Review

Cellular Basis of Bronchial Inflammation in Chronic Obstructive Pulmonary Disease(COPD)

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ABSTRACT: Chronic obstructive pulmonary disease (COPD) is a major health problem, characterized by a progressively and irreversible change in lung function. This is associated with chronic airways inflammation, structural remodeling, and alveolar wall destruction. Inflammatory cells may contribute to mucus hypersecretion, the airways remodeling by the secretion of proteases, cytokines, and fibrotic or mitogenic growth factors. Neutrophils, macrophages, and T cells have implicated in many studies, indicating their roles in the chronic inflammatory process of airways. The identification of risk factors, inflammatory mediators, and understanding their interactions are important for the development of anti-inflammatory treatments, that may reduce the inflammation and alleviate the clinical symptoms of COPD.

KEYWORDS: *inflammatory cells, inflammation, Chronic Obstructive Pulmonary Disease (COPD)*

Introduction

Nowadays, it is accepted that bronchial inflammation occupies the central position in the destruction of the pulmonary parenchyma and in the bronchial remodeling of the COPD.**(1)** The earliest and most constant pathological modification of the airways, common in smokers, is represented by the cellular inflammatory infiltration in the bronchial wall.**(2)**

This inflammatory process can be held responsible on its own for moderate bronchial obstruction.**(3-5)** Also, bronchial inflammation can lead to bronchoconstriction by releasing mediators that can act directly on the airway smooth muscle.**(6)** The persistence of the inflammation will produce other modifications such as bronchial fibrosis as well as the hyperplasia of the airway smooth muscle, either directly as an effect of the inflammation, or indirectly as a result of the contraction airway smooth muscle. All of these modifications lead to the thickening of the bronchial wall which will generate a narrowing of the bronchial lumen and a airflow limitation. On the other hand, the inflammation of the airway can play an important part in the destruction of the alveolar walls which are normally anchored on the bronchioles, this process causing the decrease of the lung recoil elastic which will furthermore contribute to the limitation of the air flow by deforming and narrowing the bronchial lumen.**(7)** Even though the stimuli that induce the inflammation of the airway are partially known, it is considered that altering the bronchial epithelium by the cigarette smoke, promotes and perpetuates the inflammation of the bronchial wall.**(8)** Cigarette smoke activates the macrophages of the bronchial tract which releases the neutrophil chemotactic factors (IL8 and LT B4). Neutrophils release proteases which produce the destruction of the connective tissue of the pulmonary parenchyma generating lung emphysema and stimulating the secretion of mucus. The neutrophil proteolythic enzymes are counteracted by the protease inhibitors (α1-antitripsine, secretory leukocyte proteinase inhibitory, SLPI and tissue inhibitor of metalloproteinase 1, TMP-1). Also, cytotoxic T cells (CD8) may be recruited and these cells are involved in the destruction of the alveolar wall. Fibroblasts can be activated by the macrophage and epithelial growth factors.

Bronchial epithelial cells are an example of structural cells which act as inflammatory cells, belonging to the un specific defense immune system. Under the action of certain inhaled irritant substances, the bronchial epithelium can produce a series of mediators which recruit leukocytes, contributing to the initiation and maintainig of the bronchial inflammatory process.**(10)** An additional indicator of the inflammatory activity of the bronchial epithelium is represented by the growing expression of the transcription factor associated with inflammation (NF-kb). Also, the bronchial epithelium can produce profibrotic factors (β-TGF; tissue growth factor - β). **(11)** Mucipar cells produce mucus which has the role

of removal of bacteria and inhaled particles. The mucocilliary escalator ensures the removal of the microorganisms contained in the mucus. Moreover, the bronchial epithelial cells secrete a series of enzymes which have antimicrobial effects and interfere in the local defense processes. Epithelial cells secrete protective antioxidants and antiproteasic factors, which limit effects exercited by the protolithic granulocyte enzymes.

