

Review article

J. DOMAGALA-KULAWIK

EFFECTS OF CIGARETTE SMOKE ON THE LUNG AND SYSTEMIC IMMUNITY

Department of Internal Medicine, Pneumology and Allergology,
Warsaw Medical University, Warsaw, Poland

The influence of tobacco smoke on human health is still an important problem worldwide. Complex inflammatory processes and changes in the immune system are crucial in the pathogenesis of smoking related disorders like chronic obstructive lung disease (COPD), lung cancer, atherosclerosis. The objective of this review is to present the alterations in the immune system in smokers. The main affected system by cigarette smoke (CS) is the respiratory tract. In bronchial epithelium metaplastic and dysplastic changes are accompanied by elevated expression of adhesion molecules and secretion of many cytokines capable of stimulation immune cells influx. In the population of pulmonary macrophages an elevated proportion of cells, changes in expression surface markers with impaired phagocytic and antigen presenting function are observed. Chronic exposure to CS causes increased production of metalloproteinases (MMP) by macrophages and proteolytic enzymes by neutrophils. These enzymes cause destruction of alveolar wall. Increased apoptosis of lung tissue results in augmentation of foreign material which may play a role of autoantigen and which is a target for cytotoxic/suppressor cells. The role of regulatory T (Treg) cells in this process is recently postulated. Smoking cessation is the most effective method of prophylaxis and treatment of diseases related to tobacco smoking. However many immunological changes in smokers are not completely reversible after quitting smoking.

Key words: *immunity, lung, lymphocytes, macrophages, neutrophils, smoking*

INTRODUCTION

Tobacco smoking is one of the main health problems of the 19th and 20th century. It is a causative agent of such serious diseases as lung cancer and

chronic obstructive lung disease (COPD) (1). Currently about 1 billion males and 250 million females across the world smoke. Smoking is said to be associated with about 50% of deaths due to cardiovascular and pulmonary diseases (1). In a year 2000 smoking was responsible for 3.84 million deaths in males and 1 million in females worldwide (2). The most striking pathological changes related to tobacco smoke are observed in the respiratory tract and, among them, the influence on immunological status may be considered as essential. These processes are crucial in the pathogenesis of COPD and cancer spread. The aim of this article was to review the main immunological changes caused by tobacco smoke.

Characteristic of cigarette smokers and distribution of smoke particles in the respiratory tract

Thousands of substances which are the compounds of tobacco smoke are inhaled directly or as the products of high temperature combustion on the end of the cigarette. The final effect of the influence of smoke depends among others, on environmental conditions. Local homeostasis, genetic predisposition, local character of cytokine network, and pathological conditions should be taken into account during analysis of cigarette smoke (CS) influence on the structural elements of respiratory tract.

In vivo studies in healthy, asymptomatic smokers are not numerous and the investigated groups consist only of 10-20 persons. The results of the studies on immune changes in COPD patients should be interpreted with caution and can not be identified with those in smokers without airflow limitation: not all elements of the inflammation in COPD are related only to smoking (3). Additional data concerning influence of tobacco smoke on the changes in the respiratory tract were achieved from the studies performed in lung cancer patients. The impairment of host defense and an enhanced immunosuppression are the common features of the immune status of patients with lung cancer. We found that healthy smokers have more intense signs of activation and suppression of some immune reactions than patients with malignant disease (4, 5). *In vivo* studies are complex and are affected by many agents: genetics, environment, treatment, infections, and others (pollutants, drugs), etc. In these studies the number of mean pack/years smoked is used in statistical analysis. For the analysis of the metabolism of smoke constituents the following biomarkers may be measured: exhaled carbon monoxide (CO), blood nicotine level, level of cotinine in the urine or biomarkers which evaluate the biologically effective dose on molecular level: DNA, RNA aberrations (6).

