TNF)-alpha, IL-8, other CXC chemokines, monocyte chemotactic peptide (MCP)-1, LTB₄, and so on as reviewed by Barnes et al.⁷⁰ Alveolar macrophages also secrete elastolytic enzymes, including MMP-2, MMP-9, MMP-12, cathepsins K, L, and S.^{71,72} The increased recruitment of monocytes from the circulation in response to monocyte-selective chemokines that are increased in COPD

airways⁷³ and local proliferation of lung macrophages may contribute to increase in lung macrophages' numbers. Macrophage actions might be conducted by nuclear factor (NF)-kappaB, which is activated in alveolar macrophages of patients with COPD, particularly during exacerbations.^{35,74,75} However, other pathways are also described, ie, activator protein-1 and tyrosine kinase c-Src.^{76,77}

Tissue resident macrophages are now recognized as a multifunctional cell, central for tissue maintenance. It is proven that macrophages contribute to tissue regeneration, compensatory lung growth and may recruit progenitor cells.^{78,79} They are equally important in fighting the infection and cutting/resolving inflammatory signaling, removing apoptotic cells, and inducing certain regenerative processes.

Participating cells: T lymphocytes

It is becoming increasingly evident that T-cells reside at the epithelial surfaces throughout the human organism, including lungs, and mediate the host defense. Their contribution in so-called T-cell-mediated diseases is being revisited now. A recent study demonstrates abundant resident T-cells in human lung (more than 10 billion).⁸⁰ Detected T-cells are of effectors' memory phenotype; some of them are central memory T-cells and some are T-regulatory cells. T-helper cells (CD4) can be further subdivided into Th1 and Th2 cytokine types. Th1-type cytokines participate in perpetuating autoimmune responses with interferon gamma as the main cytokine and resulting excessive proinflammatory responses that can lead to uncontrolled tissue damage. Lung emphysema is generally accepted as a type Th1 disease.⁸¹ In support for this, an increased level of Th1 response-associated molecules was found in peripheral blood from subjects with COPD.⁸²

T-cell roles in COPD and relevant T-cell fractions are only partially elucidated. As described by Cosio et al, all T-cell phenotypes are increased in smokers with COPD.⁸³ Despite the fact that neutrophils are the predominant cell in the lung parenchyma of non-COPD smokers, with the signs of emphysema comes also an increase in T-cells (CD3 and CD8).⁸⁴ Moreover, T-cell numbers correlate with the smoking history. Study suggests that the development of emphysema might be mediated by T lymphocytes, mainly CD8 cytotoxic T-cells, and that apoptosis might be one of the mechanisms of lung destruction leading to the development of emphysema. Cytotoxic (CD8) cells are a subgroup of cells that kill infected or damaged cells, while T-helper (CD4) cells upon activation release cytokines and orchestrate the activity of other inflammatory and related cells. In emphysema,

CD8 T-cell numbers correlate with the severity of the tissue destruction, and their accumulation continues even after smoking cessation.⁸⁵ This correlation is not linear according to other authors. As disease progresses, T-cytotoxic cell numbers may even decline.⁶⁹ These cells require antigen to accumulate and produce their damaging effects. However, the subtle interrelation between regulatory T-cells and CD8 cells might become disrupted in autoimmune process, and regulatory T-cell-released factors might become inadequate to keep cytotoxic T-cells silent.⁸³ Interestingly, a subset of T-cells, ie, CD56 lymphocytes, can kill own lung structural cells spontaneously, as it was shown in the recent study. This natural cytotoxicity was increased in subjects with severe COPD in support of their potential role in autoimmune process-driven emphysema progression.⁸⁶ All this complex data map suggests that differential interrelationship among T-cell subtypes in COPD may be important for the progression of inflammation.⁸⁷

The importance of T-cells in emphysema development was recently proven in mice model with the demonstration that T-cells from cigarette smoke-exposed mice are able to transfer the diseased process, ie, tissue destruction, to unexposed mice. Both CD8 and CD4 T-cells were required for that, and the process was antigen recognition dependent.⁸⁸ These results further support and prove cigarette smoke-induced T-cell-driven autoimmune mechanism in COPD development.

Inflammatory mediators, ROS, and environmental factors

Tens of cytokines and chemokines have been found associated with COPD but overall interplay and leading role players remain unclear. Locally produced proinflammatory molecules come not only from the host cells but also from microorganisms, ie, bacterial lipopolysaccharide (LPS), fMLP, and other mediators contribute to the inflammation at the alveolar setting. Pulmonary epithelium when exposed to noxious environmental substances releases TNF-alpha, IL-1beta, granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor (TGF)-beta1, MCP-1, LTB₄, IL-8.⁸⁹⁻⁹¹ Macrophages also release chemokines, such as CXCL9, CXCL10, and CXCL11, which are chemotactic.⁹⁰ In addition, proteolytic enzymes, eg, MMP-2, MMP-9, MMP-12, and cathepsins, are also secreted in response to ROS-rich environment.⁹² Transcription factor NF-kappaB orchestrates these responses in the cells of patients with COPD.⁹³ What cytokines and at what times are the major players on the constantly evolving stage of COPD remains

unclear. There was an attempt to target TNF- α and specifically to silence its signaling in patients with COPD. Three studies on chimeric monoclonal antibody against TNF- α were performed in patients with mild-to-severe COPD. However, none of the clinical parameters assessed, ie, exacerbation rate, dyspnea, or FEV₁, were improved.^{94–96} Even opposite, functional blockage of TNF- α has led to an increased rate of infectious complications and malignancy.⁹⁶ Therefore, it is clear that cytokines in COPD may have several important and opposing roles and eliminating one may lead to even deeper deregulation of the inflammatory process.

