Chapter 13

Different Modulators of Airways and Distal Lung Parenchyma Contractile Responses in the Physiopathology of Asthma

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http://dx.doi.org/10.5772/48347

1. Introduction

Asthma outcomes from an allergen-driven Th2 (T helper 2) response in which airway hyperresponsiveness (AHR) is associated with chronic airway inflammation and airway remodeling have crucial clinical importance (1-3).

Recent investigations have emphasized the importance of lung tissue alterations in the pathophysiology of this syndrome. Additionally, current investigations have shown that patients who died of asthma presented important alterations in the lung parenchyma (4-7) that could also be observed in animal models of chronic allergic inflammation (8-11). In this regard, the importance of the mechanical properties of the lung parenchyma has been characterized as one of the major determinants of physiological function (8, 12-15).

Asthma physiopathology is highly complex and involves a diverse immune response and the release of different types of mediators. The bronchial and tissue inflammation is caused by eosinophils, mast cells and T lymphocytes (16), and the persistence of inflammation induces changes in the structural components of the airway and alveolar walls (5, 8, 17).

The airway smooth muscle (ASM) has been considered the main effector of the AHR in asthma (17-19) and is also believed to contribute to airway remodeling and inflammation due to its increased sensitivity to different bronchoconstrictor stimuli.

The continuous bronchial inflammation process associated with the release of various mediators is thought to be responsible for asthma symptoms directly and indirectly by inducing the constriction of the ASM, enhancing airway responsiveness to different stimuli,

and inducing changes in the structural components of the airway wall, leading to airway remodeling.

Inhaled corticosteroids, which are the gold-standard treatment for asthmatic patients, are more involved in counteracting the airway inflammation than in acting in the ASM. Although some studies have shown the potential of corticosteroids in causing bronchodilation, their role in airway smooth muscle relaxation is controversial. In its formulation (hydrofluoroalkane-HFA), this inhaled corticosteroid is delivered to the distal airways more effectively (68.3%) than chlorofluorocarbon formulations (19.7%) (20, 21). Although eosinophilic infiltration could be adequately controlled in the distal airways, whether both distal lung parenchyma eosinophilic infiltration and extracellular matrix remodeling may be sufficiently modulated by this new treatment is not clear (8, 20).

We discuss in this chapter the role of different mediators and modulators in the contractile responses of the airways and lung distal parenchyma. These studies contribute to the understanding of the mechanisms involved in asthma physiopathology and in smooth muscle contraction and also open opportunities to develop new therapeutic tools to treat asthma. In this regard, we will address the importance of the modulation of iNOS, arginase and Rho kinase pathways, the impact of inducing oral tolerance and the effects of exercise. In addition, aspects of neuroimmunomodulation, including stress effects, will be discussed.

2. Airway and lung parenchyma hyperresponsiveness and smooth muscle alterations in asthma

AHR is the hallmark of asthma, and it is characterized by an increase in the airway response to bronchoconstrictor stimuli. There are two components of AHR. AHR has a variable component that mainly reflects the current airway inflammation (22, 23) and an irreversible component that probably reflects pulmonary remodeling (24).

As described above, the ASM is the major effector of the AHR in asthma (17-19). There are two phenotypes of ASM cells in asthmatics: the contractile, which is responsive to contractile agonists and has an increased expression of contractile proteins, and the synthetic-proliferative, which lacks the responsiveness to contractile stimuli and has a reduced expression of contractile proteins (17). Both phenotypes can coexist or not in the airways of the same person (25-29). Depending on the triggers, it can also induce the proliferation of the synthetic-proliferative cells or induce the maturation of these cells into contractile cells (17, 19).

In patients with asthma, the ASM was thought to generate more force and consequently a greater extent of contraction in response to different stimuli (30). Cultures of ASM cells isolated from lung tissue (trachea, bronchi) were used to study the contractile responses and the mitogenic and synthetic responses, which revealed that these cells are active players in inflammation (25, 31, 32).

In addition, ASM can contribute to lung inflammation. Many studies showed that there was an increased number of mast cells in the asthmatic ASM layer (33-38). Brightling et al. (32) evaluated patients with asthma and eosinophilic bronchitis and observed that both groups showed an increase in eosinophils but that the patients with eosinophilic bronchitis were not hyperresponsive to bronchoconstrictor stimuli. The analysis of the ASM layers in these patients showed that only the asthmatics showed a higher number of mast cells and a worsening of respiratory function, suggesting that the mast cells present in the ASM of asthmatics are responsible for the enhancement of airway narrowing.