Changes observed in peripheral blood represent mainly systemic inflammation. Local changes in respiratory tract may be evaluated by spontaneous or induced sputum (IS), bronchoalveolar lavage (BAL), or bronchial, surgical biopsy. Cells in the BALF and IS represent exfoliated

population and it is not sure if they are representative of inflammation in lung tissue. More accurate and representative data may be obtained from the examination of lung biopsy. However, it is difficult to find a reason to perform invasive investigations in asymptomatic, healthy smokers. Surgical biopsies from the 'healthy' lung in patients with lung cancer are a doubtful model of changes occurring in healthy smokers. Many studies have shown that both lungs form an integral immunological body.

We use the term 'asymptomatic smoker' to distinguish healthy smoker (without clinical symptoms of chronic bronchitis and with normal values of pulmonary function tests) from smoker with signs of COPD (7). However, the line of division is difficult to define because of the possibility of asymptomatic bronchiolitis (8). The role of tobacco smoke in the pathological processes of small airways was documented (9, 10). In many chronic smokers bronchiolitis may develop without symptoms and/or airflow limitation. In a large study on 110355 subjects (64% were of them were active smokers, 25.1% ex smokers and only 10.9% never smokers) Zielinski *et al* (11) noted that one third of the subjects with airflow limitation did not report any respiratory symptoms. Egberg-Jansson *et al* (7, 13-15) presented series of very interesting articles on a large group of 60 years old 'healthy' smokers and found a significant correlation between discrete symptoms and the number of pack/years smoked.

CS consists of about 4000 substances known to be antigenic, cytotoxic, mutagenic, and carcinogenic (16). These substances are mainly dispersed in the gas phase. The particle phase contains important constituents of CS, such as tar, nicotine, aromatic hydrocarbons, phenol, and cresol. The mean size of those particles is 0.1-0.5 μm , so they are capable of reaching small airways. 10-30% of the particles are deposited in the lungs. 40% (as documented in inhaled radiolabeled particles measurements) to 90% (in mathematical models) of these are deposited in the gas exchange region. CS contains a high concentration of reactive organic radicals (RORs) and substances producing RORs. RORs are formed on the top of the cigarette in high temperature and are present in the side stream of smoke. The side stream of smoke contains not only the gas phase of exhaled smoke, but also the products of combustion on the top of a cigarette. Therefore, persons exposed to environmental tobacco smoke (ETS) are exposed to up to 50 times higher concentration of some chemicals than smokers themselves. The effects of CS are synergistic of other pollutants, what should be noted, as many manual workers are smokers. The synergistic effects of CS and asbestos on oxidant induced changes in the cascade of cell signaling pathways leading to phenotypic and functional endpoints in bronchial cells were recently presented by Mossman *et al* (17). Also drug addicts are often smokers and it was documented that marihuana has similar effects on respiratory tract and is synergistic of CS as showed by Wallace *et al* (18).

Bronchial epithelium-loss of integrity

In normal circumstances glandular epithelium cells form an integrated structure with specific and functionally proper proportion of ciliated cells, goblet cells, and basal cells. An appropriate ciliary movement enhances effective host defense and is necessary in the cleansing processes of the epithelium. Injury of bronchial epithelium was widely described in many studies concerning the CS influence on the respiratory tract (19). In structural changes of bronchial epithelium, a very important role play basal cells, a population serving as potentially capable of differentiating and which may proliferate in many pathological circumstances. Additionally residual basal cells are capable of releasing such inflammatory cytokines as interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), and of upregulating the function of transforming growth factor- β (TGF- β) (20, 21).

Bronchial epithelium serves not only as a structural barrier in the respiratory tract, but also has a functional role in the defense against foreign agents. This is ensured by the antigen presenting function, expression of adhesion molecules, and capability of differentiation. CS enhances excretory function of bronchial epithelium. An elevated concentration of peptides, amines, expression of MHC, and proinflammatory cytokines: IL-8, IL-6, GM-CSF was noted (19). Augmentation of inflammatory cells in bronchial wall was observed as a result of an increased expression of adhesion molecules and increased permeability and loss of cellular integrity (12, 22). Recently, a role nuclear factor kappa beta (NF- κ B, transcription factor) in the activation of lymphocytes at the site of inflammation was documented (23).

