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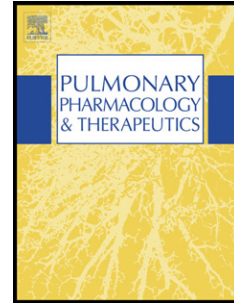
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Mast cells and COPD

Esmail Mortaz^{1,2,3}, Gert Folkerts¹ and Frank Redegeld¹

¹*Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands*

²*Chronic Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, P.O.BOX 19575/154, Tehran, Iran*

³ Corresponding author:

Dr. E. Mortaz

Tel : +31-6-20248216

Email: e.mortaz@gmail.com

Key Words: Mast cells, COPD, Cigarette smoke, inflammation

Abbreviations

COPD: Chronic Obstructive Pulmonary Disease

CSE: Cigarette smoke extract

GOLD: The Global Initiative for Chronic Obstructive Lung Disease

iPLA₂: Calcium-independent Phospholipase A₂

FEV: Forced Expiratory Volume

MC-C: Mast cell-chymase

MC-T: Mast cell-tryptase

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Abstract

The pathogenesis of chronic obstructive pulmonary disease (COPD) is based on the innate and adaptive inflammatory immune response to the inhalation of toxic particles and gases. Although tobacco smoking is the primary cause of this inhalation injury, many other environmental and occupational exposures contribute to the pathology of COPD. The immune inflammatory changes associated with COPD are linked to a tissue-repair and -remodeling process that increases mucus production and causes emphysematous destruction of the gas-exchanging surface of the lung. The common form of emphysema observed in smokers begins in the respiratory bronchioles near the thickened and narrowed small bronchioles that become the major site of obstruction in COPD. The inflamed airways of COPD patients contain several inflammatory cells including neutrophils, macrophages, T lymphocytes, and dendritic cells. The relative contribution of mast cells to airway injury and remodeling is not well documented. In this review, an overview is given on the possible role of mast cells and their mediators in the pathogenesis of COPD. Activation of mast cells and mast cell signaling in response to exposure to cigarette smoke is further discussed.

1- Chronic obstructive pulmonary disease (COPD)

Both asthma and COPD are characterized by chronic inflammation of the airways. Cigarette smoking is considered as an important risk factor for COPD [1-3] and it has been reported that 15–20% of smokers develop clinically significant COPD, suggesting that genetic factors most likely modify each individual's risk [1,4-6]. COPD has been redefined in the GOLD guidelines as a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases [7]. Although COPD affects the lungs, it also produces significant systemic consequences. Loss of elastic recoil - airway collapse, increase smooth muscle tone, pulmonary hyperinflation, gas exchange abnormalities, hypoxemia and/or hypercapnia are important manifestations of COPD [8].

1.1. Inflammation in COPD

Several inflammatory cells and their mediators, both of the innate and adaptive immune system, participate in the inflammatory response in COPD. CD8+ T cells, macrophages and neutrophils are the cells usually considered the prime effector cells in pathogenesis of COPD [1,9-11], but also mast cells may be important [12-14].

The pulmonary inflammation is accompanied by increased mucus production and an excessive propensity of the airway smooth musculature to contract in response to exogenous stimuli, a phenomenon that is termed airway

hyperreactivity [8,11,15]. It has long been speculated but not clearly shown that viruses are involved in the development of these diseases [16].

As mentioned before, COPD is mostly associated with cigarette smoking and thereby cigarette smoke is defined as major risk factor [17,18]. Cigarette smoke is a complex medium containing approximately 4700 different constituents separated into gaseous and particulate phases [19]. Carbon monoxide, carbon dioxide, ammonia, hydrogen dioxide, hydrogen cyanide, volatile sulphur-containing compounds, nitrogen oxides (including nitric oxide, NO), water, and tar are part of the gaseous and/or particulate phase of cigarette smoke [19,20]. A major contributory factor to the development of COPD is the inflammatory response to cigarette smoke. The cellular and molecular mechanisms that are involved in the pathogenesis have not yet been fully described. Thus, understanding the role of inflammatory cells will help to explore the pathogenesis of this disease.

