REVIEW ARTICLE

MECHANISMS OF DISEASE

Immunologic Aspects of Chronic Obstructive Pulmonary Disease

Manuel G. Cosio, M.D., Marina Saetta, M.D., and Alvar Agusti, M.D.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) IS A MAJOR CAUSE OF illness and death throughout the world. It affects about 10% of the general population,[1] but its prevalence among heavy smokers can reach 50%.[2] COPD is the fourth leading cause of death in most industrialized countries, and it is projected to be the third leading cause of death worldwide by 2020.[1] Tobacco smoking is the primary risk factor for the development of COPD, but other factors, such as burning biomass fuels for cooking and heating, are important causes of COPD in many developing countries.[3,4]

A principal feature of COPD is a limitation of airflow that is not fully reversible and is associated with an abnormal inflammatory response in the small airways and alveoli. The principal abnormalities in small airways are the presence of an inflammatory cellular infiltrate and a remodeling that thickens the airway wall, thereby reducing the airway diameter and increasing resistance to flow[5] (Fig. 1). Additional features are prominent inflammatory infiltrates in the alveolar walls, destruction of alveoli, and enlargement of air spaces. These anatomical hallmarks of emphysema reduce the elastic pressure that generates expiratory flow[6] (Fig. 2). Chronic bronchitis, a condition that according to some authors has little to do with the development of airflow obstruction,[7] develops in approximately 50% of smokers.

Several mechanisms may contribute to these abnormalities. In 1964, researchers reported that a deficiency of alpha$_1$-antitrypsin was associated with emphysema.[8] A few years later, neutrophil elastase was reported to be the target of alpha$_1$-antitrypsin.[9] These findings, together with the observation of increased numbers of neutrophils and macrophages in the lungs of smokers, pointed to a connection between neutrophil elastase and macrophage proteinases as the primary effectors of lung destruction in COPD, a premise that still prevails.[10,11] Other, complementary mechanisms have been proposed more recently, including oxidative stress due to cigarette smoking or to the increase in activated neutrophils and macrophages,[12] as well as apoptosis of endothelial and epithelial cells. The apoptosis results in part from the down-regulation of vascular endothelial growth factor (VEGF), which is required for the growth and survival of endothelial and alveolar cells. These events interfere with lung maintenance and repair, which are necessary to overcome the sustained injury imposed by cigarette smoke.[13] In addition, residual apoptotic debris can promote further inflammation and an immune reaction.[14] Another possible mechanism involves viral infections, which are common in smokers. Viruses can add to the inflammatory milieu promoted by smoking.[7,15] Age-related alterations, such as epigenetic changes, low-grade chronic inflammation, and cell senescence, can also contribute to the development of emphysema by enhancing inflammation and impairing tissue repair.[16] All these mechanisms point to a central role of the inflammatory response to inhaled particles and pollutants in the pathogenesis of COPD.[1]

From the Respiratory Division at Royal Victoria Hospital and the Meakins-Christie Laboratories in the Department of Medicine, McGill University, Montreal (M.G.C.); the Department of Cardiac, Thoracic, and Vascular Sciences, Section of Respiratory Diseases, University of Padua, Padua, Italy (M.S.); and Hospital Universitario Son Dureta, Fundación Caubet-Cimera, and the Centro de Investigacion Biomedica en Red (CIBER) Enfermedades Respiratorias, Mallorca, Balearic Islands, Spain (A.A.). Address reprint requests to Dr. Cosio at McGill University, Respiratory Division, Royal Victoria Hospital, 687 Pine Ave., W., Rm. L4.11, Montreal, QC H3A 1A1, Canada, or at manuel.cosio@mcgill.ca.

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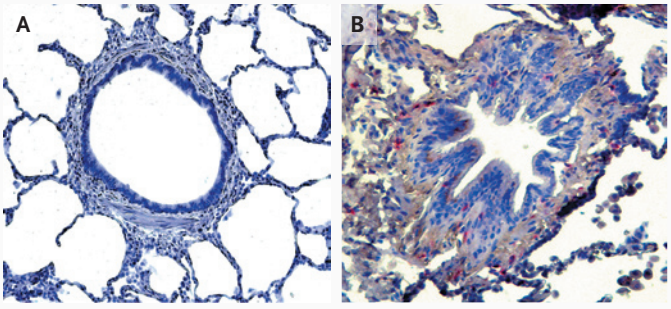

Figure 1. Specimens from the Small Airways in the Healthy Lung of a Nonsmoker and the Lung of a Smoker with COPD.