ROS are oxygen-rich unstable molecules that can become donors or recipients of free electrons. The subsequent products of their reactivity are even more unstable. Intracellular ROS can induce functional and structural changes, such as membrane lipid peroxidation, DNA strand breaks, changes in enzymatic activity, and modulate intracellular signaling cascades.⁹⁷ The effect of ROS depends on their concentration, ie, structural changes are associated with high ROS concentrations,⁹⁸ while lower levels of ROS decrease cell proliferation capacity, induce apoptosis, and necrosis.⁹⁹ Effects of petrol exhaust particles on cell viability, oxidative stress generation, DNA damage and inflammation in human lung epithelial cells, and murine macrophages are described.¹⁰⁰ Increasing awareness on air pollution-related lung destruction process stimulates to study ROS-induced processes closer. Recently, several studies were published in this field, including report on ozone and diesel exhaust particles-modulated transcription factor NF- κ B activity¹⁰¹ and LPS-induced production of inflammatory cytokines that is redox sensitive.^{102,103}

Intracellular redox state is defined by the oxidant load in the breathable air and a pooled capacity of lung-protective mechanisms to absorb the oxidative agents. Alveolar lining fluid, epithelial cells in the alveoli, local macrophages, and pulmonary fibroblasts are all primary cellular targets for ROS. In turn, they may also become a secondary source of the ROS. According to data available, ROS production might be detected or induced in almost any cell, and all lung cell types might be implicated in redox state shifts in COPD.¹⁰⁴ Generation of novel epitopes for autoimmune process might also be ROS exposure associated. Indeed, some findings suggest that ROS can modify the conformation of structural proteins leaving them exposed and immunogenic.¹⁰⁵ Sophisticated detection methods that address lung tissue architecture and assess cells in situ may reveal some complex redox state fluctuations in COPD lung.

An indoor air should also be regarded as a source of environmental ROS. It is sometimes chemically very close to outdoor air with combustion gases and exhaust particles, while sometimes it is much cleaner. However, in some cases, indoor air can become an independent pool of hazardous particles and gases. This is especially true for office spaces in high-rise buildings with parking garages or loading docks underneath. Ventilation system may become a carrier of carbon monoxide, second-hand cigarette smoke (if smokers stay at the intake openings), and other pollutants. Printers and other machines emit ozone; many types of organic chemicals are detected in the workplace as well.^{106,107} A recent study suggests¹⁰⁶ that laser printers significantly increase indoor air pollution with ozone and volatile organic compounds (VOCs), and that appropriate filters may reduce this but not the formaldehyde emissions. Authors state that there is no (and would not be) clear distinction between “dangerous” and “harmless” concentrations. The crucial preventive action should be to use low-VOC-containing materials if possible. Proper ventilation and aeration are also essential.

Moreover, office buildings are layered with pesticide-sprayed carpets; aggressive cleaning products are used creating a pool of chemicals and particles that are inhalable. In addition, VOCs and other toxic substances may be delivered by aerosol spray products, air fresheners, chlorine bleach, detergents, dry cleaning chemicals, furniture, and floor polish.^{108–111}

Building a healthier house/office also involves certain construction decisions, such as avoiding revolving door that may suck in outdoor pollutants, refusing too tight insulation, installing proper ventilation and aeration possibilities, and so-called sick buildings expose inhabitants and office workers to disease-inducing conditions that last long. Sick building-associated diseases are COPD, asthma, easy-spreading viral infections, and cancer. Understanding of “sick building” problem has started with the first outbreak of legionellosis when 182 cases of pneumonia were diagnosed to American Legion members staying in one building. Since then, understanding and policies have improved a lot, although increasing indoor work intensities, duration of time-spent indoors, outsourcing office jobs to countries with lower regulatory standards for indoor air quality, novel unsafe office supplies and materials, and several other factors contribute to increasingly poor situation with indoor air quality and morbidity caused by it. As declared by the American Lung Association in its bulletin State of Lung Disease in Diverse Communities, COPD should now be recognized as a common occupational disease.¹¹²

Unbalanced proteolysis

Unbalanced proteolysis is a long-lived and simplified explanatory mechanism to explain processes within emphysematous lung. It is becoming increasingly clear that this theory explains COPD development only partially. Proteolytic enzymes in the healthy lung are counterbalanced by antiproteases, but the balance shifts toward proteolysis when cigarette smoke exposure recruits neutrophils and macrophages able to produce large quantities of proteases and destroy the tissue and, particularly, elastin. Indeed, lung specimens from patients with emphysema have significantly decreased elastin content.⁸³ The levels of elastin degradation products may therefore serve as a good index of lung damage. In corroboration, an increased plasma and urine levels of elastin-derived peptides have been reported in patients with COPD compared with those in nonsmokers.^{113,114} However, it is also reported that elastin breakdown products are also elevated in smokers without COPD and varies independently of smoking or COPD state.^{115,116}