Smoking can induce an inflammatory local process, only if the protective mechanisms are affected. At non-smokers, smokers without bronchial obstruction and those with COPD , the majority of the cells in the bronchoalveolar lavage fluid are alveolar macrophages. **(12)** Still, smokers have much higher concentrations of **macrophages** in the bronchoalveolar lavage liquid, in contrast with nonsmokers. Exposure to by products of cigarette smoke will determine their accumulation in the lungs and the growth of the macrophagial activity at this level.**(13)** The growing number of the pulmonary macrophages in smokers and patients with COPD is a result of the recruitment of monocytes from the circulation as a response to the chemotactic chemokines of the monocytes.**(14)** Thus, in sputum and bronchoalveolar lavage fluid of the patients with COPD there have been registered growing levels of monocyte chemotactic protein-1 (MCP-1α) and growth related oncogene α (GRO-α).**(15)** Monocytes are derived from undifferentiated haematopoietic stem cells of the bone marrow. Myeloid progenitors in the bone marrow differentiate into promonocytes and the into blood monocytes. From the bone marrow, the monocytes go into the bloodstream where they remain for approximately 60 hours and then by diapedesis go into the connection tissue of the airway and alveoli, where they transform into pulmonary macrophages. Blood monocytes which present peroxidase-positive granulations, whose enzyme content is similar to the asurophilic granulations of the neutrophils, represent almost 15% of the total of the sanguine monocytes and are named proinflammatory monocytes. These monocytes synthetize serine proteases as, neutrophilial and G cathepsine elastase. Also, circulating monocytes can produce significant quantities of matrilysine-1 (MMP 7), reduced quantities of collagenases 1 (MMP 1) and gelatinase B (MMP 9) and cannot synthetize stromelysin-1 (MMP 3) or metalloelastase (MMP12). **(16)** Macrophages play an important part in the COPD pathogenesis, so long as they are activated by the cigarette smoke and secrete a series of inflammatory proteins which can orchestrate the inflammatory process in COPD. Neutrophils can be attracted by IL-8, GRO-α, LTB4, MCP-1α, and the lymphocytes CD8 by the IP-10, Mig and I-TAC. By releasing protolithic enzymes (MMP and cathepsines) they produce lysis of the connective tissue with the release of TGF-1β and CTGF (connective tissue growth factor). Releasing TGF-β by the alveolar macrophages will determine the activation of epidermal growth factor receptor (EGFR) which will stimulate the hypersecretion of mucus. Moreover, macrophages will generate free oxygen radicals and nitric oxide, which together will form nitric peroxyde, contributing to the resistance at cortisone. When monocytes differentiate in macrophages, they lose their serine proteases and gain the ability to synthetize and secrete matrix metal proteases. Human alveolar macrophages release multiple proinflammatory mediators, such as MCP-1α, IL-1β, IL 6, IL 8, GM-CSF, and TNF α. The release of MCP-1α and IL 8 will favor the later recruit of macrophages in the lungs and the monocytes of the patients with COPD will express a high capacity of migration when exposed to different by products of macrophages (GOR-α). The other proinflammatory factors (IL 8, LT B4 and IL 1β) are important for the recruitment of neutrophils in the places of pulmonary aggression.

Moreover, neutrophils answer to TNF-α including activation, production and degranulation of chemokine. Also, TNF-α plays a role in the adhesion to neutrophils and their migration through the vascular endothelium. Some of the macrophage cytokines formed in the lungs will exert systemic effects , such as the stimulation of the liver in the production of proteins of acute phase (TNF α, IL 1β) and/or influencing the bone marrow to release leukocytes (IL 6, GM-CSF). Macrophages have a potential role in the pathology of the emphysema and COPD, first of all by producing MMP which can degrade matrix proteins. Thus it has been shown that alveolar macrophages are capabale of synthetizing: macrophagial elastase (MMP 12), collagenase 1 (MMP1), gelatinase B (MMP 9) and reduced quantities of stromelysine (MMP 3) and matrillysine (MMP 7). Although macrophages cannot synthetize neutrophilelastase, these cells can entrap and release further on these enzymes, amplifying the matrix degranulation induced by MMP. (17, 18)