COPD consists of mixture of diseases - bronchitis, small airways disease and emphysema - that exhibit different patterns of inflammation and different pathology [1]. It is widely accepted that the proteolytic potential of neutrophils and macrophages is important for the destruction of the extracellular matrix in emphysema [11]. This is supported by increased numbers of neutrophils and macrophages in both airways and parenchyma of patients with COPD [11,21]. Moreover, animal studies have demonstrated that macrophages and their proteolytic activity are a prerequisite for the development of cigarette smoke-induced emphysema [22,23]. In addition, Churg and coworkers [24] and others

demonstrated that neutrophil elastase, is essential for cigarette smoke-induced emphysema in mice and human [25,26]. Moreover, neutrophils are present in the conducting airways, whereas macrophages are the major cell in secretions from the small airways and parenchyma [27,28]. Macrophages are increased throughout the respiratory tract airway lumen and epithelium in COPD and are positively related to severity of disease, airway obstruction and degree of alveolar wall damage in emphysema [29]. There is controversial data reported on the number and type of T cells in human disease and animal models of COPD, however generally it is accepted that during COPD there is either an increase in the CD8⁺/CD4⁺ ratio of T cells, or an increase in the in total numbers of both CD8⁺ and CD4⁺ T cells, in the tissue [30]. Until now, the mechanism for this process is not well documented. Maeno and coworkers described a critical role for CD8⁺T cells in inflammatory cell recruitment and lung destruction in a murine model of cigarette smoke-induced COPD [30]. In this line, we recently showed that CD8⁺ T cell proliferation was increased in the presence of cigarette smoke extract (CSE) primed conventional dendritic cells (cDC) [31]. CD8⁺ cytotoxic/suppressor T cells release cytotoxic perforins and granzyme B, which cause cell death and apoptosis, a feature of emphysema [30].

Epidemiological studies indicated that smoking can induce the incidence of many diseases such as heart diseases [32], lung diseases [33] and cancer [34]. Further, a link between cigarette smoking and allergic reactions has been published [35-37]. For instance, maternal smoking, particularly *in utero*, is clearly associated with an increased risk for the later development of childhood atopy and asthma

[38,39]. Limited pathological data in asthmatic individuals who smoke, suggest that cigarette smoking may modify the airway inflammatory process [40]. On the other hand, a population-based cohort study indicated that personal or parental smoking reduces risk of allergic sensitization in people with a family history of atopy [41]. Cigarette smoke affects both suppressor T cells and T helper cells [42], but its final effect on allergic sensitization is not well understood.

1.2. Mast cells and COPD

Mast cells are playing a critical role in pathogenesis of allergic [43,44] and non-allergic disorders [45-47]. Studies are accumulating on the distribution, type of mast cells in lungs and tissues of smokers and COPD patients and their potential role in pathogenesis of disease. Mast cells normally reside close to epithelia, blood vessels, nerves, smooth muscle cells, and mucus-producing glands [48]. Recently, it has been demonstrated that the numbers of mast cells were significantly increased in sputum of smokers compared to ex-smokers [49]. Moreover, CXCL-10 is elevated in the airways of smokers compared to control groups [50]. CXCL-10 has been implicated in mast cell migration to the airway smooth muscle cells bundles [51]. In this line, TNF- α and IFN γ synergistically enhance transcriptional activation of CXCL-10 in human airway smooth muscle cells via STAT-1, NF- κ B, and the transcriptional coactivator CREB-binding protein [50].

Mast cells in the airways are exposed to inhaled, environmental challenges. As mast cell activation results in the coordinated release of proinflammatory