The role of proteolysis is proven and recognized from the very beginning, when emphysema was first described as an antiprotease deficiency-associated disease.¹¹⁷ As reported, cigarette smoke or polluted air-derived irritants attracts inflammatory cells and create an overwhelming proteolytic environment mainly comprised of neutrophil-derived protease 3 and elastase, macrophage-derived MMP-12 or so-called macrophage metallo-elastase,^{118,119} cathepsins L and S^{120,121} and collagenases MMP-2 and MMP-9.¹²² The antiproteolytic shield is in response created by AAT, secretory leukocyte protease inhibitor, tissue inhibitors of MMPs (TIMPs), and other inhibitors as reviewed by Abboud and Vimalanathan.¹²³ In addition to host inflammatory cells-derived proteolytic and inhibiting substances, bacterial enzymes and inhibitors should be considered. Accumulating data suggest that bacterial proteases contribute to inflammatory process within the lung tissue, eg, *Pseudomonas aeruginosa*-delivered elastase activates epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase (ERK) signaling pathway and enhances IL-8 production by upregulating NF-kappaB in lung fibroblasts.¹²⁴

Unbalanced proteolysis results in extracellular matrix (ECM) proteolytic degradation and fragments that may act as chemokines and promote inflammation locally.^{125,126} Specifically, laminin and fibronectin fragments are chemotactic to human neutrophils and monocytes.^{125,127} In that way, proteolysis of ECM generates fragments that may perpetuate inflammation even after smoking cessation.

Epigenetic changes in COPD development

Imbalanced proteolysis theory is also backed by data from genomic studies such as genome-wide association studies (GWAS) and gene expression studies. Recent COPD GWAS studies have identified following loci at genome-wide significance: *FAM13A* on 4q22, at the upstream enhancer of *HHIP* on 4q31, *IREB2* and nicotinic acetylcholine receptors (*CHRNA3* and *CHRNA5*) on 15q25, the 19q13 locus with genes *RAB4B*, *EGLN2* and *CYP2A6*, *RIN3* on 14q32, *MMP12* on 11q22, and *TGFB2* on 1q41.^{128,129} Yu et al have also performed a meta-analysis and indicated that *MMP12* as well as *COX2* genetic polymorphisms may be strongly implicated in the development of COPD.¹³⁰ The meta-analysis has revealed an association of six loci at disintegrin and MMP33 (*ADAM33*) gene with the risk of COPD.¹³¹ Population-scale genetic analysis revealed that the *IL1 β* (-511), (-31), and *IL1RN* (*VNTR*) polymorphisms are associated with COPD risk in East Asians¹³², *IREB2* (rs13180) is associated with COPD in Hainan population (People's Republic of China),¹³³ TT genotype of *MDR-1* gene is significantly more frequent in patients with COPD from the Aegean part of Turkey.¹³⁴ The second-generation analytical approach is a combined analysis of GWAS together with gene expression meta-analysis data. Recently, Lamontagne et al combined genome-wide genotyping and gene expression in 1,111 human lung specimens to map expression quantitative trait loci (eQTL).¹³⁵ They found that upregulated cystatin C (*CST3*) and *CD22* were associated with worse lung function in COPD. Gene expression microarray studies allow genome-wide assessment of transcriptional activity as a means of exploring the biologic pathways involved in COPD pathogenesis. Hobbs and Hersh have reviewed gene expression profiling studies performed in respiratory and nonrespiratory tissues, including lung tissue (nine studies), airway epithelium (seven studies), and peripheral blood (four studies).¹²⁸ Depending on the severity of emphysema, different pathways were found affected. Among differentially expressed pathways in the lung tissue inflammation, ECM-, and TGF-beta-related signaling were stated in two and/or more studies. In the airway epithelium studies, there was no matching in the pathways affected except oxidation (in two studies). Inflammation pathway was differentially expressed in blood samples in three of four COPD studies. Zeskind et al have compared several lung tissue transcriptome studies using computational approaches and have found some fundamental similarities and identified common biological processes underlying