Lung epithelium, as a functionally active unit, once activated by CS and RORs promotes systemic inflammation by cytokine release, stimulation of the liver to produce CRP and of bone marrow to produce leukocytes and platelets. Increased expression of membrane attack complex (MAC1, CD11b+/CD18+) on bronchial epithelial cells and increased expression of its ligand intracellular adhesion molecule (ICAM 1) on vascular endothelium were observed in smokers and in patients with ischemic heart disease (24). Hypoxia induces loss of alveolar capillaries, and a reduction of vascular endothelial growth factor (VEGF) and its receptors was observed in the emphysematous lung (25). These mechanisms may explain the possible participation of lung epithelium in the cardiovascular disorders observed in smokers.

Bronchial epithelium plays an important role in the defense against infections. This is accomplished due to inflammatory mediators, chemotactic mediators, and antimicrobial substances, which are identified in airway epithelium. Their release may be modified by CS. Many receptors are involved in the recognition of microorganisms. Toll-like receptors (TLR), which are expressed on bronchial epithelial cells, belong to such family (26). Disruption of the cellular integrity,

changes in the profile of cytokine network, and increased inflammation facilitate the invasion of microbes through the bronchial wall in smokers.

Most of 'healthy' smokers complain of expectoration. Increased mucus production is caused by structural changes, such as goblet cell hyperplasia, glandular metaplasia, and chemical composition of the mucus in smokers. It has been found that CS activates the epidermal growth factor receptor (EGFR) which regulates mucin production in smokers (27).

Macrophages-depression of function

Macrophages are the main lung cell population which serves as the first line of cellular defense against pollutants due to their antigen presenting function and phagocytic properties. Lung macrophages are divided into the population of alveolar macrophages (AM) and pulmonary macrophages (PM). CS particles (the main component of particles is kaolinit) are visible in a light microscope in the cytoplasm of AM, even after a short period of tobacco use and this feature persists after smoking cessation, up to 2 years (28). CS causes an influx of AM into the airways lumen and a 2-3 times increased number of AM in the BALF of smokers is commonly observed (4). Apart from changes in the morphology and the number of AM, impaired function of these cells has been observed in smokers (29). In general, macrophages obtained from tobacco smokers are less mature, have elevated expression of CD14 (monocyte marker), have a condense cytoplasm, and are hyperdense. Antigen presenting function of smokers' AM has been proved to be impaired (29) and the metabolic activity to be weak (28). AM in smokers have been found to have an increased suppressive effect on natural killer (NK) cells (30). Different effects of CS were reported depending on the dose and time of exposure. Decreased secretion of IL-1, IL-6, IL-8, and TNF- α by AM (31, 32) and impairment of AM phagocytic function has been observed (33). Chronic effect of CS seems to result in an increased secretion of cytokines and chemoattractants to other inflammatory cells (34). Among others, phagocytosis of apoptotic material by AM gives a signal to the secretion of IL-8, leukotriene B4 (LTB4) and TGF- β (27). *Fig. 1* presents the role and place of a macrophage in the series of inflammatory pathways caused by smoking.

As bronchial epithelial cells, macrophages play an important role in the defense against microorganisms. A high level of lipopolisaccharyde (LPS) endotoxin is inhaled during active smoking. ETS also contain LPS: Larson *et al* (35) showed that smokers' environment contains 120 times higher level of endotoxin than the smoke free indoor air. Such circumstances cause a continuous stimulation of AM. It may explain a reduced immune response to infectious agents in smokers, which was noted many years ago (36). Droemann *et al* (37) demonstrated that AM from smokers had a decreased surface expression of TLR2, compared with nonsmokers. Recently, it has been found that maternal smoking during pregnancy can change the toll-like-receptor innate neonatal immune response (38).