The ASM cells release chemotactic agents for mast cells, such as CCL11 (25), CXCL10 (34) and CX3CL1 (35). Because the mast cells are in the airways, they adhere to the ASM cells and produce, together with the eosinophils, contractile mediators, such as prostaglandins (PGF2α, PGD2, and thromboxane TXA2) (39).

Clinically, the AHR symptoms are described as cough, tightness of the chest and wheezing after exercise or exposure to cold air or other environmental irritants (40). Some studies suggest that monitoring of the AHR in asthmatic patients can serve as a guide to asthma therapy (24).

In clinical and experimental studies, AHR is evaluated by the aerosol administration of bronchoconstrictor agonists, such as histamine, methacholine or carbachol. This methodology considers that the ASM in asthmatics exposed to exogenous bronchoconstrictor stimuli showed an increased tonus and a concomitant bronchoconstriction. The hyperresponsiveness occurs due to an increase in both the sensitivity and/or reactivity of the airways (Figure 1). The increase in sensitivity is a reduction in the minimal dose that is necessary to induce bronchoconstriction, whereas the increase in reactivity is described by an increase in the intensity of the bronchoconstriction.

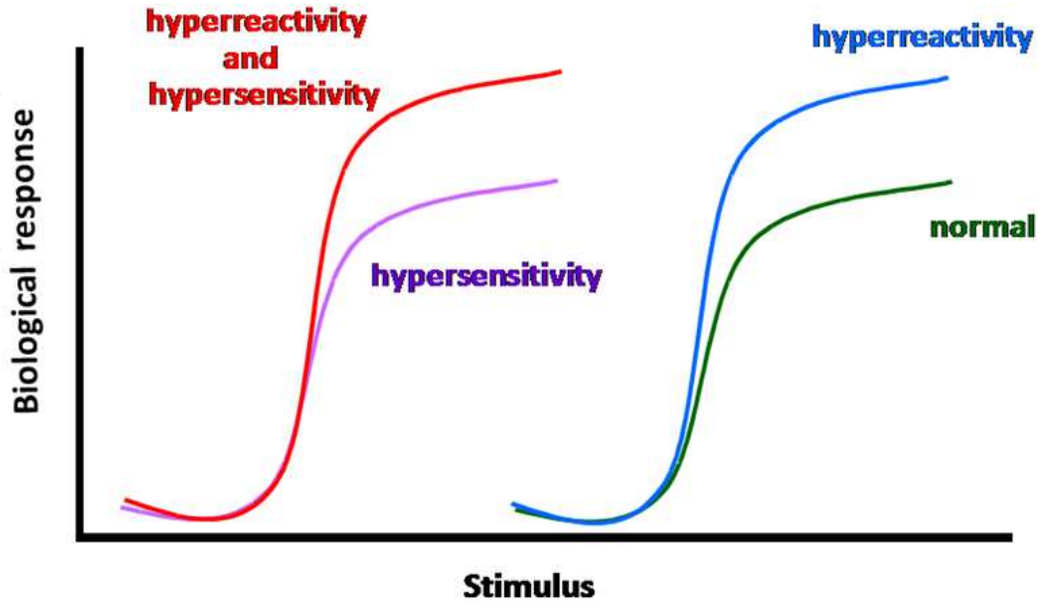

Figure 1. Airway hyperresponsiveness.

Considering that lung parenchyma strips have long been used to study the behavior of the peripheral lung, they are commonly used to evaluate the mechanics and pharmacological properties of the lung periphery (41). Dolhnikoff et al. (15) concluded that human lung tissue strips respond to an acetylcholine (ACh) challenge with changes in their dynamic mechanical behavior. In addition, Lanças et al. (10) have recently shown that the lung tissue is involved in the late asthmatic response in guinea pigs with chronic allergic lung inflammation, which is correlated to lung tissue eosinophilic recruitment and extracellular matrix remodeling.