COPD, despite each study having identified largely nonoverlapping lists of differentially expressed genes.¹³⁶ Computational approaches to identify gene regulatory networks are also likely to provide insights into the primary causes and secondary consequences of the complex biology that underlies COPD. Regardless of the complexity of multifactorial analysis, a promising tool would be the implication of the epigenomics into already complexed COPD data networks of genetics and transcriptomics. Examples of epigenetic changes are post-translational modifications of histones, DNA methylation, and the expression of noncoding RNAs (microRNAs [miRNAs]), which modulate gene expression without altering the sequence of the target genes. Ezzie et al screened lung samples from smokers with and without COPD for miRNA and messenger RNA (mRNA) profiles.¹³⁷ They found 70 miRNAs and 2667 mRNAs differentially expressed and proposed several miRNAs, including members of the *miR15/107* family, which deserve further investigation in the regulation of TGF-beta signaling in COPD. DNA methylation is an important regulator of gene transcription, which is strongly modulated by environmental factors. In the large-scale gene-specific investigation of DNA methylation marks that associate with COPD and limited lung function, Qiu et al have identified genes both studied in COPD in the past (such as *SERPINA1*) as well as new candidate genes (such as *FUT7*).¹³⁸ Vucic et al have performed DNA methylation and gene expression arrays on small airway epithelium from COPD subjects and controls.¹³⁹ They found 1,120 differentially methylated genes, mostly hypermethylated, which showed enrichment for three pathways: G-protein-coupled receptor signaling, aryl hydrocarbon receptor signaling, and cAMP-mediated signaling. Methylation state in 144 of these genes was negatively correlated with gene expression in three pathways: phosphatase and tensin homolog (PTEN) signaling, nuclear factor erythroid-derived 2-related factor 2 (also known as Nrf2) oxidative stress response, and IL-17F effects in allergic inflammatory diseases. A total of 15 of these 141 genes (11%) have been previously associated with COPD, at either the DNA or mRNA level, but none has been previously associated with differential DNA methylation in COPD.

Future directions from large-scale epigenetic assessments like mentioned above focus on the genes and pathways to interrogate for functional impact on COPD susceptibility and severity. The emerging role of epigenetics in COPD development stimulates new approaches. Knowledge in this field would enable reprogramming, minimizing risks, explaining etiology, and creating novel treatments for COPD.

Lung microbiome variations in COPD

Human microbiome concept pursued by National Institutes of Health-sponsored Human Microbiome Project have contributed to our knowledge about the human microbiome, and its methodologies have opened new horizons in our understanding on many human/microbial interaction aspects, including lung microflora and its physiologic and pathologic fluctuations.^{140,141} Suddenly scientists, clinicians, and educators have realized that dogma about lungs being a sterile space below the larynx is untrue and that we have denied the holistic picture of the life in the respiratory space for ages and only the pathogens were identified and studied at the certain conditions.

Today scientists are able to test and identify microbial content of human lung without getting the microorganisms out and culturing them in the laboratory. With powerful PCR technology and specific primers, the entire microorganism pool in a certain location can now be amplified. Technologies enable not only the global identification of microbiome but also potential functional abilities of the microbes identified. Together with such metagenomics, one can also expect metatranscriptomics and metaproteomics to emerge, where RNA and protein content would be selectively identified for each area of interest as suggested by Chambers et al.¹⁴² However, what works perfectly for skin or gut may fail in the lung. Lung tissue presents challenges due to its anatomy (not easy to reach), high risk of contamination from upper parts of respiratory system or mouth, and low availability of tissue explants from healthy donors. Thus, data on healthy lung microbiome composition are still under construction.¹⁴³ It is clear that multiple factors, such as anatomical architecture, varying epithelial defense system abilities, nonuniform influx of immune cells, subject's age, and sex, determine nonhomogenous microbiome within the human lung. Microbiome evolution dynamics, uniqueness, and seeding routes, ie, aspiration, inhaling, and hematogenous route, are intensively investigated by scientists worldwide. It is known that microbiota in the lower airways do not come entirely via mouth.¹⁴⁴ It is also accepted now that lung microbiota are acquired and constantly build up perinatally, ie, within the womb (under influence of placenta microbiome), during and after the birth (affected by delivery route, feeding, etc.). And the more diverse microbiota become, the better it is for the health of the lungs later in life.^{145,146} The baseline of the healthy lung microlife forms was set, and the upper and lower airways microbiota were declared indistinguishable recently.¹⁴⁷

Authors also stated that airways are inhabited by rather homogenous microbiota and that biomass decreases from upper to the lower tract. In healthy smokers and healthy nonsmokers, only small part of the upper airways, ie, mouth, is inhabited differently.¹⁴⁴ This statement comes from lung microbiome group where the largest collection of the lower respiratory tract microbiome in healthy individuals was studied and the lung microbiome was compared with the mouth for the first time. Study results show that the lung microbiome is originally separate from the mouth, that mouth microbiome differs in nonsmokers and smokers, while lung communities are not significantly altered by smoking.

Our improving understanding of lung microorganisms' ecosystem brings new explanations for microbe-related lung diseases. Apparently, we need to review the pathologic process not as a consequence of overwhelming bacterial intervention but rather as an increasing failure of normal lung ecosystem.¹⁴⁸ Lung ecosystem's capacity to adapt is based on heterogenous complex entities with complex inter-relationships and joint actions. Moreover, in the same study, lung biogeography was explained by "island model", and the environmental gradients were described together with previously unexplored positive feedback loops in pneumonia development. It is now accepted that exacerbations of COPD should be regarded as an increasingly pronounced local dysbiosis with the pathogenic bacteria focally disrupting the airway ecosystem. Host immune responses at the dysbiosis site might also become divergent and perpetuate in the so-called vicious cycle.