In the specimen of the small airways (membranous bronchioles) from the healthy lung of a nonsmoker (Panel A), the airway walls are thin, and intact alveoli are attached along its circumference. In a comparable specimen from the lung of a smoker with COPD (Panel B), the diameter of the airway is narrowed, the airway wall is thickened, and many of the alveolar attachments are broken. CD8+ T lymphocytes (in red) infiltrate the airway wall in the specimen from the smoker with COPD (Panel B) but not in the specimen from the nonsmoker (Panel A) (immunostaining with antihuman CD8; counterstained with hematoxylin). Images courtesy of Dr. Fiorella Calabrese.

Inflammation mediated by T cells in the lung, which persists for years after cessation of smoking, has been identified as a key component of COPD.[17,18] These findings suggest that the immune response is involved in the pathogenesis of COPD and that COPD could be an autoimmune disease triggered by cigarette smoking.[19-21] In the 5% of patients with COPD who are nonsmokers, the disease seems to be associated with organ-specific autoimmunity.[22]

In this article, we review the proposed mechanisms leading to COPD and the emerging evidence that progression of the disease is the result of T-cell–mediated inflammation. T cells could be activated by antigens released during smoking-induced lung injury. We have divided these mechanisms into three steps that may be involved in the development of COPD.

MECHANISMS LEADING TO COPD

STEP 1 — INITIAL RESPONSE TO CIGARETTE SMOKE

Innate and adaptive immunity are components of an integrated system in which cells and molecules function cooperatively. The innate immune response relies not on antigen-binding receptors but on pattern-recognition molecules, and unlike the adaptive immune response, it is not associated with long-lasting protective immunity. Innate immunity is a rapid, nonspecific response to microbes and tissue injury that stimulates and influences the relatively slow development of specific adaptive immune responses.[23]

How irritants such as cigarette smoke trigger an innate immune response is unknown, but the "danger hypothesis" of Matzinger[24] is a plausible explanation. Matzinger has proposed that it is not the presence of a microbe itself that alerts the immune system to respond but rather the cellular stress or tissue damage (danger signals) resulting from infection. Toll-like receptors (TLRs), of which there are about 15 different types, are sensors on cells of the innate immune system that recognize molecular patterns displayed by pathogens. Some TLRs can also initiate immune responses against injured tissue.[25] Each puff of a cigarette contains more than 2000 xenobiotic compounds and 10^{14} free radicals that injure lung epithelial cells to a degree that is directly proportionate to their concentration.[26,27] Breakdown of connective tissue also occurs in humans who smoke,[28,29] as it does in mice exposed to cigarette smoke.[30] Products derived from epithelial-cell injury can act as ligands for TLR4 and TLR2; when either is cross-linked by its corresponding ligand it will activate nuclear factor κB (NF-κB),[31,32] inducing epithelial cells to produce mediators of inflammation. These mediators activate alveolar macrophages and neutrophils,[33,34] which in turn secrete proteolytic enzymes and, together with reactive oxygen species, damage lung tissue further.[35,36]

Exposure to infectious or environmental insults, tissue trauma, oxidative stress, or cell death[37] could release sequestered autoantigens,[38] modify proteins, damage mitochondria, and release DNA from apoptotic cells.[39] The adaptive immune system can recognize these products as foreign antigens and trigger an immune reaction.[37,40-43] These and other similar mechanisms have been implicated in rheumatoid arthritis, atherogenesis, multiple sclerosis, and systemic lupus erythematosus.[37,38,40] In smokers, such antigens could be released as a result of necrosis and apoptosis of epithelial and endothelial cells and extracellular-matrix injury.[41-43] Self-antigens are not by themselves sufficient for the development of an immune response or an autoimmune disease; however, TLRs will link innate and adaptive immunity, enhancing the pathogenic potential of those antigens.[44] Inflammatory lung injury