Although the *in vivo* apparatus of oscillatory mechanics permits the evaluation of large and small airways, the oscillatory mechanics *in vitro* provide a tool for the specific evaluation of the lung periphery with minimal interference with the compartment represented by the small airways (10, 15). In addition, this *in vitro* methodology permits the specific analysis of the effects of several mediator/modulators in the lung periphery while avoiding other compensatory mechanisms that could be activated in *in vivo* studies. Lung parenchyma strips exclusively represent the distal units of the lung tissue and offer a better assessment of pure tissue properties. Thus, studies using this technique have been performed to evaluate the mechanical and pharmacological properties of the lung periphery (10, 42, 43).

Several authors have discussed the importance of these structures in the mechanical behavior of lung tissue, including the consequences of stiffening the extracellular matrix network and of elastin and collagen digestion in these responses (44, 45). In the subpleural region, there was a small number of bronchial and blood vessels (less than 30%). Romero et al. (46) concluded that pneumoconstriction significantly modifies the intrinsic mechanical properties of the connective matrix via a mechanism differing from that of passive stretching. In fact, the contractile cells could be accepted as being able to modulate the mechanical properties of the connective matrix.

3. Mediators involved in airways and distal lung parenchyma contractile responses

A large quantity of extracellular agonists (inflammatory mediators or neurotransmitters) released in an inflammatory milieu can stimulate the contraction of ASM in asthma. Mediators that are found in high concentrations in asthma, including leukotrienes (produced by inflammatory cells) (47), prostaglandins such as PGF2α, PGD2, and thromboxane TXA2 (produced by mast cells and/or eosinophils) (39) and endothelin (produced by epithelial or endothelial cells) (48, 49), are direct contractile agonists of ASM. Neurotransmitters, such as ACh or neurokinins, are highly present in asthma and are also potent contractile messengers of ASM (50, 51).

To increase the release of the contractile mediators, there is also a lower release of relaxant mediators, such as vasoactive intestinal peptide (VIP), PGE 2, adrenaline and NO (35, 52). These mediators are involved in the mechanisms responsible for many of the structural and functional lung alterations observed in asthmatic patients and in animal models of chronic pulmonary allergic inflammation (53-55).

3.1. Excitatory non-adrenergic non-cholinergic mediators: Neurokinins and Substance P

Neurokinins and substance P are involved in the excitatory NANC responses and modulate several histopathological alterations observed in asthmatics, such as airway smooth muscle contraction, peribronchial edema formation and airway mucous secretion. In this regard, substance P (SP) and neurokinin A (NKA) play significant roles in priming and recruiting eosinophils and lymphocytes in models of allergic lung inflammation (56-58).

Asthmatic patients are hyperresponsive to the SP and NK1 expression that is augmented in their bronchi (59). Tibério et al. (60) showed that capsaicin infusion induced an increase in the respiratory system resistance that was attenuated mainly by a NK2 receptor antagonist. The NK receptors are also involved in eosinophil recruitment, which contributes to the hyperresponsiveness. Using a model of experimental asthma in guinea pigs, Tibério et al. (57) evaluated the airway inflammation induced by repeated exposure to ovalbumin and the effects of neurokinin depletion on these responses. These authors showed that neurokinin depletion reduced the peribronchial edema, CD4 lymphocytes and the hyperresponsiveness to the antigen challenge. In addition, Prado et al. (61) showed that the bronchodilation observed after 14 days of capsaicin infusion could be related to the increase in NO produced by nNOS, which counteracts the bronchoconstriction.

Emphasizing that SP has a preferential affinity for NK1 receptors and that neurokinin A has a preferential affinity for NK2 receptors is important (58). However, each neurokinin also exhibits activity at other NK receptors. In this regard, Regoli et al. (62) showed that NKA has 25% of the affinity of SP for the dog carotid artery, a preparation that contains only NK1 receptors. Tibério et al. (60) investigated the role of substance P (SP) and neurokinin A (NKA) and their receptor antagonists (RAs) SR140333 and SR48968 (respectively for the NK(1) and NK(2) receptors) in the pulmonary eosinophil influx induced by the stimulation of capsaicin (CAP)-sensitive nerve terminals. Both SP and NKA contribute to eosinophil lung recruitment in the distal airways and the alveolar wall, and these findings suggest that neurokinins may contribute to the development of eosinophilic inflammation in both allergic asthma and hypersensitive pneumonitis.

3.2. Cysteinyl leukotrienes

Cysteinyl leukotrienes (cysLTs) are synthesized *de novo* from arachidonic acid, and most of their actions are mediated by the CysLT1 receptor, a G protein-coupled receptor (63). CysLTs have many pulmonary actions, including human airway smooth muscle contraction, chemotaxis, mucous secretion, smooth muscle proliferation and increased vascular permeability (64-66).

