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Review Articles

Human Pulmonary Mast Cells: Review

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The aim of the current work was to overview the knowledge regarding the mast cell origin, morphology, mechanisms of mast cell activation and the localization of these cells in normal lung.

In the human respiratory system, the role of mast cells has been examined in two aspects: firstly, as these cells participate into innate and adaptive immunity they are considered to be responsible for lung health, and secondly, mast cell mediators cause and modulate inflammation, and structural and functional remodeling of airways, parenchyma, and vasculature in the respiratory diseases.

The knowledge of mast cell heterogeneity in different lung compartments contributes to clarify the role of these cells in maintaining the homeostasis. Mast cells number in normal lung may be used as referent values in diagnosing lung diseases.

Key words: mast cells, lung, human

Introduction

Mast cells (MCs) play dual role in lung functions. These cells release mediators that contribute to the lung homeostasis and mediators involved in the pathogenesis of lung disorders [7, 25, 32]. Upon activation, MCs release their granule contents, which include proteases, vasoactive amines, proteoglycans, and growth factors [7, 25, 32]. In addition, activated MCs are able to produce a variety of cytokines, chemokines and lipid mediators [7, 25, 32]. The variety of molecules produced by MCs might explain their functions: the recruitment, activation, and differentiation of inflammatory cells [32] and the regulation of vascular permeability [32], smooth-muscle cell contractility [1], and fibroblast growth [32]. Thus, MCs have been found to be involved in the pathogenesis of allergic, chronic inflammatory, and fibrotic diseases. Apart from the mentioned pathological conditions, these cells also play a role in normal physiological processes. The mast cell mediators such as histamine and heparin have been shown to enhance vascularization and endothelial cell proliferation [51]. Furthermore, the bidi-

rectional communication between MCs and sensory nerve cells may provide a homeostatic function such as the regulation of blood flow [32].

Mast cells heterogeneity in human lung has been evaluated concerning their localization, ultrastructure, granule content, receptor expression, mechanisms of their activation and pharmacologic responsiveness [7, 11, 41, 47, 51]. The role of human MCs in the respiratory system has been studied in two aspects: on the one hand, their participation in the maintenance of the healthy lung [4, 7] by contributing to innate and adaptive immunity, and on the other hand, the involvement of mast cells in pathogenesis of lung deseases characterized by allergic, acute and chronic inflammatory, fibrotic and neoplastic processes [2, 7, 41, 51]. The variety of mast cell phenotype is important for development of new effective drugs for respiratory disorders [14].

The aim of this study was to discuss the current knowledge regarding the mast cell origin, morphology and localization in normal lung.

I. Development of mast cells

Mast cell origin

The knowledge regarding the mast cells origin has been undergone considerable evolution over the years. Originally, Combs [12] supposed that mast cells are derived from undifferentiated mesenchymal cells. More precise information about the mast cell progenitors was given by Kirshenbaum et al. [30], who revealed that $CD34^{+}$ $CD117^{+}$ $CD13^{+}$ cells are common precursors for both mast cells and monocytes. Later, Dahlin et al. [15] added that $Fc\varepsilon RI^{+}$ cells development from the Lin^{-} $CD34^{hi}$ $CD117^{int/hi}$ progenitors separates the mast cell lineage from the monocyte lineage and in this regard the Lin^{-} $CD34^{hi}$ $CD117^{int/hi}$ $Fc\varepsilon RI^{+}$ blood cells are considered to be a specific mast cell progenitor. Drew et al. [19] specified that CD34 is lost during mast cell maturation. Therefore, CD34 can be used as a reliable marker to distinguish immature from mature mast cells.

Growth factors involved in mast cells differentiation

Kirshenbaum et al. [29] have found that IL-3 alone and in combination with SCF promoted the growth and survival of human mast cells from bone marrow progenitors. Recently, Toru et al. [46] reported that Interleukin-4 promotes the development of both human tryptase positive mast cells (MCT) and chymase positive mast cells (MCC), whereas stem cell factor (SCF) and IL6 – only MCT.