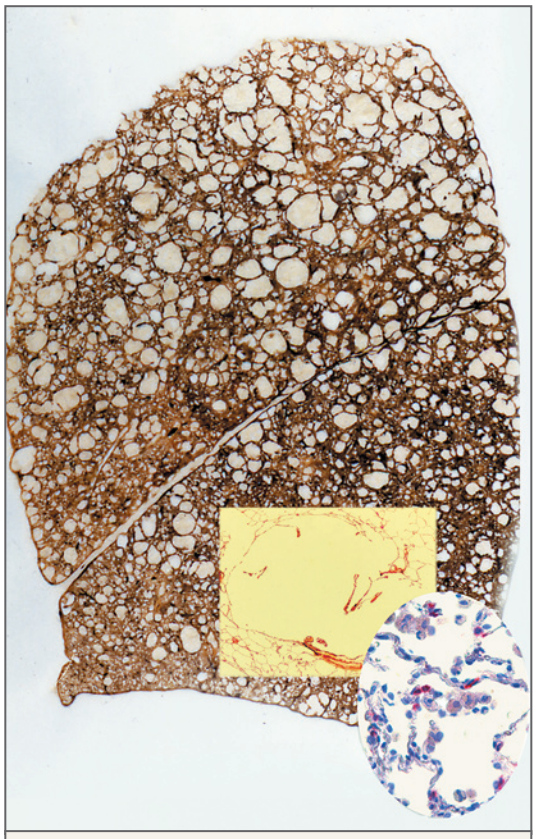

Figure 2. Lung Specimen from a Patient with Emphysema and COPD.

A 100-μm slice from the whole lung shows centrilobular emphysema, which is more prominent in the upper lobes (no staining). The square inset shows centrilobular destruction and enlargement (hematoxylin and eosin). The oval inset shows a severe inflammatory infiltrate, consisting of alveolar macrophages, CD8+ T lymphocytes (immunostaining with antihuman CD8; counterstained with hematoxylin), and other cells, involving the alveolar wall and surrounding air spaces.

can disrupt the extracellular matrix, and breakdown products such as hyaluronate[45] and biglycan[46] can ligate TLR2 and TLR4.[47] The end result is that macrophages and dendritic cells produce cytokines and chemokines that orchestrate the inflammatory conditions required to activate the adaptive immune system.[44,47] In mice, lung inflammation induced by cigarette smoke depends on the presence of TLR4 and myeloid differentiation factor 88, an adapter protein that stimulates NF-κB.[48]

We refer to this chain of events as step 1 in the progression of COPD and propose that in most smokers the disease process will not advance if innate inflammation is minimized and the events described in step 2 and step 3 do not take place (Fig. 3). Patients at step 1 are most likely smokers with normal lung function or stage 1 COPD as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).

STEP 2 — T-CELL ACTIVATION AND PROLIFERATION

Immature dendritic cells alert the adaptive immune system to the presence of incoming pathogens or to tissue injury.[25,44,49,50] These cells mature when the TLRs they display bind to a ligand. Mature dendritic cells express high levels of class II major-histocompatibility-complex (MHC) proteins and the costimulatory molecules CD80 and CD86, which direct them to local lymph nodes, where they present antigens to T cells.[51] Expression of interleukin-12 by dendritic cells activates the signal transducer and activator of transcription 4 (STAT4), which induces T cells to differentiate into type 1 helper (Th1) T cells, which in turn produce interferon-γ. In smokers with COPD, there is a marked increase of mature dendritic cells in the peripheral airways[52,53] that is most likely related to the high expression in the lungs of the dendritic-cell chemoattractant CCL20.[52] There is also an increase in CD4+ T cells expressing STAT4 in the lungs. Consistent with these events in the chain of causation is the observation that the expression of STAT4 and interferon-γ correlates with the degree of airflow limitation in COPD.[54]

It is likely that material in the lungs of smokers from stressed, injured, and necrotic cells[55,56] and from apoptotic cells[57] is taken up by dendritic cells and presented by dendritic cell–MHC class I molecules to CD8+ T lymphocytes. Such T cells are abundant in the lungs in COPD.[58]