The cysteinyl leukotrienes (LTC4, LTD4, LTE4) produced by inflammatory cells and endothelin, produced by epithelial or endothelial cells, are increased in asthma. They are also potent contractile agonists of ASM (48, 67).

Leukotriene antagonists have been shown to reduce sputum and mucosal eosinophils in subjects with asthma (68, 69). However, recent long-duration trials have evaluated the impact of CysLT receptor antagonists compared with glucocorticoids and showed that spirometry, symptoms, β2-agonist use and the quality of life were improved to a greater extent with glucocorticoids (70-72). Corroborating this idea, the blockade of leukotriene activity does not cause an improvement in airflow as intense as that obtained with glucocorticoids (70, 73).

Considering studies in animal models, Gardiner et al. (74) observed that the inhibition of leukotriene synthesis resulted in an attenuation of OVA-induced airway contraction in sensitized animals. Liu et al. (75) demonstrated that the CysLT1 receptor antagonists pranlukast and zafirlukast inhibited OVA-induced mucus secretion in the trachea of a sensitized guinea pig. Comparing the effects of montelukast and corticosteroid treatments in a guinea pig model, Leick-Maldonado et al. (76) showed that although montelukast, an antagonist of leukotriene, reduced some aspects of inflammation, this treatment was not able to attenuate the changes in lung mechanics.

3.3. Complex NOS-arginases

Nitric oxide derived either from constitutive isoforms (nNOS and eNOS) or from other NO-adduct molecules (nitrosothiols) modulates bronchomotor and vascular tone. In addition, NO derived from inducible isoenzyme (iNOS) is mainly involved in the immunomodulation (77-80).

Prado et al. (81) tested the differences between chronic and acute nitric oxide inhibition by *N*-nitro-L-arginine methyl ester (L-NAME) treatment in lung mechanics, inflammation, and airway remodeling in an experimental asthma model in guinea pigs. Both acute and chronic L-NAME treatment reduced the exhaled nitric oxide in sensitized animals. Chronic L-NAME treatment increased the baseline and maximal responses after an antigen challenge (ovalbumin) of the respiratory system resistance and reduced peribronchial edema and airway infiltration by mononuclear cells. Acute administration of L-NAME increased the maximal values of respiratory system elastance and reduced the mononuclear cells and eosinophils in the airway wall, supporting the hypothesis that, in this model, nitric oxide acts as a bronchodilator in the airways.

iNOS enzyme activation has been found in many types of inflammatory cells, such as eosinophils, neutrophils and macrophages, as well as in respiratory epithelial cells. In fact, NO produced from this isoenzyme is related to the amplification of the inflammatory and remodeling responses (54, 78, 79, 82). Considering these aspects, a specific inhibition of iNOS-derived NO has been considered to be a future therapeutic strategy for several diseases, such as asthma, sepsis and acute lung inflammation (82-85).

Considering the smooth muscle responses, NO mainly derived from cNOS relaxes the airway smooth muscle. Many studies have focused on the role of NO in the modulation of airway smooth muscle contraction in different models of experimental pulmonary allergic

inflammation (78, 81, 85-87). NO that is mainly derived from the constitutive isoforms of NOS has been shown to attenuate the bronchoconstriction induced by allergens in sensitized experimental animals (54, 85, 88). In contrast, others have observed that nNOS-derived NO could contribute to airway constriction (61). We previously evaluated the effects of NO in respiratory system resistance using a guinea pig model of asthma and compared the cNOS and iNOS inhibition. We showed that chronic treatment with L-NAME, a false substrate that nonspecifically inhibits the production of NO, increased the respiratory system resistance in sensitized animals, whereas the iNOS-specific inhibition by 1400W reduced this response (54). Our results suggested a protective effect of NO derived from cNOS. In addition, we showed that iNOS contributes to the airway hyperresponsiveness in this model. Interestingly, in naïve animals, we observed that both L-NAME and 1400W treatments increased the resistance of the respiratory system. Because the role of iNOS is more pronounced in inflammatory situations, few studies have evaluated the effects of iNOS inhibition in physiologic situations. We have previously shown that there is a basal expression of iNOS in resident cells around the airways in guinea pigs not exposed to an inflammatory stimulus (54, 78). In addition, Guo and colleagues (89) showed that iNOS is continuously produced by the airway epithelium in normal humans. These data suggested that NO produced by iNOS under physiological conditions can also contribute to the control of the airway smooth muscle responses.