On the oth er hand, macrophages can play the part of antigen presentation cells. Alveolar macrophages function as phagocytes in connection with the innate immune response to foreign particles and microbes, by swallowing and processing extracellular particles and by expressing antigenic complexes on the cellular surface. LT CD4+ can assist the microbe degradation by acknowledging these antigens and the secretion of IFN-γ. This interaction between the macrophage and the lymphocyte potentiates the immune response.

The nuclear polymorphous neutrophil is one of the granular cells which play an important role in the COPD pathogenesis. This cell interferes in the initial inflammatory response as well as in the late stages of the immunologic defense mechanisms. The roles of the neutrophils in the pathogenesis of COPD are not completely elucidated. Although a growing number of neutrophils in sputum and in the bronchoalveolar lavage fluid has been observed in these patients, the number of neutrophils is equally sensitive in the bronchial wall and the pulmonary parenchyma in patients with COPD as well as in smokers without the bronchial obstructive phenomena. This can be explained by the very short passage time of neutrophils through the bronchial wall to the lumen of the airway. **(19)** The implication of neutrophils in the pathogenesis of COPD is suggested by other clinical observations such as: the existence of a correlation between the number of circulant neutrophils and the decrease of FEV1; the demonstration of a direct relation between the number of neutrophils in the sputum and bronchoalveolar lavage fluid of patients with COPD and the decline rate of the pulmonary function; **(20)** the growing percentage of neutrophils along with the activation of bronchial obstructive disease, which correlates to the limitation of the air flow through the airways.

The neutrophil is formed in the hematogenous marrow, where it differentiates between 7 and 10 days, starting at the premyeloblastic stage. In this period, the neutrophil produces his enzymatic equipment of neutrophilial elastase and 3-proteinase and deposits enzymes in its primary and asurophilic granulations. The synthesis of these enzymes begins early during the differentiation, in the premyelocytic stage and stops in the myelocyte stage. Mature neutrophils are released in the circulation, where they stay for 6-8 hours. After this interval, they are recruited to different tissues where they develop their activity and are destroyed, being phagocytated by macrophages. The recruiting of neutrophils in the airways and the pulmonary parenchyma appears, at least in part, as an answer to cigarette smoke, has the effect of growing the circulant neutrophils number and the promotion of their kidnap in the pulmonary capillaries. Also, smoking exerts a direct effect on the bone marrow, stimulating the production of neutrophiles by a mechanisms which seems to involve granulocyte and neutrophil colonies stimulating factor (FSC-GM) release by the macrophages. **(21)**

The primary chemotactic signs whose effect is to recruit neutrophils in COPD are: LT B4, IL 8 and GOR-α, mediators which come from the alveolar macrophages and the alveolar epithelial cells. Near the stimulus, the circulant neutrophil adheres firmly to the endothelial cells and will migrate through the vascular wall to the injury zone. The attachment and moving along the vascular wall is possible because of the reversible connections which are established between the neutrophils and endothelial cells at the level of transmembranary glycoproteins. Neutrophils contribute to the production of COPD by growing the mucus secretion and by demage the pulmonary parenchyma. These effects are mediated by the release from activated neutrophil granules of such substances as: neutrophil elastase, cathepsine G, matrix metal proteases (MMP 8, MMP 9), myeloperoxidase, human neutrophil lipocalin and lactoferin. **(22)**