Naive, quiescent T cells cannot enter the lung parenchyma outside blood vessels,[59] but once activated by antigen-bearing dendritic cells, they can be drawn into the lung by means of their tissue-specific chemokine receptors.[58,59] In the lungs of smokers with COPD, CD8+ and CD4+ T cells express the tissue-specific chemokine receptors CXCR3, CCR5, and CXCR6,[60-62] but they do not express CCR3 or CCR4, both of which are chemokine receptors expressed by CD4+ Th2 T cells in asthma.[61,62] The ligands for CXCR3–CXCL10 (or interferon-inducible protein-10 [IP-10]) and CXCL9 (or monokine induced by interferon-γ [MIG]) are strongly expressed by struc-

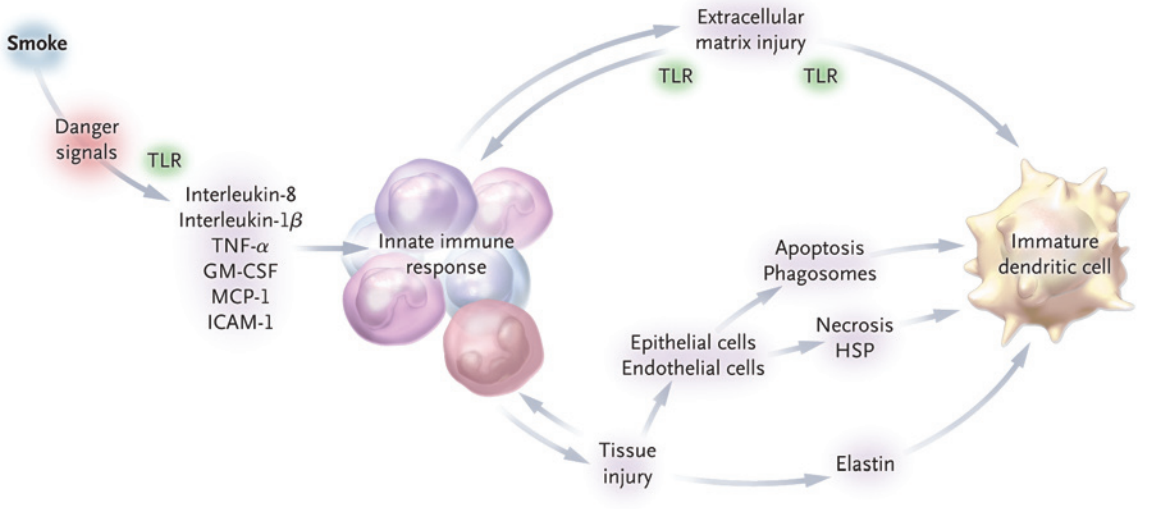

Figure 3. Initial Response to Cigarette Smoke — Step 1 in the Process Leading to COPD.

Cigarette smoke injures epithelial cells, which release "danger signals" that act as ligands for toll-like receptors (TLRs) in the epithelium. These actions trigger the production of chemokines and cytokines, which results in an innate inflammation. Products from the inflammatory cells may injure the extracellular matrix, leading to the release of TLR ligands and consequent TLR activation, which will promote further inflammation, tissue injury, and the production of antigenic substances. This chain of events may cause dendritic cells to mature and migrate to local lymph organs, where, if the conditions are favorable, T-cell activation may result, with progression of the disease. If the innate inflammation in step 1 is minimized or controlled, the inflammation will not progress to adaptive immunity, and the disease may be arrested. These processes are typical of smokers who have neither COPD nor Gold stage 1. GM-CSF denotes granulocyte–macrophage colony-stimulating factor, HSP heat-shock protein, ICAM-1 intercellular adhesion molecule 1, MCP-1 monocyte chemoattractant protein 1, and TNF tumor necrosis factor.

tural cells in the airways and pulmonary arteries in patients with COPD but not in those of smokers and nonsmokers without COPD. The expression of these receptors and their ligands correlates with the severity of the disease.[60-62]

We refer to this chain of events as step 2 and propose that disease progression and severity at this point are determined by the ability of dendritic cells to stimulate T cells. This ability is modulated by immunoregulatory mechanisms: mild failure of these regulatory mechanisms results in GOLD stage 1 or stage 2; severe failure allows progression to what we call step 3 and GOLD stage 3 or stage 4 (Fig. 4).