mediators into the surrounding tissue, exposure to environmental challenges may result in chronic inflammatory pathology [52, 53]. The mechanisms by which mast cells can be activated in the airways of patients with COPD are not well described. However, it has been shown that IgE-mediated mechanisms presumably do not play a primary role. On the other hand, it has been shown that mast cells have many other receptors that can possibly be activated in patients with COPD. For example, mast cells express Fc-gamma receptors that can be engaged by immune complexes, complement receptors that can be triggered by C3a and C5a [54] and c-kit, the receptor for stem cell factor [55]. Finally, activation of Toll-like receptors (TLR), which are abundantly expressed by mast cells [56, 57] can be activated by e.g. bacterial products during COPD exacerbations which results in production of leukotrienes, cytokines and chemokines, but generally not by the release of preformed mediators (degranulation) [58, 59]. Activation of mast cells can lead to production of a wide array of effector molecules including prestored mediators (serotonin, histamine, proteases), and actively synthesized mediators released within minutes (prostaglandins, leukotrienes) and a large variety of cytokines and chemokines at several hours after activation. The role these mediators play in tissue remodelling is poorly understood. Mast cells are a source of IL-4 and IL-13 that can influence T cell responses, mucus gland hyperplasia and smooth muscle hypertrophy/hyperplasia [60-63]. Angiogenesis is another feature of tissue remodelling and mast cells can be a major source of angiogenic factors such as VEGF [64- 66]. Besides, mast cell-derived mediators such as histamine and

cysteinyl leukotrienes can activate lung macrophages to generate nitric oxide, lysosomal enzymes and proinflammatory cytokines [67].

In the following sections, recent studies on the potential role of mast cells in COPD will be summarized.

1.3. Mast cells and its regulatory effects on the immune system

Mast cells arise from pluripotent stem cells, mature in tissue, and have the ability to generate inflammation following exposure to a variety of receptor-mediated signals initiated by both innate and acquired immune response mechanisms [68, 69]. Tissue mast cells can be activated in wound healing, fibrosis, cardiovascular disease and autoimmunity in addition to allergic inflammation [70-72].

Mast cells are easily identified by the presence of prominent granules within their cytoplasm and are heterogeneous in morphology and staining characteristics.

Mast cells originate from myeloid stem cells and before full maturation they leave the bone marrow as immature committed progenitor cells and undergo their final differentiation in connective tissues such as the skin, and in the mucosa of the respiratory tract and gut under the influence of stem cell factor (SCF) and other locally produced cytokines [73-75]. Many different factors such as interleukin IL-3, IL-4, IL-9 and IL-10, nerve growth factor (NGF), chemokines and retinoids can influence mast cell maturation and differentiation [76]. Mast cell number within connective tissue is constant, whereas their numbers in e.g. respiratory and gastrointestinal tracts can vary considerably. For example, in inflammatory conditions such as allergy, asthma, rheumatoid arthritis and inflammatory bowel

disease, mast cells numbers may increase [72,77-79]. In this line it has been reported that during inflammation the number of immature progenitors mast cells in the circulation are increased [76].

2. Does the mast cell play a role in COPD?

2.1. Animal models of lung emphysema

To gain insight into the underlying pathophysiological mechanisms of human disease and to investigate and develop new compounds with therapeutic activity, animal models of human disease were designed. *In vivo* animal models can help to unravel the molecular and cellular mechanisms underlying the pathogenesis of emphysema and COPD. COPD due to the complexity in pathogenesis needs to be validated by *in vivo* models. The *in vivo* modeling of emphysema started in 1965 by Gross et al. who described the first reproducible animal model of lung emphysema by instilling the proteinase papain intratracheally into rats [80].

Inhalation of noxious stimuli, such as tobacco smoke, sulfur dioxide, nitrogen dioxide, may also lead to COPD-like lesions in mice, bases on the concentration, exposure time and strain specific genetic susceptibility.

Many labs, developed cigarette smoke exposure-induced emphysema models in animals featuring either acute phase neutrophilic influx [17,71] or chronic disease [82-86].

Studies in rodent models of COPD revealed that cigarette smoke-exposure induces chronic inflammation in the lung associated with the development of emphysema, lung remodelling and decreased local immunity [87-89].

D'hulst et al, described a chronic model of developing lung emphysema by cigarette smoke. In this protocol, mice are exposed to the smoke of five cigarettes, 4 times a day with 30 min smoke-free intervals, 5 days per week for 24 weeks in total [90]. An increased number of cells has been shown after 3 days and the end of 6 months with an accumulation of monocytes/macrophages, neutrophils, lymphocytes and DCs in BAL fluid. Moreover, microscopic analysis of lung tissue sections revealed a significant degree of emphysema after 6 months of smoke exposure.