The degranulation mechanisms are incompletely studied. The release of the granular content is dependent upon a C protein-kinases or a 3' 5' GMPc-kinases, depended of 3' 5'GMPc **(23)** The neutrophils granules contain peptides with toxic effect upon the bacteria, the fungi and the viruses, and also enzimes with bactericidal effect. Once the granules released the content those substances will produce a major effect upon the airway and the pulmonary parenchyma, responible for an important destruction of the alveolas and a bronchial remodeling characteristic for COPD. The neutrophilic elastase represents a signal for the epithelial cells to release IL8, which has as an effect the recruting of several inflamatory cells at the place of the injury. This elastase induces also the mucus hypersecretion of the bronchial glands, an epithelial degradation, and a decrease of activity of mucocilliary elevator. On the other part this elastase demage the secretory immunoglobulin A and of an important receptor

that takes part into the opsonization (C3b1), contributing to the alteration of the defence mechanisms, which goes in favour for the bacterial colonization of the airways, even for the patients which are in the stable stade of the disease, determining a supplementary recruting process of the neutrophils. The degradation of the conjunctive tissue determined by the neutrophilic elastase could be reduced by the α1 antitripsin. Under normal circumstances, the beginning and the spreading of the lesions till the appearance of the emphyzema is influenced by the nature and the number of the neutrophils recruted in the lungs. In the case of the α1antitripsina congenital deficiency the imbalance between the prothease and the antiprothease will go in favour for the appearance more precocious and more spread of the pulmonary emphysema. In the same time the neutrophilas contribute to the increase of the oxidative stress which appear as a direct reponse to the cigarettes smoke. This is due to the fact that the activation of those cells determines the raise of the extracellular level of the active oxigen and of the nitric oxide production by the stimulation of the nitric oxide sinthetase. The oxidative stress can produce not only the tissular destruction but it can amplify the inflamatory reponse and inhibit the antiinflamatory effects of the steroids (by the reduction of the histon-deacetilase activity: HDAC activity).

The role of the lymphocytes in the production of the COPD is vague but intensive studing the last years. It was observed that an immune chronic stimulation is accompanied by the increase of the lymphocytes number at the level of the airways and of the pulmonary parenchyma. This fact is sustained also by exploration of the lower airways of the COPD patients which emphasise an increase in the infiltration of the bronchial mucosa with B lymphocytes together with the hyperplasia of the bronchial lymphatic follicles (BALT) which shows the fact that at the smokers having COPD it could be observed an activation of the immune adaptive (gained) reactions followed by an infiltration with LT CD4+ and CD8+. of the alveolar and bronchial walls. **(24)**

The recruting and the activation of these cells at the level of the airways and of the lungs is preceded by a sustained innate immune reponse, caracterized by the growth of the number of the neutrophils and macrophages in the bronchical mucosa. These immune reactions suppose the migration of the cells presenting the antigen from the bronchical epithelium in the bronchical limphatic follicles, with the presence of the limphocytes T antigenic substances, followed by a clonal proliferation of the LT CD 4+ and of LT CD 8+. **(25)**

For that lymphocytes T arrive and proliferate in the lungs, they must be first activated by an antigen and only after that they arrive in the organ that produced the antigen. The lymphocytes T non-activated (naive) don`t stay for long time in the lung, they pass in the bloodstream or they are destroyed by the alveolar macrophages. (26) The presence of a great concentration of macrophages in the lungs and the significant correlation, in the smokers, between the number of LT CD3 and the pulmonary macrophages, suggests that in this process they are involved both the LT CD4+ and the LT CD8+. γIFN will supplementary stimulate the alveolar macrophages, which in turn will also secrete IL-12, these producing a feed back on the lymphocytic call line, that promote the differenciation LTh1, in this way stimulating LT CD4+ and CD8+ to produce citokines.