STEP 3 — ADAPTIVE IMMUNE REACTION

The CD8+ cytotoxic T cell is the predominant cell in large airways,[63] small airways,[64] and the lung parenchyma[19,65-67] in COPD. The number of CD8+ T cells in the lung correlates with the degree of airflow obstruction and emphysema, suggesting that these cells cause tissue injury in COPD.[68] Any cell that displays MHC class I molecules can be a target for CD8+ cytolytic T cells. After a cytolytic attack, target cells die of apoptosis or necrosis from the damage done by perforin, granulysin, or granzyme A or B, all of which are proteolytic enzymes released by CD8+ T cells[69] in the lungs of patients with COPD.[70,71]

In the lungs of smokers with COPD and emphysema, epithelial and endothelial cells undergo apoptosis,[72,73] and the number of these apoptotic cells increases with the extent of smoking and correlates with the number of CD8+ T cells in the lung.[19] Furthermore, cell survival seems to depend on signals from the integrin family of adhesion receptors, which continuously sense the extracellular milieu. Another signal for apopto-

sis in COPD could be the loss of cell contact with the extracellular matrix induced by breakdown of the matrix by proteases.[74-76]

The apoptosis and necrosis of epithelial and endothelial cells induced by CD8+ T cells and possibly proteases, which cell proliferation does not compensate for,[65] most likely contribute to lung destruction in COPD.[13] Furthermore, phagocytosis of apoptotic cells by alveolar macrophages is deficient in COPD,[77] an abnormality that can increase the amount of antigenic material and that has been implicated in some autoimmune disorders.[14,39,47,78]

CD4+ T cells are also found in large numbers in the airways and parenchyma of smokers with COPD.[7,67] These cells are activated and are oligoclonal; clones of CD4+ T cells appear in the lungs but not in the blood, suggesting that the accumulation is the result of stimulation by antigens distributed throughout the lung.[79] CD4+ T cells in the lungs of smokers with COPD express STAT4 and interferon-γ, further suggesting antigenic stimulation.[54] The number of CD4+ T cells expressing interferon-γ correlates with the degree of airflow obstruction,[54] supporting the suggestion that these cells, along with CD8+ T cells, play a role in the pathogenesis of COPD. The effector functions of the CD4+ T cell are mainly mediated by Th1 cytokines, which promote transendothelial migration of inflammatory cells to the site of injury.[23] The recruitment and activation of inflammatory cells, macrophages, neutrophils, eosinophils, CD4+ and CD8+ T cells, and B cells progress as COPD worsens.[7,67,80] In COPD, the interaction between the chemokines CXCL10 (or IP-10) and CXCL9 (or MIG), secreted by T cells, and their receptor, CXCR3, found in alveolar macrophages, up-regulates the production of matrix metalloproteinase-12 by these cells, thereby facilitating lung destruction.[62]

The presence of B cells in lymphoid follicles has been reported in the airways and parenchyma of patients with COPD and of mice exposed to cigarette smoke.[7,81] Sequence analysis of rearranged immunoglobulin genes in individual B-cell clones in these lymphoid follicles has revealed the presence of immunoglobulin-variable-region mutations in clonally related B cells, suggesting an antigen-driven selection process. The absence