II. Mast cells heterogeneity

The mast cells heterogeneity in human lung has been evaluated regarding their ultrastructure, granule content, receptor expression, mechanisms of their activation and pharmacologic responsiveness, localization in healthy lungs and in specific lung diseases.

Ultrastructural features

Human MCs possess one nucleus, one or two nucleoli, mitochondria, ribosomes and endoplasmic reticulum, Golgi apparatus, membranous secretory granules with a diameter of 0.5-0.7 μm [11, 47, 50]. Caulfield et al. [11] observed that unstimulated MCs possess about 2 μm long folds of their plasmalemma. Intermediate filaments were described predominantly in perinuclear region, but a few filaments were located in the surface folds and under the plasma membrane. According to the presence of the contents two types of granules were identified - crystalline (0.5 Å in diameter) and amorphous (1mm in diameter) [11].

After immunoglobuline E (IgE)-dependent stimulation, Caulfield et al. [11] observed only amorphous granules. The mentioned authors revealed two ways of granules discharge: firstly, by fusing with the plasma membrane, and secondly, by fusing with other granule membranes to form deep channels or labyrinths consisting of membranes that are involved in the exocytosis. This kind of discharge was described as typical for mast cells and distinguish them from other cell types [51].

Size and shape of mast cells

The size and shape of human lung MCs are described in detail by several authors [3, 11, 47]. According to these authors, human MCs are large cells with a diameter of 10 to 20 μm. Andersson et al. [3] observed mast cells with different size depending on the protease content of their granules. With respect to their size, MCT localized in bronchi and small airways are significantly larger than MCT in pulmonary vessels. Mast cells, containing both tryptases and chymases (MCTC) in pulmonary vessel walls are larger than MCTC in bronchial and small airway walls. Alveolar MCTC are smaller than MCT.

With respect to their shape, MCTC are significantly more circular in all compartments (except the alveolar septa) than MCT in the bronchial wall, small airways and pulmonary vessels [3]. With respect to the FcεRI expression by lung MCs, only the alveolar MCs express no FcεRI [3]. The physiological function of alveolar MCs has been still uncleared [3]. In this respect, the large alveolar mast cells number deserves attention in order to clarify their biological and pathological role.

Mast cells in fetus

Morphological studies on mast cells in human embryo and fetus tissues are scarce. For example, Kitamura et al. [31] described mast cells in human embryo between 15th to 60th days. MCs with metachromatic granules were observed in the tissue of human embryos after the 2nd month. In the 5th and 6th month old human fetus mast cells were defined in the kidney, liver, spleen, skin and muscles, but were of smaller size than in adults [31]. However, we did not find the data regarding mast cells localization in fetal lung.

In normal lung of infants, dos Santos et al. [16] observed larger number of MCT in outer layer than in epithelial layer of airways. However, the authors did not present the data about chymase-positive mast cells. In the airways, the role of these cells has been assumed to be associated with the promotion of the vascular permeability, airway obstruction, and leukocyte recruitment [32].

Mast cells mediators. Histochemical and immunohistochemical features

The heterogeneity of MCs involves differences in morphology, histochemical [36] and immunocytochemical [42] characteristics of mediators in their granules [7, 32]. Mediators secreted by mast cells are usually subdivided into two types: preformed and secretory granule-associated, and newly generated after activation. Preformed mediators include histamine, proteoglycans (heparin, chondroitin sulfate E), serotonin, proteases (such as tryptase, chymase, β-Hexosaminidase, β-Glucuronidase, β-D-galactosidase, cathepsin G and carboxypeptidase), some cytokines such as IL-4 and IL-15, and growth factors (basic fibroblast growth factor, bFGF, tumor necrosis factor alpha (TNF-α), nerve growth factor (NGF), transforming growth factor-b (TGFb), vascular endothelial growth factor (VEGF) [7, 32]. The newly synthesized mediators releaseing after activation are the lipid mediators (prostaglandin D_2 and leukotrienes, generated from arachidonic acid), thromboxanes ($TXAB_2$), 5, 12-hydroxyeicosatetraenoic acid, nitrogen radicals, oxygen radicals, inflammatory cytokines and chemokines [7, 32].