The bronchial epithelial cells and the alveolar macrophages, after their stimulated by the existing antigenes in the cigarettes smoke or the neoantigenes appeared because of the local degenerative processes induced by the macrophage and the neutrophils enzimes, can attract LT in the lungs by the expression of a high concentration of CXCL-10 (protein-10 induced by α-interferon; IP-10).**(27)** It was observed that the bronchial epithelial cells at the patients with COPD express high concentration of CXCL-10 and this is associated with a rise of the concentration of LT CD 8+ that expresses receptors for CXCL-10 (CXCR-10). Other examples of chemoattractants which act on receptors CXCR10 from the surface of LTc1 are CXCL-9 (MIG, monokine induced by γ-interferon) and CXCL-11 (I-TAC, α-chemoattractant of the T cells induced by interferon). The alveolar activated macrophages exerts a range of functions: the generation of reactive oxygen species (nitric oxide), initiates an acute inflamatory reaction by secretating short life inflammatory mediators(eicosanoids) and they become antigen presenting cells, much more effective after activation. The high concentrations by the macrophages and LT from the smokers lungs with COPD, suggest that in this process are involved both LTh 1and LT CD8+.

LTh1 CD4 + produce γ-IFN which is the strongest cytokine that activates the

macrophages. It can also be produced by LT CD8. γIFN will further stimulate alveolar macrophages which will secrete IL-12, which exerts a feedback on the lymphocytic cell line which promotes the differentiation LTh 1, stimulating the production of cytokines by LT CD4 + and CD8 + and the differentiate CD8 + . The concentrations of LT CD4 + and CD8 + increase significantly in the lungs of smokers only after 30 years of smoking, suggesting that LT CD4 plays a key role in the inflammation process. **(28)** To maintain the immune memory and ensure a long survival of LT CD4 +Â cells, these cells must receive responses from LT CD8 +. So, in smokers with COPD, for appearance the inflammatory infiltrate with LT CD8 + T is obviously essential to exist small concentrations of LT CD4 + in the lungs of these subjects. On the other hand, activated LT can alter the pulmonary microcirculation.

Under the action of cytokines secreted by activated LT antigen, the endothelial cells of the pulmonary capillars provides a number of features which enhance the inflammation. Vasodilatation in pulmonary microcirculation will provide a greater influx of neutrophils in the lung inflammation places through prostacyclin and nitric oxide. **(29)** Also, endothelial cells will express high levels of adhesion proteins, contributing to leukocyte joining the postcapillary venules. These endothelial cells under the influence of activated LT will produce IL8 and chemotactic protein 1 for monocytes (MCP-1), favoring leukocyte extravasation. Cytokines produced by activated LT will cause changes in the shape of endothelial cells and the modellation of basement membrane, followed by leakage of macromolecules and cell extravasation into the interstitial spaces.

Knowing that one of the effector functions of LT CD8 + is the cellular apoptosis is not surprising that apoptosis may play an important role in the destruction of lung parenchyma in patients with pulmonary emphysema. At least theoretically, LT CD8 + may have a destructive effect on lung tissue by release of lytic substances such as perforin and granzyme B. In this regard, it was observed that cytotoxic CD8 + T cells in sputum of patients with COPD, express higher levels of perforin than in controls. Majo and his collaborators have shown that the degree of apoptosis and the number of alveolar LT CD8 + increase simultaneously with the amount of smoke inhaled by smokers with emphysema**. (30)** This suggests that smoking can cause lung parenchymal destruction via structural apoptosis following cytotoxic proliferation LT. Other authors have shown a significant correlation between the number of T cells and expansion of alveolar wall emphysema, as in other articles overestimate the role of LT in the pathogenesis of this disease. However the proposed role for LT in the pathogenesis of COPD in smokers, does not exclude the role of macrophages and neutrophils. Cosio and his collaborators consider that smoking causes initially a neutrophilic and macrophage inflammation which through various mechanisms (proteolytic and oxidative) damage pulmonary parenchyma (elastin, collagen and proteoglycans). The lung injury may lead to structural alterations of own antigens that creates new cross-reactive antigens and release off sequestered antigens anatomically to be recognized by autoreactive T, inducing their activation and proliferation. Through their effector functions, LT CD4 + and CD8 + can destroy the lungs by stimulating further inflammation cellular (neutrophilic and macrophage inflammation), destroying the target cells directly by necrosis induced by perforin or apoptosis induced by perforin or Fas-Fal and possibly cause tissue damage and remodelation observed in patients with COPD. If LT alone or together with other inflammatory cells are responsible for lung injury and progression in patients with COPD, this mechanism seems to resemble a response to an antigenic stimulus originating in the lungs that is induced by smoking. Thus, we can consider COPD as an autoimmune disease that is triggered by smoking. In terms of inflammatory and immunological, seems that after years of reduced pulmonary inflammation of lung components, some smokers produce an acquired immune response to lung antigenic stimuli that constituting from exposure to smoke. In this sense, there is a lung infiltration with LT only in patients with COPD, but not in smokers without disease. If neutrophils were previously considered to participate in the production of emphysema alone, given that neutrophils were the main cellular components of bronchoalveolar lavage fluid, currently there are evidence that LT are involved in producing emphysema and that they cooperate with neutrophils and macrophages to produce lesions of emphysema.