Attributing COPD and its exacerbations to dysbacteriosis-type diseases opens new treatment possibilities, somewhat similar to those used in other organ systems where preserving and restoring microbiome could protect against disease developing and/or exacerbating. Still too few studies are available today, and data reported need proofs and expansion. To date, several groups have started to evaluate lung microbiota content in COPD.^{141,149,150} Their main findings suggest that patients with COPD have distinct microbiome in comparison with healthy individuals with the higher prevalence of *Afipia*, *Brevundimonas*, *Curvibacter*, *Moraxella*, *Neisseria*, *Undibacterium*, *Corynebacterium*, *Capnocytophaga*, and *Leptolyngbya* genera. Significant decrease of the bacterial diversity associated with *P. aeruginosa* presence was detected in some cases.^{151,152}

In light of the above-mentioned findings, it is not surprising to see reports on corticosteroid use-associated infectious complications in COPD.¹⁵³⁻¹⁵⁶ One can also speculate that the use of antibiotics might be associated with the changes in

microbiome content in the lungs and also lung health overall, although data on this are lacking. It was reported that treatment with antibiotics decreases the abundance of Proteobacteria and suppression of other microbiota follows.¹⁵⁷ Data from other organ systems and overall understanding also suggest and antibiotic-treatment contribute as a major factor to the decreasing diversity of lung microbiome. Indirectly, this notion is also supported by the very recent data on decreasing diversity of the bronchial microbiome with the increasing severity of COPD and increasing antibiotic exposure of the patients.¹⁵⁸ A loss and replacement of microbiota by pathogenic species are also reported in the study. Similarly, in patients with cystic fibrosis, there was an increase in the total bacterial count, while microbial diversity was significantly decreased. Authors speculate that such shift might be caused by the repeated antibiotic exposure.¹⁵⁹ Data from other human organ systems, eg, gut, suggest that treatment with antibiotics significantly modulates microbial ecosystem and its interactions with host metabolism.¹⁶⁰ However, proofs of antibiotic-related lung microbiota changes would require specifically designed trials, sensitive assessment techniques, and frequent sampling. Assuming that antibiotics are most often prescribed and targeted toward various airway infections, the impact of these treatments on lung microbial ecosystem might be devastating. In addition, reports show the importance of gut microbiome on lung health, ie, antibiotic-exposed intestinal flora modulates immunity in the respiratory mucosa via the activation of inflammasomes.¹⁶¹ Moreover, airway hyper-responsiveness was reported in antibiotic-treated mice in comparison with untreated,¹⁶² specifically, when antibiotics were given perinatally, gut flora was found affected, and, subsequently, specific predisposition to hyper-responsive lung diseases was also detected. In corroboration, similar effects were proven in humans by large-scale retrospective and prospective epidemiological studies and meta-analyses, ie, children exposed to antibiotics early in life were more prone to develop bronchial asthma and transient wheezing.¹⁶³⁻¹⁶⁵ However, some confounding familial factors and confounding respiratory infections might have been involved, and the association might have been biased as a recent study that involves siblings has reported.¹⁶⁶ To further prove a link between lung and gut health and healthy microflora, several studies report COPD and inflammatory bowel disease correlation in patients and, possibly, common pathways.^{167,168} COPD-associated comorbidities, such as inflammatory bowel disease, atherosclerosis, cardiac failure, diabetes, osteoporosis, cachexia, gastroesophageal reflux disease, and depression, affect the whole human organism. Some of them are consequential

(eg, following steroid use), some etiological and, probably, dysbiosis related. The latter might be illustrated by the results from the study where smoking was shown as a modulating factor for the human gut microbiota, and potential pathogenetic link between smoking and inflammation in the gut was discovered.¹⁶⁹ It illustrates how COPD affects person's multiple body systems rendering them inflamed, dysbiotic, and metabolically disturbed.

Authors of the recent intelligent review also underscore the meaning of microbiome and virome.¹⁷⁰ The fungal composition of the respiratory tract biomass appears to be more environment- and dietary habits-related than bacterial is. In addition, fungal participants are important for the post-antibiotic treatment rehabilitation when colonization may take place due to diminished colonization resistance followed by inflammatory reactions, alteration of microbial metabolic networks, and disturbed quorum sensing.¹⁷¹ Another entity of microbiome, virome, is distinct and considerably stable between patients, including upper respiratory tract,¹⁷² and it is partially bacteriome integrated since a large number of viruses are bacteriophages.¹⁷³ Since recently, it is accepted that viruses can also be found beyond lungs and gut, ie, in the circulation of healthy individuals.¹⁷⁴ The importance of virome in disease development, expanding an auto-epitope repertoire, influencing microbial counterparts and host cells is being recognized now. Retamales et al have found that lung destruction correlates with the number of alveolar epithelial cells expressing adenoviral proteins,¹⁷⁵ which suggests that latent expression of the adenoviral protein may have a role in exaggerating inflammation. Moreover, persistent viral infection in animal model is shown to amplify lung destruction and increases cigarette smoke exposure-induced effects.¹⁷⁶

Mechanisms of microbiome–host interactions that are important in COPD remain unclear. One of the mechanisms might be dependent on the potential inhibition of macrophage immunological functions induced by cigarette smoke.¹⁷⁷ In addition; microbial colonization-related COPD exacerbations might be realized via toll-like receptor (TLR) signaling. Indeed, TLR expression on CD8 T-cells from subjects with COPD was found to be increased compared with subjects with normal pulmonary function.¹⁷⁸ Furthermore, authors show how upon stimulation with TLR ligands, COPD patients-derived T-cells increase production of cytokines, cytotoxic molecules, and chemokines compared with controls.