The mast cells were identified as cells which are stained metachromatically with methylene and toluidine blue, and fluorescent binding of berberine to the granules of mast cells due to the presence of glycosaminoglycans [20, 46, 52]. The first classification of MCs into two subtypes: connective tissue mast cells and mucosal mast cells was made in rodents and was based firstly on distinct staining characteristics, fixative properties and anatomical location but later on morphology, granule content and function of the mentioned cells [25, 52].

Based on their protease content human mature MCs were divided in two phenotypes. For example, MCTC concist of tryptases, chymases, and carboxypeptidases, whereas MCT contain tryptases only [25]. A third phenotype of MCs expressing tryptase and carboxipeptidase A3, but not chymase, was described in the airway epithelium in asthmatic subjects (17).

Bradding et al. [6] reported that human MCs are also heterogeneous regarding the cytokine content. IL-4 is expressed predominantly by the MCTC (85%), whereas MCT - 15%. The authors reported that MC phenotypes contain tryptase, heparin, chondroitin-sulfates A and E, histamine and IL4. In contrast, MCT contain also IL-5 and IL-6. This suggests that the different biological functions of mast cell types also depend on their capacity to generate and release different cytokines.

Pulmonary MCs were found to be heterogeneous with respect to both size and granule content [39]. The histamine content in normal human lung ranged from 2.5 ± 0.5 pg/MC for the smallest diameter MCs (up to10 μm) to 10 ±2.5 pg/MC for the largest (16-20 μm). Later, Van Overveld et al. [49] revealed that in lung, histamine content of MCs depends on their formalin sensitiveness.

In lung, the mast cell heterogeneity based on formalin sensitiveness was described by Van Overveld et al. [49]. According to mentioned authors, MCs cells have been defined as formalin sensitive and formalin insensitive. For example, formalin-sensitive MCs release leukotriene C release, whereas formalin-insensitive MCs showed no release of leukotriene C.

Unlike in rodent MCs, histochemical heterogeneity, based on the presence or absence of heparin proteoglycan, is not a useful marker for distinguishing different subtypes of human mast cells. In this respect, Craig et al. [13] revealed that all human MCs contain heparin. Mast cells heterogeneity in humans is based mainly on the protease content and in this relation two subtypes have been identified: tryptase containing mast cells or mucosal mast cells and mast cells containing both tryptase and chymase or connective tissue mast cells [25]. Immunohistochemical studies using antitryptase antibody proved that tryptase is a selective marker for human MCs [25, 32]. The protease content of human MCs is distributed in a tissue-specific manner and it has been suggested that mast cell phenotype is controlled by microenvironmental factors [25].

Mast cell tryptases, a tetrameric neutral serine protease with a molecular weight of 134 kDa, hydrolyzes protein and peptide bonds on the COOH-terminal side of residues [40, 82]. The genes encoding mast cell tryptase are located on the chromosome 16 [40, 42]. There are two main types of mast cell tryptase, α-tryptase (subdivided into α I- and αII-tryptases) and β-tryptase (subdivided into β I-, β II-, and β III-tryptases) [34, 40]. βII-tryptase is stored in the secretory granules of MCs, whereas α-protryptase secreted from MCs as an inactive proenzyme was found in the blood of normal subjects.

Protease activated receptors (PAR)

Mast cell tryptase activates protease activated receptors (PAR) receptors which belong to G-protein-coupled receptors. β-Tryptase activates the PAR-2 receptor [27] icreasing intracellular Ca^{2+} [5]. PARs were observed in airway epithelial and smooth mus-

cle cells, terminal bronchial epithelium, type II pneumocytes, endothelial and vascular smooth muscle cells, and MCs within the respiratory tract [5].

Biologic role of tryptase

Tryptase stimulates the release of high-molecular-mass kininogen (HMMK) – peptide histidine methionine (PHM) and calcitonin gene related peptide (CGRP) which increase bronchial muscle contractility [8; 44]. In epithelial cells, the tryptase has been shown to stimulate a catalytic site dependent release of IL-8, which was also associated with increase of intercellular adhesion molecule-1 (ICAM-1) expression. Thus tryptase may stimulate epithelial repair and in the recruitment of granulocytes following mast cell activation [7].