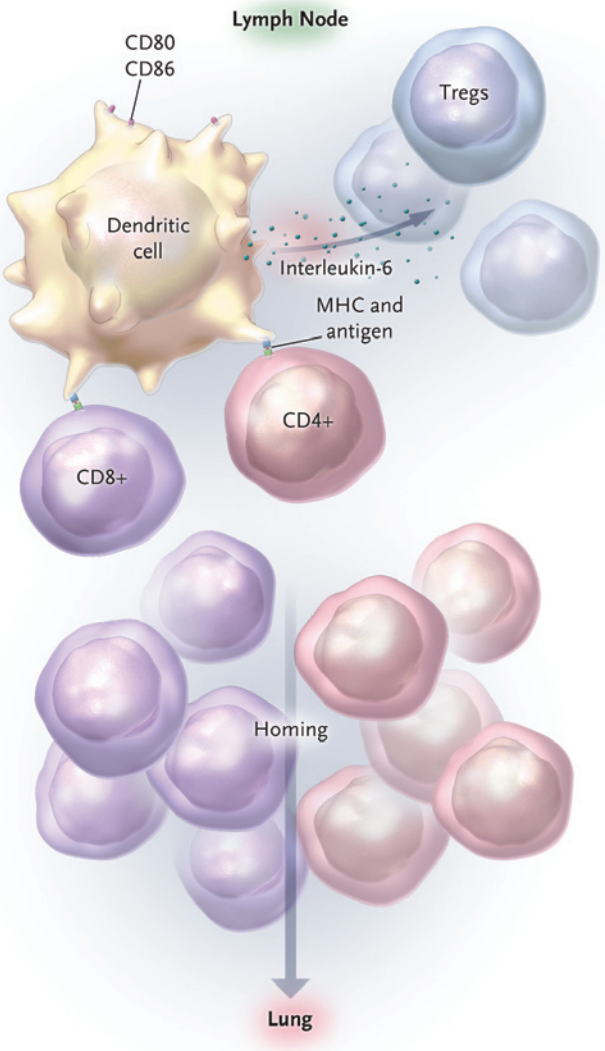

Figure 4. Proliferation of T Cells — Step 2 in the Process Leading to COPD.

When step 1 is successful, mature dendritic cells migrate to local lymphatic organs, whereupon stimulation by toll-like receptors (TLRs) leads to the expression of CD80–CD86 and cytokines, creating a propitious milieu for T-cell antigen presentation and proliferation into effector CD4+ type 1 helper (Th1) T cells and cytolytic CD8+ T cells. Interleukin-6, secreted by the dendritic cells, favors the production of effector T cells by overcoming the signals from regulatory T (Treg) cells. Upon activation, effector T cells express tissue-specific chemokine receptors. Immune regulation or tolerance mechanisms will determine at this stage the degree of proliferation of T-cell effectors, homing, and eventually, disease severity. An absence of tolerance is associated with Gold stage 3 or stage 4, moderate tolerance with Gold stage 2, and full tolerance with Gold stage 1. MHC denotes major histocompatibility complex.

of bacterial and viral products in the follicles suggests that these oligoclonal B cells arise in response to lung antigens.[81] Nevertheless, viral and bacterial infections could be important in perpetuating the inflammatory process and are regarded as the main cause of the exacerbations in COPD.[15,67] Such infections might trigger an immune response that culminates in pulmonary damage.[42]

We refer to this chain of events as step 3 in the progression of COPD. We propose that enhanced dendritic-cell function, genetic predisposition, and failure of immune regulation result in adaptive immunity and severe disease (GOLD stage 3 or stage 4) (Fig. 5).

Pulmonary inflammation in severe COPD includes large numbers of activated oligoclonal Th1 T cells,[62,79] B cells,[81] and CD8+ T cells, which persist for years after cessation of smoking,[82] suggesting a self-perpetuating process that is a feature of autoimmune diseases. This chain of events suggests that the adaptive immune response in COPD, along with its persistence after