Major inflammatory lung diseases appear to have distinct microbiome shifts, ie, healthy airway microbiota are diverse and balanced, asthma might be characterized by dysbiosis with an outgrowth of Proteobacteria, and a shift in the proportion

of streptococci in the Firmicutes phylum, while COPD microbiome manifests with an increase in staphylococci and streptococci, in addition to an outgrowth of Proteobacteria phylum.¹⁷⁰ Knowing the microbial ecology of human lung will help us understand the pathogenesis of COPD exacerbations and disease progression in general. We still need to know what normal and abnormal lung microbiota are and if it is possible to repopulate diseased airways with beneficial microbes the same way that we manage gut dysbiosis. Hygiene hypothesis, which focuses on the deteriorating effects of nonbiodynamic diet, improved sanitary conditions, and increased use of antibiotics and anti-inflammatory agents on adequate immune maturation and balanced T-cell system leading to immune diseases, merits some reevaluation and recognition. Indeed, data from two studies (PARSIFAL – Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle) and GABRIEL (Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) reveal that children from families living in farms and adherent to anthroposophic lifestyle (low vaccination rates, low use of antibiotics and antipyretics, biodynamic food, etc.) have lower prevalence of asthma and atopy and are exposed to a greater variety of environmental microorganisms than city children.¹⁷⁹

Autoimmune COPD component

Autoimmunity is described as an excessive immune response prompted by antigens of the host organism (autoantigens). Vast variety of mechanisms are known today that expose or modify host molecules rendering them antigenic, including oxidized, nitrosylated, polymerized, citrullinated, or otherwise chemically and structurally affected proteins and peptides, mutated under the influence of environmental insults proteins, aberrantly exposed molecules (eg, in the event of abundant apoptosis) and viral or bacterial invasion-affected cells. All of those might be important in emphysema. In the review by Cosio et al, cigarette smoking is represented as a major promoter of parenchymal inflammation, and possible multiple mechanisms for the expression of novel epitopes are discussed.⁸³ Consequences of such self-attack induce lung tissue damage and further lead to structural alterations and further creation of neoantigens.¹⁸⁰ The same study reports that adoptive transfer of CD8 cytotoxic T lymphocytes specific for an alveolar neoantigen (influenza hemagglutinin) leads to progressive lethal injury and lung destruction in transgenic mice. Similarly, proofs that T-cells mediation is essential for the lung tissue injury were reported long ago, when

experiments were carried out in influenza model in T-cell-deficient mice. Experimental influenza was significantly milder and histologically less pronounced than in wild-type animals.¹⁸¹

Recognition of the autoimmune component importance in the COPD development calls for epitope(s) identification.¹⁸² Whether it is unusually exposed protein, eg, neutrophil degranulation products,¹⁸³ viral epitopes on host cell surface, apoptotic cell content exposed, or a mix of several remains to be elucidated. There is an old observation that patients with COPD blood sera reacts with bronchial secretions and the so-called mucus antibody, and this is followed by mucus accumulation and disease progression.¹⁸⁴ More recently, several reports have demonstrated that alveolar epithelium or small vascular endothelium might be the target, eg, global antiepitheial antibodies (against type II respiratory epithelium) were found in all patients with COPD in comparison with minority of controls.¹⁸⁵ Some of those antibodies were associated with decreased body mass index. And furthermore, cytotoxicity of pulmonary epithelial cells by allogeneic mononuclear cells also increased after activation by patients' with COPD blood plasma, compared with controls. Such results prove that vast array of pulmonary epithelium-directed autoantibodies are prevalent in COPD and participate in the disease development. Next to epithelium, endothelium of the lung vasculature is also exposed to autoimmune attacks. Indeed, in the unique animal model, Taraseviciene-Stewart et al have shown that xenogeneic endothelial cells injected induce autoantibody production that subsequently leads to lung emphysema in rats, involving an accumulation of CD4 T-cells in the host lungs.¹⁸⁶ Moreover, adoptive transfer of pathogenic, spleen-derived CD4 cells into naive immune-competent animal also results in emphysema. The study further proves that tissue destruction in emphysema depends (in part) on immune system and that CD4 cell-dependent mechanisms can induce emphysema development without other insults. Corroboratively, another study demonstrates that patients with COPD have significantly higher serum antiendothelial cell antibodies than subjects in the reference population.¹⁸⁷ Recently, antineutrophil antibodies directed against granule content – lactoferrin were detected in the circulation of AAT deficiency carriers, a subset of patients with COPD.¹⁸⁸ Supplementation therapy did reduce the levels of autoantibodies and the overall proinflammatory content in the patients' plasma and neutrophils. Other studies report more candidate epitopes and autoimmune activity, including antihuman cytokeratin 18 protein antibodies that were detected in patients with COPD sera in correlation with lung

functional parameters.¹⁸⁹ Antielastin antibodies have been proposed to drive COPD progression, but the data accumulated today are conflicting.^{190,191}