In another model, sub-chronic cigarette smoke exposure in mice has been reported [91]. In this protocol, mice are exposed to cigarette smoke in dose-response experiments from 3, 6, or 9 cigarettes/day for 4 days, delivered three times per day 3, 6, and 9 cigarettes/day were very well tolerated. In this model a dose-dependent increase in the total number of PMN and macrophages with low percent of CD4 and CD8 T cells was found. Further an increased amount of MMP-9 in BALF with low amount of lymphocytes has been shown. In this model, mice exposed to 9 cigarettes/day for 4 days showed a low level of mononuclear peribronchial inflammation within the alveolar space. The epithelium showed inflammatory activation as indicated by Clara cell capping. Similarly, S100A8-positive neutrophils were observed in alveoli as well as intravascularly, with some peribronchial infiltration. Mucus metaplasia was observed in larger airways,

where AB-PAS-stained sections from cigarette smoke-exposed mouse lungs showed acid and neutral mucins and goblet cell metaplasia.

In time course experiments, mice were exposed to cigarette smoke generated from 9 cigarettes/day for 1, 2, 3, or 4 days. Nose exposure of cigarette smoke for development of lung emphysema has also been employed [92]. In this model, mice are exposed to two 1R3 reference cigarettes daily for 5 days per week using a smoke exposure system. In this model, isolated cells from the BAL of nose-only smoke- or sham-exposed mice are greater than 95% mononuclear cells (sham: 99.13 ± 0.50 ; smoke: 97.90 ± 0.57 ; $n = 6$ with 5 animals pooled per experiment). The remaining cells were neutrophils with no eosinophils present. No difference in cellular composition in the BAL was observed between the groups. Isolated cells from the BAL of whole-body smoke- or sham-exposed mice are greater than 95% mononuclear cells (sham: 99.88 ± 0.29 ; smoke: 96.96 ± 4.82 ; $n = 5$ per group). Similar to nose-only exposure, the balance of the remaining cells was neutrophils, and no difference in cellular composition in the BAL was observed between groups

In an initial lead-up period, animals are accustomed to 1 cigarette in the first and to two cigarettes in the second week. To control for handling, groups of mice are placed into restrainers only and exposed to room air (sham-exposure).

At present the role of mast cells in the development of chronic obstructive pulmonary disease is not studied in detail. The described animal models for COPD could be helpful to delineate a possible involvement of mast cell in the

pathogenesis of this disease [93, 94]. For instance, employing (conditional) mast cell-deficient mice may shed more light on a participation of mast cells in COPD.

2.2. In vitro studies using cigarette smoke extract

Extracts from cigarette smoke bubbled through water have been used extensively to show that cigarette smoke can directly activate or inhibit cellular activation pathways possibly influencing an inflammatory outcome. Such studies have been performed predominantly with macrophages or monocytes [95-98]. Thomas et al studied the role of extracts of tobacco smoke on the activation of mast cells isolated from canine [99] and found that cigarette smoke extract solution induced the release of the preformed mediators histamine and tryptase in an energy- and temperature-dependent, non-cytotoxic manner. In another study, exposure of RBL-2H3 with tobacco-derived materials induced overproduction of proteinases, but attenuated degranulation via the release of NO [100]. In line with this, our studies demonstrated that CSE suppressed IgE-mediated degranulation and cytokine release, while no effect was observed on leukotriene release [101] whereas in human cord mast cells, CSE increased the release of mediators [101]. It is presently not known if the type of mast cells and/or the condition of activation may account for this discrepancy. The role of mast cells in non-allergic diseases such as COPD is just starting to become unraveled. Likely, mast cell accumulation and activation in such processes is related to IgE-independent pathways involving for example releases of proteases, interleukin-8 [102] and chemokines [103] and TNF- α [47,104] which play an

important role in COPD as discussed before. Recently, we have demonstrated that CSE induces production of chemokines by mouse mast cells [103]. TNF- α or macrophage inflammatory protein-2 (mouse analogue of human IL-8) may induce the influx of neutrophils. Thus, smoking may cause the recruitment of inflammatory cells via mast cell-derived chemokines. On the other hand, exposure to cigarette smoke may lead to a reduced allergic (IgE-mediated) activation of mast cells without affecting their response to activation via e.g. bacterial derived LPS.