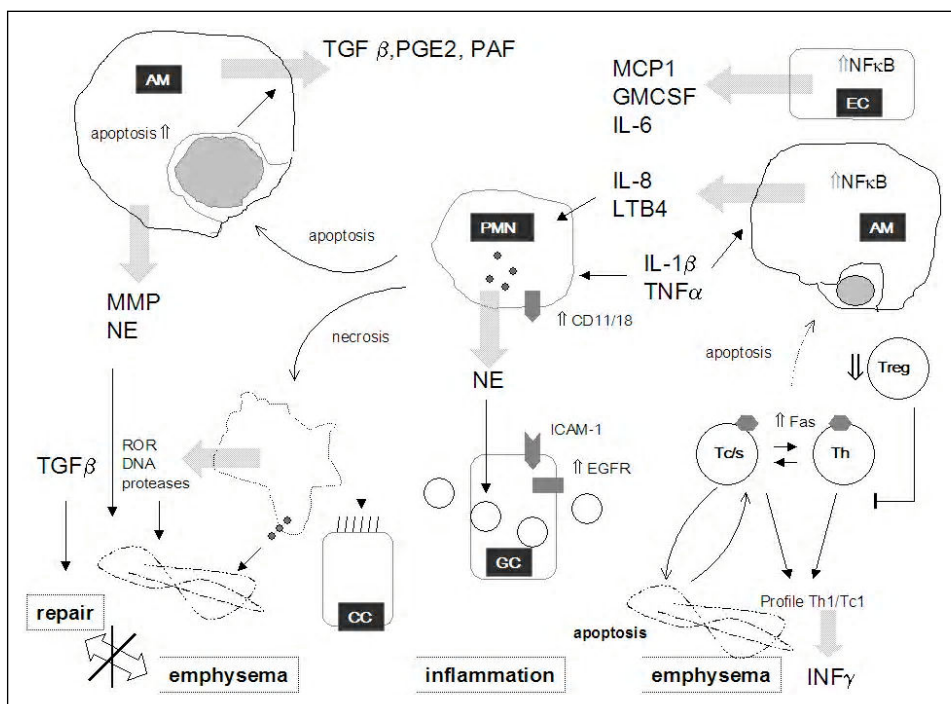


Fig. 1. The main pathways and mechanisms of altered immunological reactions in smokers; modified from (84). \uparrow, \downarrow - changed expression, wide arrows indicate the influence of cigarette smoke. Abbreviations: AM-alveolar macrophage, CC-ciliated cell, EC-epithelial cell, EGFR-epidermal growth factor receptor, GM-CSF-granulocyte macrophage colony stimulating factor, GC-goblet cell, ICAM-intracellular adhesion molecule, IL-interleukin, LTB4-leukotriene B4, MCP-monocyte chemoattractant protein, MMP-metalloproteinases, NF-κB-nuclear factor kappa beta, NE-neutrophil elastase, PAF-platelet activating factor, PMN-polymorphonuclear neutrophils, PGE-prostaglandin, ROR-reactive oxygen radicals, TGFβ-transforming growth factor-β, TNF-tumor necrosis factor, Th-T helper cell, Tc/s-T cytotoxic/ suppressor cell, and Treg-T regulatory cell.

Phenotypic characteristics of AM are difficult, since these cells are labile and are observed *in vivo* in very different stages of activation, maturation, and function. This may cause different results in the studies of macrophage phenotypes with the use of immunocytochemistry and flow cytometry, which calls for special attention in data interpretation (39). It should be mentioned that the antigenicity of AM may be changed by phagocytosis of other live, apoptotic, or necrotic cells and fragments of cells. CD68⁺ was commonly used as a marker of mature AM. Elevated proportions of CD68⁺ cells which correlate with epithelial desintegration and the proportion of cells positive to IL-1β in the bronchial biopsies of healthy smokers has been observed (12, 40). In one study, a proportion of AM with expression of CD11b⁺, CD54⁺, and CD71⁺ in induced sputum has been increased in asymptomatic smokers when compared with

nonsmokers (41). Moreover, we found that these changes are stable and persisted after smoking cessation in smokers with COPD (42). AM of healthy smokers are characterized by elevated CD14⁺ expression, which may indicate an influx of young form of cells from the circulation (41).