Environmental insults, particularly oxidative stress inducers, induce structural protein carbonylation in exposed lungs. It was demonstrated that carbonylated proteins serve as auto-epitopes in patients with COPD and in an animal model of chronic exposure to ozone.¹⁹² Cigarette smoke is also clearly associated with citrullination of proteins and contributes to the developmental mechanisms of rheumatoid arthritis. In COPD, levels of anticitrullinated peptide antibodies are also increased.¹⁹³ This response is even more exaggerated in wood smoke-induced-COPD compared with tobacco smoke-induced COPD. The important role in the disease process should be attributed to the other autoimmune disease-related autoantibodies, eg, rheumatoid factor. Recent study suggests that in COPD, rheumatoid factor antibodies and autoantibodies to heat shock protein (HSP) 70 are detectable and elevated in comparison with controls. Moreover, they remain elevated after smoking cessation.¹⁹⁴ In a complex disease as COPD, systemic autoantigens are also involved. Patients with COPD were found to have high titers of circulating antinuclear and antitissue antibodies in Spanish Phenotype and Course of Chronic Obstructive Pulmonary Disease study.¹⁹⁵ The relationship between antibody titers and lung function was also demonstrated. There is a category of nonspecific autoantibodies, which are present in everyone's circulation and may play a role in disease development as suggested by recent study.¹⁹⁶ Moreover, the amount of IgG antibodies recognizing lung tissue components was significantly lower in the circulation of COPD compared with nonsmoking control subjects, and the target for some of those antibodies was identified as collagen-1. Thus, authors speculate that naturally occurring antibodies or rather lack of them is caused by lower availability due to their increased reactivity or abundance of their epitopes. Whether their role is destructive or beneficial remains to be elucidated.

All these data stimulate discussion if autoimmune process in COPD is a causative process or just a consequence or even a coincidence. Lymphoid organ neogenesis and persistence in smoke-exposed mice lungs suggest that autoimmune process is causative rather than simple coincidence.¹⁹⁷ This notion is further supported by the fact that the subset of lymphocytes, particularly CD56 lymphocytes, is able to rapidly kill autologous lung cells. It was a spontaneous process, and it was upregulated in subjects with severe COPD.⁸⁶ There is an individual variability and, probably, individual COPD-related epitope array in every case. In the future, revealing

specific autoantibodies for each patient or patients' group might initiate possibilities to diagnose and treat etiologically distinct COPD entities individually.

At the crossroads of microbiota and autoimmunity theories, virus- and other microorganism-associated epitopes emerge. Viral epitopes, ie, viral peptides presented on the cell surface by major histocompatibility complex molecules, signal for the destruction of the infected cells. Chemical modifications of viral peptides and proteins may be crucial for their inadequate proceeding by host cells, generation of new epitopes, and increase in antigenic insult. Human organism as a habitat for numerous micro-roommates goes into disease as a whole system, and two main hypotheses are proposed to explain the interrelationships and predisposition to the disease. The hygiene hypothesis suggests that extensive microbial exposure in early childhood is essential for proper immune-regulatory mechanisms to engage and control auto-immune reactions and/or allergy. And in parallel, the triggering hypothesis suggests that specific microbes, particularly, viruses, may trigger autoimmunity.¹⁹⁸ Similarly, enteroviral infections cause autoimmune diabetes in animals and are associated with an increased risk of autoimmune diabetes in epidemiological and other studies.¹⁹⁹ And opposite, the same review discusses protective effects of microbes that have been studied in animal models and in epidemiological studies, ie, hepatitis A virus and *Helicobacter pylori* persistence is associated with lower risk of autoimmune diabetes.

Inefficient repair, current therapeutic approaches, and study models

It is increasingly clear that slowing down the progression of the disease might be very complicated due to all ongoing chronic inflammatory, proteolytic, autoimmune, and dysbiotic processes reviewed earlier. In addition, it is generally accepted that repair mechanisms in emphysematous lung are deficient. The exact reasons for that are not known. Some compensatory lung tissue regrowth is possible, and several factors, such as, thyroid transcription factor 1, vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1, and keratinocyte growth factor, are known to be responsible for it.^{200–203} Central process for the repair according to some authors is epithelial–mesenchymal transition (EMT).²⁰⁴ Particularly, epithelial cells participate in EMT and restores integrity directly.²⁰⁵ In the elegant study by Barkauskas et al, a reparative behavior of type II epithelial cells in the alveoli was studied.²⁰⁶ They show that type II epithelial cells can self-renew in culture and differentiate into alveolar-like