Analyzing the nitrergic nerve density, there appears to be a progressive reduction throughout the bronchial tree (90). In fact, Prado et al. (54) demonstrated that the inhibition of NO by chronic L-NAME treatment amplified the elastance responses. Considering that the respiratory system elastance responses are related to alterations in the distal airways and lung tissue, the authors suggested that NO could also be involved in the modulation of lung tissue constriction. Dupuy et al. (90) proposed that inhaled NO only affects the distal airways at high doses, suggesting that, although less intensive, NO can also modulate the responses of the distal airways and/or lung tissue.

Angeli et al. (11) evaluated the effects of chronic L-NAME treatment, a false substrate for all nitric oxide enzymes, on the modulation of lung tissue mechanics, eosinophilic inflammation and extracellular matrix tissue remodeling in guinea pigs with chronic lung inflammation. The authors suggested that nitric oxide plays an important role in lung tissue constriction and elastic fiber deposition within the alveolar septa in this animal model of chronic pulmonary inflammation. The activation of the pulmonary oxidative stress pathway, mainly via 8-iso-PGF2α, may contribute to these responses.

Starling et al. (9) demonstrated that iNOS activation contributes to lung parenchyma inflammatory and remodeling alterations in guinea pigs with chronic pulmonary allergic inflammation. 1400W, an iNOS-specific inhibitor, diminished the lung tissue elastance and resistance as well as the eosinophilic infiltration, collagen and elastic fiber content and volume proportion of actin in lung tissue. To our knowledge, this study has provided the first evidence of the effects of iNOS inhibition on the distal lung parenchyma.

In addition, the authors showed that specifically blocking iNOS reduced 8-isoprostane expression in the alveolar septa, which had previously been increased by repeated ovalbumin exposures (9). These findings suggest that the effects of iNOS-derived NO in the lung parenchyma depend, at least partially, on the activation of the oxidative stress pathway. The inhibition of NO production derived from iNOS activation also reduced the actin content (9). These results suggest an iNOS-derived effect on the myofibroblasts, which were believed to be the major cells responsible for the production of the extracellular matrix and the contraction of the parenchyma (91).

Another pathway to be discussed involves the arginases. These enzymes convert L-arginine into L-ornithine and urea and are the key enzymes of the urea cycle in the liver (arginase 1) but are also expressed in cells and tissues that lack a complete urea cycle, e.g., arginase 2 expression in the lung (88). Arginases are involved in cell growth and tissue repair via the increased production of L-ornithine, a precursor of polyamines and proline (88).

Que et al. (92) demonstrated the expression of arginase in the bronchial epithelium and in peribronchial connective tissue fibroblasts. In addition, Meurs et al. (87) showed that arginase appears to modulate the tone of the airway smooth muscle and potentiates methacholine-induced airway constriction. Arginase accomplishes these actions by forcing the common substrate L-arginine away from epithelial cNOS to diminish the agonist-induced production of NO. Arginases and NOS compete for the bioavailability of the same substrate, L-arginine, and are involved indirectly in the regulation of NO synthesis (53, 88). Corroborating this idea, Morris et al. (93) showed that there is a reduction in the levels of plasma arginine in asthmatic patients compared with patients without asthma but with increased serum arginase activity. Together, these results suggest that increased arginase activity in asthma may be a contributing factor to the decrease in the circulating levels of L-arginine and the consequent NO deficiency. Thus, blocking NO production could be a tool to study the indirect involvement of arginase in various pathophysiological processes (82, 87).

Several powerful drugs have been used to investigate the role of arginases in the pathophysiology of asthma, including nor-NOHA (Nω-hydroxy-nor-L-arginine), which is one of the most potent inhibitors of arginase (88). Meurs et al. (87), studying in vitro tracheal ring-sensitized guinea pigs, demonstrated that treatment with nor-NOHA reduced the hyperresponsiveness to methacholine, and this effect was reversed by treatment with L-NAME.