VIP is known as a mediator of nonadrenergic relaxation of airway smooth muscle [9]. It is known that mast cell tryptase hydrolyzes VIP at two sites: Arg14-Lys15 and Lys2'-Lys21 [9]. On the other hand, there is an evidence that tryptase does not hydrolyze the bronchoconstricting neuropeptide substance P of airway sensory neurons [8]. These studies show the important role of mast cell proteases in modulating neural control of airway tone, which can be achieved by tryptase impact on bronchodilator VIP but not on the bronchoconstrictor substance P.

Mast cell chymases

Chymases are mast cell specific serine proteases with a chymotrypsin-like specificity. Humans express only one mast cell chymase which is encoded by CMA1 gene [51]. The presence of chymases in mast cells was detected originally by histochemical studies. Gomori, 1953 using a-naphthol chloroacyl derivatives as histochemical substrates, defined strong esterase reactivity in MCs. According to Wong et al., [53], the mentioned histochemical technique was used to identify MCs in human skin, whereas for human airway the technique was less useful because only a part of mast cells of human lung contains chymase like esterolytic activity. In this regard, using antibodies against chymase Irani et al. [25] established that only 10% of MCs in the lung contain chymase, but in skin 90% of mast cells are chymase positive. The same authors also found that in the lamina propria of airways, the MCC number is the largest. Therefore, in human lung, the release and actions of chymase depends on the microenvironment.

Biologic role of chymase

Sommerhoff et al., (1989) revealed that more than 70% of the MCs within bronchial submucosal glands contain chymase, suggesting the role for chymase in the physiologic regulation of airway gland secretion [43]. Chymase was found to stimulate cultured serous cell secretion, whereas tryptase had no effect. The findings suggest a potential role for chymase in airway hypersecretion, as in asthma or bronchitis, but the molecular mechanisms for these secretagogue effects are not known [43]. This role of chymase was confirmed by Caughey [10] who also reported other possible roles for chymase including inactivation of sensory neuropeptides, and an increase of histamine dependent vascular permeability [10, 15].

Other role of human chymase is to convert angiotensin I to angiotensin II independent of angiotensin converting enzyme in vivo and in vitro playing a role in blood pressure regulation [23]. Chymase can inactivate bronchoactive peptides such as bradykinin and kaffidin [38] as well as neuropeptides, such as VIP [22]. Thus chymase, unlike tryptase, can inactivate some bronchoconstrictors, resulting in limitation of mast cell degranulation. Mast cell chymase, like tryptase, participate in activation of metalloproteinases MMP-1 and MMP-3 [7, 32].

Histamin leads to smooth muscle contraction

Pietra et al. [37] established that histamine causes increased mucous secretion, pulmonary vasoconstriction and induces bronchial venular permeability resulting in pulmonary edema [37]. Histamine exerts its effects on lung by interacting with two cell membrane-associated receptors: H1 and H2 receptors [18, 48]. H1receptor is responsible for vasoconstriction in lung, bronchial smooth muscle contraction, and systemic vasodepression [48], whereas H2 activation leads to pulmonary vasodepression [18].

III. Activation of mast cells

Mast cells can undergo activation by antigens, superoxides, complement proteins, neuropeptides, and lipoproteins [32]. Since mast cells activation in the airways results in the release of proinflammatory mediators into the surrounding tissue, exposure to environmental stimuli may result in chronic inflammation [32]. The most established involvement of MCs is their activation by surface-bound IgE leading to rapid degranulation, mediator release and development of allergic reaction [32]. Released histamine and serotonin lead to endothelial gap formation in post capillary venules and extravasation of plasma into the airway wall and lumen, causing airway oedema [35]. According to Kennedy et al. [28] alveolar parenchyma has mechanisms for local downregulation of IgE receptor Fc-epsilon-RI which prevent an anaphylactic degranulation in these regions.