T lymphocytes "natural killer" represent a distinct population of cytotoxic T lymphocytes specialized in destroying transformed or infected cells with viruses. There

are severeal studies that showing the presence of an increased number of natural killer lymphocytes in the submucosa of the large airways of smokers with COPD, arising the hypothesis that increased accumulation of these cells in patients with COPD is due to repetitive viral or bacterial infections. A recent study evaluated lymphocyte subpopulations in non-smokers, healthy smokers and smokers with emphysema have not showed an increased level of NK cells in patients with pulmonary emphysema.

Dendritic cells that form a large cellular network set on the bronchial epithelium and mucosa in the airways and lung parenchyma, play an important role in immune homeostasis of the bronchial tract, because of their ability to activate native immune LT **(31)**. As antigen presenting cells, the dendritic cells act as monitor for inhaled antigens that are deposited on the surface of the bronchial mucosa and then be remove by endocytosis. Also, dendritic cells can activate a several inflammatory and immune cells such as neutrophils, macrophages, T-and B-lymphocytes. Recently, a strong immunostimulating glycoprotein has been isolated in the cigarette smoke. It would appear that the dendritic cells play an important role in the lung response to the cigarette smoke and other harmful agents. There was an increase in the concentration of dendritic cells in the airways and alveoli of smokers with COPD. Granulocyte- macrophage colony-stimulating factor produced by macrophages in the respiratory mucosa of patients with COPD is the main factor stimulating and activating dendritic cells. In turn these cells expressed some adhesion molecules that mediate their interaction with T cells. At the moment there are known five combinations of receptors dendritic cell and equivalent receptors on T lymphocytes: MCH class I and CD8, MCH class II and CD4, CD 54 and CD11a, I CAM and CD11a , 2FA-3 and CD2. So, dendritic cells play a central role in initiating innate and adaptive immune response due to interaction with T cells.

Eosinophilic inflammation of the bronchial mucosa has been described also at some patients with COPD. People with this disease, which have airflow limitation partially reversible to corticosteroids show an increase in the number of eosinophils in sputum, bronchoalveolar lavage fluid and bronchial mucosa, and increased eosinophilic cationic protein levels (ECP) in sputum and bronchoalveolar lavage fluid. **(33)** Also, during periods of exacerbation of COPD (EACOPD) , the concentration of eosinophils in the bronchial mucosa is similar to that seen in asthmatics and was associated with increased RANTES expression in epithelial and subepithelial cells. **(34)** The influx of these cells in the bronchial mucosa is initiated by the inflammatory process induced by smoking induced through IL 8. This is demonstrated by the existence of a direct relationship between levels of ECP and IL 8 levels in the bronchoalveolar lavage fluid of patients with COPD. **(35)** On the other hand, asthmatics display different bronchial inflamations than patients with smoking-induced COPD. So, the two diseases appear to be two distinct diseases, more than two different expressions of the same pathogenic mechanism.

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