3. Clinical evidence

Exposure to cigarette smoke activates an inflammatory cascade in the airways, resulting in the production of a number of potent cytokines and chemokines with accompanying damage to the lung epithelium, increased permeability, and recruitment of inflammatory cells in to the lungs [1,5].

In 1982, Walter et al reported a change in the number and degranulation of mast cells in smoker lungs of monkeys [105]. In the lungs and skin of human smokers, mast cells increase in absolute numbers, and smoking may be associated with activation of mast cells [13,106]. Earlier studies demonstrated elevated histamine and tryptase levels in bronchoalveolar lavage fluid of smokers [107]. Moreover, several human studies demonstrated increased numbers of mast cells in the circulation, airways, and parenchyma of patients with COPD [108]. Since mast cells are able to produce TNF- α [47,104] proteases [109, 111] and IL-8 [102], it is tempting to speculate that these cells could contribute to the pathogenesis of

COPD. TNF- α and IL-8 contribute to neutrophilic inflammation in COPD [94-96], which is in turn associated with more severe airflow limitation. On the other hand, the release of tryptase and chymase may also be protective by prevention of epithelial cell proliferation [110] and inhibiting smooth muscle proliferation [98], respectively.

No consistent information on the involvement of mast cells in pathogenesis of lung emphysema is available. Several studies suggest that mast cells may be involved in smoking induced lung diseases. For example, Kalenderian et al [13] found that the levels of the mast cell mediators, histamine and tryptase were considerably elevated in bronchoalveolar lavage fluids from smokers. The authors concluded that these data indicated 'a greater propensity for mast cell-mediated injury in the smoker. Subsequent studies have borne out this conclusion [111]. Gosman et al reported that the distribution of tryptase- and chymase-positive mast cells in the airways is similar in patients with COPD compared to controls without airflow limitation. In contrast, the number of these cells in the subepithelial area of central airways is lower in COPD compared to controls. Besides, the mast cell populations in the lung are altered in COPD, as exemplified by a change in the MC-TC/MC-T balance, altered tissue distribution, and modified morphological and molecular characteristics. Collectively, the data show alterations in lung mast cells in COPD that correlate with lung function which may have significant pathophysiological consequences [112].

4. Conclusions, further questions and outlook

In conclusion, in the current review, we tried to summarize current information on the possible role of mast cells in pathogenesis of lung emphysema and COPD. It remains to be clarified whether mast cells are central to or only supportive in the pathogenesis of these airway diseases.

Clinical data show increased levels of mast cell-secreted tryptase and increased numbers of degranulated mast cells in the lavage and bronchial tissue of smokers [113]. Besides, proteinase-activated receptor (PAR)-2 is expressed by mast cells, and tryptase is a potent activator of this receptor [113]. Mast cells can be activated by a PAR-2 agonist to secrete IL-8, a chemokine which can contribute to the progress of inflammation [113].

Moreover, activation of calcium-independent phospholipase A₂ (iPLA₂) via mast cell tryptase could lead to arachidonic acid liberation, PAF production, cell surface P-selectin expression and increased neutrophil adherence [115] (Fig. 1). Activation of iPLA₂ also could lead to the release of IL-1 β , which is also involved in a neutrophilic response [116]. Mast cells could be a source of inflammatory mediators which induce recruitment of e.g. neutrophils (Fig.1). Taken together, present literature suggests a role for mast cells in pathogenesis of emphysema. Currently available animal models for emphysema could be employed to further address the role of mast cells and their possible value as therapeutic target in the treatment of COPD.

Figure legend:

Fig. 1. Schematic diagram of possible involvement of mast cells in inflammatory responses induced by cigarette smoke

Chemokine production (CCL2, CXCL3) by mast cells induced by cigarette smoke could lead to recruitment of polymorphonuclear cells into the lung. Cigarette smoke may also induce the increased release of mast cell tryptase. This may trigger the activation of iPLA₂, activation of inflammasome signaling and production of IL-1 β and IL-18 to induce further lung inflammation and injury. On the other hand, inhibition the IgE-mediated degranulation may be beneficial in the suppression of allergic reactions.

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