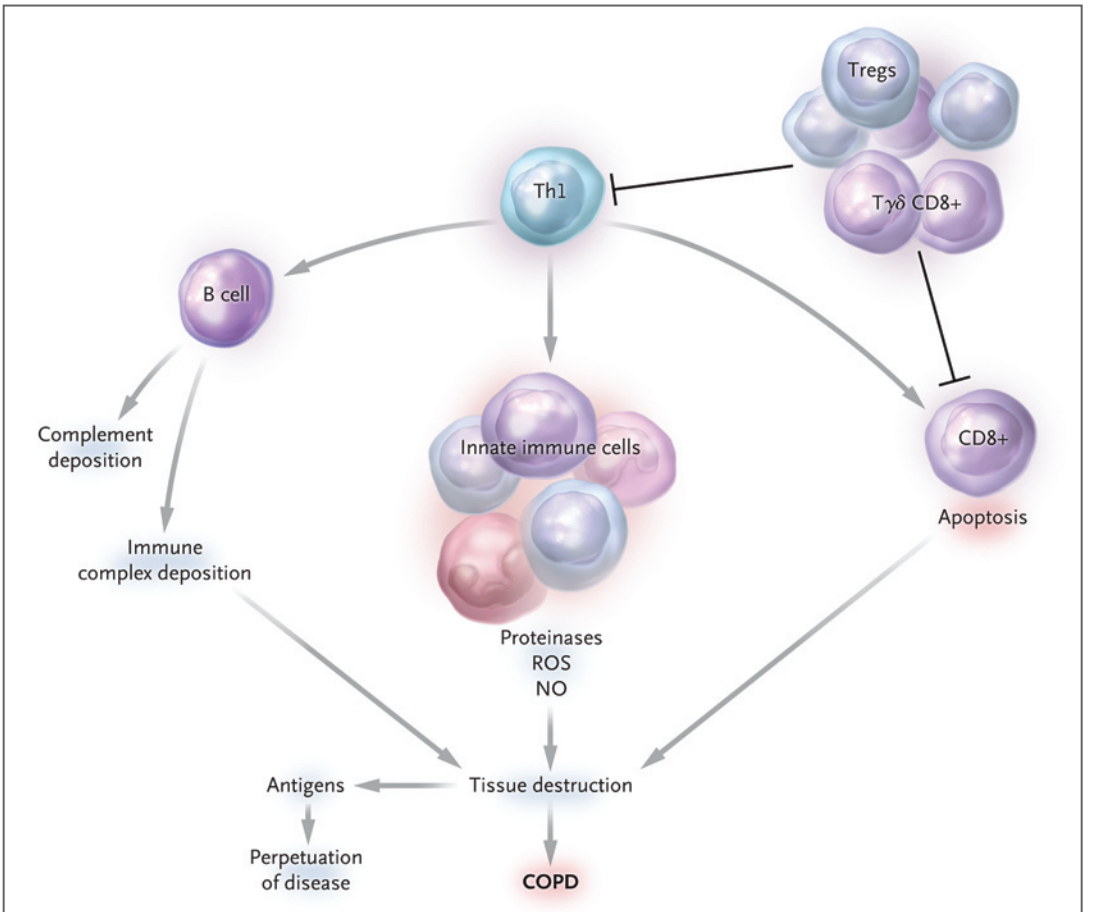

Figure 5. The Adaptive Immune Reaction — Step 3 in the Process Leading to COPD.

With the failure of tolerance or immune regulation in step 2, an adaptive immune inflammation (autoimmune) develops in the lung, consisting of CD4+ type 1 helper (Th1) T cells, cytolytic CD8+ T cells, and IgG-producing B cells. Regulatory T cells (Treg) and γδ CD8+ T cells could modulate the severity of the adaptive immune inflammation. The resulting immune inflammation, induced by CD4+ Th1 T cells and consisting of activated innate immune cells producing oxidative stress and proteinases, along with cytolytic CD8+ T cells and B cells, leads to cellular necrosis and apoptosis, immune and complement deposition, tissue injury with airway remodeling, and emphysema, as well as the release of additional antigenic material, which perpetuates the process. In step 3, the full autoimmune process has developed, producing the most severe disease (Gold stage 3 and stage 4). NO denotes nitric oxide, and ROS reactive oxygen species.

smoking cessation, might be due to a response to self-antigens.

EVIDENCE OF AUTOIMMUNITY IN COPD

Three types of evidence can be marshaled to establish that a human disease is autoimmune in origin: circumstantial, indirect, and direct.[83] In the case of COPD, all three types of evidence have been established. The increased numbers of T cells and B cells in the lungs of patients with COPD constitute circumstantial evidence. Indirect evidence of autoimmunity is provided by the presence of circulating antibodies against elastin in COPD and emphysema, along with the observation that CD4+ T cells cultured from the lungs of patients with COPD respond to elastin by secreting interferon-γ and interleukin-10. The extent of the response is proportional to the degree of emphysema, and the response can be blocked by MHC class II antibodies, which indicates that antigen presentation is involved.[84] Indirect evidence is also provided by the presence of circulating IgG autoantibodies against pulmonary epithelium and endothelium that have the ability to promote antibody-dependent, cell-mediated cytotoxicity and that participate in the antigen–antibody complexes that are deposited along with complement in the lung.[85]