Macrophages play an important role in the pathogenesis of smoke-induced emphysema. Elastolytic enzymes released by macrophages have destructive effects on the airway wall. Chronic CS exposure causes increased production of metalloproteinases (MMP) by macrophages. These are proteolytic enzymes and their augmented release may be responsible for lung tissue destruction (25,34). MMP-2, MMP-9, and MMP-12 are involved in the pathogenesis of emphysema. MMP are additionally capable of modifying the function of: TGF- β and IL-1 β -cytokines involved in the destruction of lung tissue. The proper balance between MMP and its inhibitor-TIMP is necessary for saving lung tissue structure. AM of smokers release less TIMP1 than of nonsmokers and so disrupt normal MMP/TIMP balance (34,43). As was recently shown CS also inhibits lung repair (44).

Apart from lung cancer and COPD, smokers may be affected by smoking-related interstitial lung diseases (45). Desquamative interstitial pneumonia (DIP) and respiratory bronchiolitis-interstitial lung disease (RB-ILD) are classified as a form of idiopathic interstitial pneumonias. The main histological finding in DIP is the augmentation of pigmented macrophages within the alveolar space.

Apoptosis and circulation of lymphoid cells

Augmentation of lymphoid cells in the airways of smokers indicates the necessity of the analysis of circulation of these cells. The mean life span of macrophages is about 80 days, but in smokers this period is much longer (34). On the other hand, Aoshiba *et al* (46) found that CS and oxidative stress enhance the apoptosis of AM and that antioxidants suppress CS-induced apoptosis (46). Increased expression of receptors of programmed cell death was observed in smokers on lymphocytes, neutrophils, and AM (27, 46, 67). Hodge *et al* (21, 47) found increased rate of lymphocyte apoptosis and expression of Fas receptor on CD4⁺ and CD8⁺ lymphocytes in patients with COPD. We observed an elevated proportion of Fas⁺ lymphocytes in smokers with a significant correlation of Fas⁺ lymphocytes with pack/years smoked (48).

CS is capable of induce apoptosis of the bronchial epithelial cells and of the alveolar wall (21, 49). The role of TGF- β in this process has been postulated. Apoptotic epithelial cells may act as an antigen and may stimulate the augmentation of T cytotoxic/suppressor T cells (Tc/s,CD8⁺), which play a crucial role in the pathogenesis of emphysema in smokers (50, 51). The augmentation of the apoptotic material may cause impaired phagocytosis and could change the pathway from apoptosis to necrosis causing persistent inflammation in smokers who develop COPD (21, 27). The apoptosis of neutrophils (PMN) seems protective in development of chronic inflammation. Phagocytosis of apoptotic PMN protects against the release of neutrophils' proteolytic enzymes. Increased

necrosis of PMN dominates in smokers and may cause increased levels of enzymes with destructive properties for the lung tissue (*Fig. 1*) (27).

Neutrophils, oxidative stress

Leukocytosis is the main immune alternation observed in the systemic circulation in smokers (52). A common feature is the increase in the proportion of PMN in the induced sputum, bronchial biopsies, and BALF of smokers (12, 29, 42), even after a short time of smoking (29). In many studies, increased concentration of PMN products: IL-8, TNF- α , and neutrophils human lipokaine (NHL) has been reported (12, 13). The life span of PMN is short and these cells are capable of fast migration. The influx of PMN into the airways is a nonspecific reaction. The main chemoattractants for these cells are IL-8 and LTB₄ and the adhesion molecules expressed on the bronchial epithelial cells with a crucial role of MAC1/ICAM1 axis for the PMN migration. Smokers' PMN demonstrate increased surface expression of MAC1 CD11+/CD18+ (53). Oxidative stress causes elevated concentration of cytokines which are capable of PMN activation, and which prolong the life of these cells (27).