structures “alveolospheres” containing type II and type I epithelial cells. In alveolar tissue context, MSC also has been shown to contribute to regeneration in numerous experimental settings, ie, home to sites of asbestos-induced lung injury, contribute to tissue remodeling in a rat monocrotaline model of pulmonary hypertension, decrease chronic airway inflammation in ovalbumin model of asthma, restore alveolar and lung fluid balance after endotoxin-induced acute lung injury, and modulate the fibrotic response to radiation-induced injury.^{207–214} A new study shows that transplanted MSC is capable to integrate into damaged (pulmonary fibrosis model) mice lungs and differentiate into type II lung cells expressing surfactant proteins.²¹⁵ Moreover, in the cigarette smoke induced rat emphysema model, amniotic fluid-derived MSC has generated type II alveolar epithelial cells, integrated at the sites of destruction and induced local regeneration of the lung alveolar epithelium.²¹⁶ Promising potential of MSC for the repair of the lung tissue might be harnessed in several ways: via endogenous activation and recruitment, delivery from outside via circulation, or via airways. The latter possibility was explored in the new study, and authors have demonstrated that intratracheal MSC engrafts at the hyperoxia-induced dysplasia sites in the rat lungs and expresses surfactant proteins.²¹⁷ However, authors claim that therapeutic benefit was achieved due to paracrine mechanisms, since engraftment was very low. Moreover, this and other studies show that MSC secretion products reduce structural lung cells apoptosis, accelerate healing, and enhance endothelial functions.^{217,218} Reports show that bacteria-induced tissue damage promotes the airway engraftment of heterologous bone marrow–derived stem cells and their epithelial transformation.²¹⁹ The ability of MSC of various origins to transform and express phenotypic markers of airway epithelium and to participate in airway remodeling in vivo is documented.^{219,220}

Since cures and regeneration induction strategies are still in infancy, the best treatment is prevention. In this context, healthy living, clean air, proper use of antibiotics, and adequate exposure to microorganisms become increasingly important. Physical activity was also reported as a factor associated with lower rates of hospital readmission in patients with COPD.²²¹ Also, it should be clear that current therapeutic approaches needs constant revisions and reapproval. It is particularly important for oral corticoids, antibiotics, and anti-inflammatory agents. If we picture COPD as dysbiosis, the quality and diversity of microbiome in the human airways become highly valuable. From the birth and with the every breath after birth, microbiome deserves protection since it

comprises an essential part of our own health. In addition to balanced microbial ecosystem, there is also discussion on dietary interventions, ie, increasing antioxidant content.²²² Interestingly, living in altitude might also be a therapeutic approach depending on the lung disease.²²³

To date, many COPD (both emphysema and bronchitis component) models are in use and different approaches are employed, ie, protease challenge,^{224,225} toxic gases,²²⁶ and cigarette smoke.²²⁷ In addition, numerous species have been used to study of COPD, eg, rats, mice, dogs, guinea pigs, monkeys, and sheep as reviewed by Mahadeva and Shapiro.²²⁸ Cigarette smoke-exposed mouse remains the most often used in vivo model. Relatively low costs of the animals and the molecular biology tools available make this model attractive. However, detailed local cellular involvement (migration of neutrophils, macrophages, T-cells, release of proteases, and various proinflammatory molecules), loss of epithelium and endothelium, transformation of the mesenchyme, and ongoing repair processes are easier to study and visualize in larger models in vivo or, also, in vitro. Primary cells obtained ex vivo play a crucial role in COPD research by helping to assess the differential behavior of cells from diseased and healthy persons. In addition, a number of immortalized cell lines derived from carcinomas or transformed from primary tissue are in use, eg, A549 (type II pneumocyte), several bronchial epithelial cell lines.²²⁹ These cells exhibit phenotypic characteristics of their original cell type and are easy to use and well documented. In addition, they enable to easily reproduce results. However, all ex vivo tissue cultures have their shortcomings, eg, lack of the native environment, contacts with the ECM, the inflammatory ambience, and interactions. Lung-on-chip technologies enable high throughput and convenient cytotoxicity and genotoxicology assays. However, they lack epithelium–ECM interactions and discrete spatial architecture that defines parenchymal structure and function within the lungs. There are few attempts reported to use native lung matrix, ie, decellularized and reseeded lung tissue matrix. Such modeling provides with relevant structural and biochemical scaffold for the tissue cultures and greatly resembles in vivo situation. However, native organs require specific processing and are complicated to handle. With all the possibilities available, evaluation and choice of the most relevant study model depends on the individual project's aim, capabilities, and complex questions that are going to be addressed.

Conclusion

COPD as a lifestyle and/or occupational disease should be regarded as a complex problem related not only on what

we breathe in but also on what we eat, how we move, what are we treated with, and so on. With all these factors in mind, we should direct our efforts toward prevention and regeneration, since symptomatic treatment of the disease symptoms has led us nowhere. Balancing immune response in close relation to maintaining a diverse microbiome (ie, low antibiotic exposure), elimination of air pollutants and smoke (safe smart homes and offices, air pollution management policies, etc.), full resolution of inflammatory processes (limited anti-inflammatory medication use), healthy diet with good antioxidant load, and physical activity are among the measures that need to be taken into consideration when managing COPD. Similar research lines should follow.

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