Although antibodies reflect the microenvironmental availability of antigens, they are not necessarily causal. Causality and direct evidence of immunity can be implied from the results of experiments in rats injected with human umbilical-vein endothelial cells. Antibodies against the endothelial cells were produced in the rats, and emphysema developed in association with apoptosis of alveolar cells and an accumulation of CD4+ T cells in the lung.[86] This study also showed that the antibodies against human endothelial cells in the rats induced endothelial-cell apoptosis in vitro and caused emphysema when transferred to mice. Finally, the transfer of T cells isolated from the spleens of rats that were immunized with human endothelial cells also caused emphysema in immunocompetent rats.[86]

The presence of autoantigens and antibodies in COPD is not by itself direct evidence of causality. However, the properties of the autoantigens, T cells, and antibodies in COPD, along with the findings in the animal model, support the idea that autoimmunity is important in the pathogenesis of COPD.

WHY SOME SMOKERS EVADE COPD

Immune regulation, genetic susceptibility, and the environment are factors that provide protection against or induce a predisposition to autoimmune diseases, and they may be important in COPD.[42,43] With time, the lungs of all smokers are damaged and release material with the potential for eliciting an immune response. Not all smokers have a reaction to these antigens, however, and among those who do have a reaction, its intensity varies. These differences probably account for the wide range of disease severity in response to similar amounts of exposure to cigarette smoke.[17,64,87]

An important mechanism that controls T cells is mediated by CD4+CD25+FOXP3 regulatory T cells.[88] A deficiency of regulatory T cells can impair the immune system's tolerance for autoantigens and thereby lead to immune disease.[89] As compared with the lungs of people who have never smoked, the lungs of people who smoke but have normal lung function have a greater number of regulatory T cells. However, the lungs of smokers with COPD and emphysema[90] have fewer regulatory T cells and less FOXP3 messenger RNA than the lungs of healthy smokers.[84] This suggests that regulatory T cells may regulate T cells in smokers with normal lung function, preventing development of the immune reaction.[91] Another population of T cells with immunoregulatory properties, the $\gamma\delta$ CD8+ T lymphocytes,[92] is also increased in smokers with normal lung function but not in smokers with COPD.[93] These findings point toward impaired immune regulation in smokers with COPD.

A characteristic of autoimmune diseases is their propensity to appear in families, and several studies have shown an increased prevalence of airflow obstruction among smokers who are first-degree relatives of patients with COPD.[94-96] In addition, the maximal expiratory flows in twins who smoke are matched as closely as those in pairs of twins who never smoked,[97] a finding that strongly suggests an underlying genetic susceptibility. However, environmental factors (e.g., secondhand smoke) cannot be ruled out. Genetic studies in smokers have shown associations

among genes involved in oxidative processes and several mediators of inflammation, but no genes related to autoimmunity have been directly investigated.[98]

Cigarette smoke may also promote the development of COPD through its influence on epigenetic factors, such as chromatin acetylation. A reduction in histone deacetylase activity in the lung parenchyma, bronchial-biopsy specimens, and lung macrophages, mediated by oxidative stress, has been described in smokers with COPD, and this decrease is correlated with the severity of the disease and the intensity of the inflammatory response.[99]

Finally, tobacco smoking can play an important role in autoimmune diseases, probably by inducing apoptosis and influencing lymphocyte functions, both of which synergize with genetic factors to create an important increased risk for the development of rheumatoid arthritis and systemic lupus erythematous.[100]

CONCLUSIONS

There is mounting evidence that autoimmunity has a role in the pathogenesis of COPD. Although circumstantial, indirect, and direct evidence of the role of autoimmunity in COPD has been identified, no cause-and-effect relationship between autoimmunity and the mechanisms of COPD has been established. We hope that the evidence presented will stimulate the research needed to clarify the role of autoimmunity in the pathogenesis of COPD.

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