Augmentation of neutrophils in the airways of smokers causes increased levels of proteolytic enzymes: neutrophil elastase (NE), cathepsin G, and protease-3. Neutrophil proteases stimulate mucin release by goblet cells. They also have a destructive effect on ciliated cells and are capable of destroying the extracellular matrix. In normal circumstances, PMN release small amounts of enzymes. Oxidative stress and CS directly stimulate PMN mucin production by activation of the epidermal growth factor receptor (EGFR). A second way of increasing proteolytic enzymes release is necrosis of neutrophils and a failure to phagocyte dead cells by AM observed in the lumen of smoker and COPD patient airways (27). Active smoking changes the phenotype of inflammation from eosinophilic to neutrophilic inflammation with increased concentration of IL-8 in asthma patients (54). The influence of active smoking and ETS needs to be taken into consideration in severe asthma.

Eosinophils influx

Many data suggest that eosinophils are involved in the inflammatory pathways in smokers. Their influx into the place of inflammation may be an unspecific reaction (53). The presence of eosinophils in the submucosa was documented in bronchial biopsies of smokers (55). In the author's studies, an elevated proportion of eosinophils has been found in induced sputum of active smokers with COPD compared with ex-smokers (42). Amin *et al* (12) found significantly elevated proportions of cells positive to eosinophil peroxidase (EPO) in biopsies of healthy smokers compared with non-smokers (12). Influx of eosinophils into the airways of smokers seems to be a rapid reaction and the number of these cells normalizes

after smoking cessation in asymptomatic smokers and in patients with COPD as well (42, 56)

Lymphocytes - cytotoxic phenotype

A common feature of persistent inflammation in the airways of smokers is infiltration by lymphocytes. BALF and bronchial biopsies taken from smokers are characterized by an increased proportion of Tc/s cells and a significant decrease of CD4+/CD8+ ratio (18, 19, 29, 36, 57, 58). An increase in CD8+ population in BALF is observed after a short time of smoking and correlates with pack/years smoked (58). It has been postulated that smokers with elevated proportion of CD8+ cells are a population at high risk of developing COPD, with a genetic predisposition to such disorder. In the interesting studies by Egberg *et al* (14, 15), the CD8+ cell infiltration in bronchial biopsies correlated with symptoms reported as 'trouble with breathing' by healthy smokers and with FEV₁ of these subjects.

The population of Tc/s cells has been shown to have destructive effects on lung tissue and is important in the development of emphysema (49, 50). Increased levels of proteolytic enzymes in smokers cause destruction of alveolar epithelium. CS is capable of activating the proapoptotic mediators and thus enhances the apoptosis of the alveolar wall, which is a target for Tc/s cell cytotoxic attack. A role for regulatory T (Treg) cells in this process is recently postulated (59). Increased regulation by Treg cells results in the accumulation of both CD8+ and activated T helper (Th, CD4+) cells (60). Th cells regulate the maturation, circulation, and apoptosis of Tc/s cells and the coexistence of these two lymphocyte subpopulations is indispensable in health and disease (61). Of the three subtypes of T helper cells, Th1 seems to play an important role in the pathogenesis of lung inflammation in smokers. Th1 are known to release IFN- γ , the expression of IFN- γ is regulated by transcriptional factor - signal transducer and activator of transcription (STAT4). Increased expression of nuclear and cytoplasm STAT4 in biopsies of smokers has been observed by di Stefano *et al* (40).