Other stimuli may also stimulate degranulation, such as components of the complement, neurotransmitters, osmotic changes and mechanical damage [7, 32]. For example, MCs express Fc-gamma receptors that can be engaged by immune complexes, complement receptors that can be triggered by C3a and C5a [30] and c-kit, the receptor for stem cell factor [7, 32]. Finally, activation of Toll-like receptors (TLR), which are also expressed by MC [7, 32] might be activated by bacterial products which results in production of leukotrienes, cytokines and chemokines.

Activation of MCs can lead to production 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 remodeling 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 or hyperplasia [7, 25, 32]. Angiogenesis is another feature of tissue remodeling and MCs can be a major source of angiogenic factors such as VEGF [5].

Apart from their proinflammatory actions, MCs exert their anti-inflammatory effects by releasing the antiinflammatory cytokine IL-10 [26]. Another antiinflammatory action is the ability of mast cell granule proteases to neutralise key cytokines, such as tumor necrosis factor α (TNF-α), IL-4, IL-13 and IL-33 [7, 32]. Thus, MCs are regarded as local immune modulators which regulate the balance between pro- and antiinflammatory responses.

IV. Localization of mast cells in normal lung

In normal lung, many studies described the mast cell localization and density depending on both staining with toluidine blue – toluidine blue positve (MCTB), and proteinase content: tryptase only - MCT, tryptase and chymase - MCTC, and chymase only - MCC positive mast cells. Andersson et al. [4] revealed a lack of a MCC in healthy subjects. According to Marshall and Bienenstock [33] MCT in the alveolar tissue of human lung represent 93%, whereas MCTC - 7% of the total lung MCs. In bronchial wall, the percentage of MCT (77%) is also higher than MCTC (23%). In bronchial wall, the percentage of MCTC is higher than in alveolar tissue.

The number of MCs was evaluated as mean number per square millimeter or mean number per high-power field. In this regard, MCs were found to be localized near blood vessels and within lamina propria of airways in healthy subjects [7, 36]. Hunt et al. [24] estimated mast cell density in the peribronchiolar regions (mean, 13.7 ± 3.5 cells per high-power field). In normal lung, MCs were distributed predominantly within connective tissue around airways and vascular structures. Brightling et al., 2002 revealed that in the submucosal layer of the bronchi of normal lungs, the average number of MCT is 17 cells/mm^2. The number of MCs in smooth muscle was found to be significantly smaller (from 0 to 5).

More precise description of mast cell density in normal lung was presented by Caroll et al. [7]. A few MCs were observed by these authors in the alveolar septa near the small blood vessels. In contrast to the mentioned authors, other researchers revealed that the alveolar parenchyma in the human lung contains significantly more MCs than in the rodents [3, 21, 49]. Important finding is the different mast cells distribution detected in the wall of cartilaginous and membranous airways. In cartilaginous airways, the density of MCs was highest on the smooth muscle (74 MCs/mm^2), intermediate in the inner airway wall (35 MCs/mm^2), in stromal tissue surrounding glands (40 MCs /mm^2) and outer airway wall (7 MCs/mm^2), and lowest in the epithelium (1.5 MCs/mm^2) and lumen (0.2 MCs/mm^2) [7]. In membranous airways the highest density of MCs was on the smooth muscle (201 MCs/mm^2) and in the outer airway wall area (163 cells/mm^2). Mast cell density in the inner wall, in lumen and epithelium was significantly lower (78 MCs/mm^2, 1 MC/mm^2 and 0.8 MCs/mm^2, respectively) [7].

Some authors observed intraepithelial localization of MCs in the bronchial mucosa of normal human lung [7, 36]. However, the number of intraepithelial MCs in healthy lungs is significantly lower than in lungs in pathological state [36].

Conclusion

In human lung, site-specific MCT and MCTC populations are identified in small and large airways, pulmonary vessels and the alveolar parenchyma. Due to their presence and multifunctional capacity, MCs are most probably involved in most allergic and nonallergic diseases in lung. The knowledge of mast cell heterogeneity in different lung compartments contributes to clarify the role of these cells in maintaining the homeostasis. The data regarding the number of mast cells in normal lung can be very useful as referent values in diagnosing lung diseases.

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