The influence of CS on other lymphocyte subtypes is less known. Data reported by many authors differ because of different cell types defined as activated and rather small groups of patients investigated (4, 14, 62, 63). Ekberg-Jansson *et al* (14) observed a decreased proportion of activated lymphocytes: CD28+, CD 69+ and CD 57+ in BALF of asymptomatic smokers (14). In our studies, we defined activated lymphocytes as those having expression of HLA DR or CD25 and we have found an elevated proportion of such lymphocytes in the BALF of smokers, compared with nonsmokers (*Table 1*) (4). In a study of Egberg-Jansson *et al* (14), the proportion of CD3+/CD25+ in the BALF of smokers is higher in smokers when compared with nonsmokers, which is in agreement with our results (14).

CS has been shown to have a suppressive influence on the number (63) and function of NK cells in smokers (30). Lymphocytes B are not numerous in the

Table 1. Subpopulations of lymphocytes in the bronchoalveolar lavage fluid and peripheral blood of smokers and nonsmokers (*author's results*).

Lymphocytes subpopulation	BALF smokers n= 18	BALF nonsmokers n= 12	Peripheral blood smokers n= 16
T cells	92.5	90.8	72.0
CD 3+	(81.5-93.6)	(88.1-92.5)	(67.0-76.9)
B cells	0.8	1.2	7.0
CD19+	(0.0-1.3)	(0.9-2.0)	(6.7-8.6)
NK cells	6.9	8.8	14.5
CD3+ CD16+56+	(4.9-16.0)	(5.3-10.2)	(10.9-19.4)
T helper	46.9*	62.7*	35.0
CD3+ CD4+	(33.2-54.8)	(53.5-70.6)	(26.8-42.0)
T cytotoxic/suppressor	39.4*	31.0*	36.0
CD3+ CD8+	(31.8-47.7)	(24.8-38.5)	(27.0-36.7)
Activated T cells	34.5*	17.5*	13.5
CD3+ HLA DR+	(15.5-48.6)	(9.6-21.0)	(6.8-25.5)
T cytotoxic cells	11.6*	5.9*	6.2
CD3+ CD16+56+	(6.0-16.0)	(3.8-9.3)	(2.5-13.0)
T with IL2- receptor	10.5	7.5	5.1
CD3+ CD25+	(7.0-12.4)	(3.7-10.2)	(3.0-8.0)

Data are expressed as median (p25-p75). BALF- bronchoalveolar lavage fluid, *P<0.05 (Mann-Whitney test).

bronchial lumen. Recently an augmentation of B cells in peripheral airways of smokers has been noticed (64).

The described above local changes in the lung environment, which forms an integrated and specific immune system, are not fully reflected in the systemic immunology. In general, many important differences in the same patient between the signs of inflammations in the peripheral blood and BALF or induced sputum have been reported (7, 65). Analysis of immune cells in circulation is representative for cells 'in transit'. Recently, many data confirm the role of systemic inflammation in COPD and the augmentation of neutrophils in the circulation of patients with COPD is found (66, 67). However, little is known about the role of lymphocytes in systemic inflammation caused by CS. In a recent study, we found an elevated proportion of Tc/s cells and a lower CD4+/CD8+ ratio in peripheral blood of smoking patients with COPD (48). Similar results have been reported by other investigators (62, 68). However, other authors reported an elevated proportion of T and Th cells in the peripheral blood of smokers (69, 70).

CS is one of many risk factors leading to atherosclerosis. Systemic inflammation in tobacco smokers activates macrophages and lymphocytes to produce proinflammatory cytokines like IL-1 β , TNF- α , INF- γ , and IL-6 which cause endothelial cell activation with increased expression of ICAM, vascular cell adhesion molecule (VCAM), and plasminogen activator inhibitor type 1

(PAI-1) and promote the prothrombotic state and atherosclerosis plaque production (25, 71, 72). Some similarities are found in the immune reactions in smokers with COPD and other chronic systemic diseases, such as diabetes, atherosclerosis, osteoporosis, and peptic ulcer. In these conditions, a crucial role is played by TNF- α and nuclear factor κ B (NF- κ B) to stimulation production of cytokines (72-74)

Cytokines - inflammatory pattern

A large network of pulmonary and systemic cytokines is involved in chronic inflammation of smokers. Most of them were presented above. IL-6, IL-8, IL-1 β , and TNF- α are active in the inflammation in smokers; locally, in the respiratory tract, and in the circulation as well. Chemokines exert the effects on target cells *via* binding to cell surface receptors. Each chemokine receptor has a distinct chemokine and leukocyte specificity. Chemokines and their receptors play a role in the selective recruitment of various immune cell subtypes. IL-8, an active proinflammatory cytokine in smokers, activates neutrophils *via* the CXCR1 and CXCR2 receptor. In the bronchial epithelial cells of smokers, high reactivity for CXCR3/ CXCL10 has been found. Expression of some types of CXCR3 and CCX5 characterizes the Th1 subtype. Participation of Th1 in the inflammation in smokers' lung has been described before.

An important transcription factor involved in the regulation of inflammatory genes in smokers is NF- κ B. NF- κ B regulates the genes for IL-1, IL-6, IL-8, MCP1, TNF- α , endothelin-1, and ICAM 1 (22). Di Stefano *et al* (23) showed a significantly increased proportion of T cells and epithelial cells expressing p65 (the major subunit of NF- κ B) in smokers. They concluded that CS activates bronchial epithelium and the epithelial cells are the source of NF- κ B (23). CS-induced activation of NF- κ B in human lymphocytes has been evidenced in other studies (75, 76). Moodie *et al* (77) showed that oxidative stress, H₂O₂, and TNF- α induce release of IL-6 and IL-8 and cause increased activation of NF- κ B. Activation of signal transcription pathways leads to an epigenetic change: histone acetylation. Acetylation of DNA fragments is thought to be related to the regulation of gene transcription in epithelial cells and leads to sustained gene transcription of proinflammatory genes. Histone acetylation is reversible and regulated by histone acetyltransferases (HATs) and histone deacetylases (HDAC). Decreased HDAC2 in smokers has been found (78).

Smoking cessation - what benefit?

The role of smoking cessation in slowing the detriment in airflow limitation observed in pulmonary function tests, particularly in FEV₁, in lung cancer incidence, and in reduction of cardiovascular mortality is well documented (79-81). These epidemiological observations have been made in patients and little is known about the possible benefits of quitting smoking in restitution normal

immunological homeostasis in healthy persons. Willemsee *et al.* (82) concluded that in healthy smokers subtle changes are partially reversible. The number of AM normalizes after smoking cessation (83), but some of functional features of inflammatory cells do not. We found no differences in the cell numbers and macrophage phenotype in current and former smokers, apart from the normalization of the proportion of eosinophils in induced sputum of smokers with COPD (42). Willemsee *et al.* (82) also noted normalization of the eosinophil number in ex-smokers. Other elements of inflammation in COPD remain unchanged (42, 47), which seems to confirm participation yet other than tobacco smoke agents in the pathogenesis of COPD (42, 84-87)

Concluding remarks

In *Fig. 1* are presented main pathological pathways and mechanisms of altered immunological reaction in smokers. The influence on the immune status of CS depends, to a large extent, on smoke constituents, local conditions, genetics, drugs, and host's defense mechanisms. Some of the injuries described above may be related to compensative mechanisms, some are primarily pathological, but others, unfortunately, may result from the methods of investigation used. It is the author's hope that the present review on the pathogenesis of smoking-induced inflammation shows the necessity to investigate larger groups of healthy smokers.

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Author's address: J. Domagała-Kulawik, Department of Pneumology and Allergology, Warsaw Medical University, Banacha 1a St., 02-097 Warsaw, Poland; phone: +48 22 5992562, fax +48 22 5991650; e-mail: domagalakulawik@gmail.com