We demonstrated that chronic distal lung inflammation was associated with an increase in arginase content and iNOS-positive cells (data not published). These results were associated with constriction of the distal lung parenchyma. The increased iNOS expression leads to activation of the oxidative stress pathway and formation of PGF2α, which had a procontractile effect. In addition, we showed that the mechanism involved in the activation of arginase and the iNOS pathways may be related to the modulation of NF-kB expression. Finally, we demonstrated that the association of iNOS and arginase 2 inhibitions potentiated the reduction of PGF2α and NF-kB expression in the distal lung of guinea pigs with chronic pulmonary inflammation (data not published).

Airway inflammation is accompanied by a marked upregulation of iNOS expression, particularly in the airway epithelium (94), which has been associated with the activation of nuclear factor-kB (NF-kB), a transcription factor that is implicated in the induction of multiple genes expressed during the inflammatory response (95). Ckless et al. (96) showed that the activation of NF-kB may induce an increase in NOS and arginases. Furthermore, NF-κB activity can be affected by reactive oxygen species (ROS) and by reactive nitrogen species (RNS) (97).

Several mechanisms reported in the literature have tried to explain how NO could interfere with airway tone. The ability of NO to control airway tone could be related to both GMPc-dependent and GMPc-independent mechanisms (98-100).

Although the mechanisms involving the effects of NO in airway constriction have been extensively described, the exact mechanism involved in the effect of NOS inhibition on reducing lung parenchyma constriction is not completely understood. Another pathway discussed by some authors is related to the fact that the release of NO by NOS activation also contributes to oxidative stress, amplifying the deleterious and harmful effects on the lungs (9, 77).

The potent oxidant peroxynitrite is formed by the interaction of NO and superoxide by a rapid iso-stoichiometric reaction (77). Haddad et al. (101) suggested that peroxynitrite may contribute to the injury of pulmonary surfactant. Bhandari et al. (102) demonstrated that increased peroxynitrite formation was associated with a dose-dependent increase in the apoptotic cell death of type II pneumocytes. However, in strip preparations perfused with Krebs solution, the importance of reducing pulmonary surfactant was poorly associated with the pulmonary mechanical responses.

In contrast, peroxynitrite formation leads to lipid peroxidation and the generation of iso-prostanes (8-iso-$PGF_{2\alpha}$). Jourdan et al. (103) showed that L-NAME treatment greatly inhibits 8-iso-$PGF_{2\alpha}$. Therefore, isoprostanes appear to induce airway and vascular smooth muscle contractions by acting through tyrosine kinase, Rho and Rho kinase, leading to the de-creased activity of myosin light chain phosphatase. The net response is associated with an increased level of phosphorylated myosin light chain and contraction (104).

3.4. Rho kinase pathway

The protein Rho, a member of the Ras superfamily of small monomeric GTPases, controls a variety of downstream effector proteins, including Rho kinase. Rho exhibits GDP- and GTP-binding and GTPase activity and is able to alternate between a GDP-bound inactive state and a GTP-bound active state. This alternation allows Rho to function as a molecular switch to control downstream signal transduction, influencing the level of smooth muscle tone and changes in the actin cytoskeleton, which contributes to cell adhesion, motility, migration, and contraction (105). Effects on the airway smooth muscle responses may be one of the most important factors that need to be considered for the development of new therapies for asthmatics (106).

The influence of Rho kinase on airway hyperresponsiveness is considered to be at least partly related to agonist-mediated Ca^{2+} sensitization. Ca^{2+} sensitization, which is also observed in the airways, is the increase in smooth muscle tension and/or phosphorylation of the 20-kDa regulatory light chain of myosin (MLC_{20}) at a constant Ca^{2+} concentration (107). In a variety of smooth muscles, this Ca^{2+} sensitization is mediated by a small G protein, RhoAp21, and its target protein, the Rho kinase (108), which is especially important during the sustained phase of contraction in smooth muscle (107).

Several studies have shown that the use of Rho kinase inhibitors might be beneficial for the treatment of airway diseases. Y-27632((+)-(R)-*trans*-4-(1-aminoethyl)-*N*-(4-pyrydil) cyclohexanecarboxamide, monohydrate) is one of the drugs that arose as a possible treatment for asthma. Y-27632 is a highly selective inhibitor of the Rho kinase pathway, capable of reversing G-protein sensitization and consequently relaxing the airway smooth muscle (108).

The effects of the acute inhibition of Rho kinase in sensitized animals have been analyzed by several authors. Schaafsma et al. (109) showed that the inhalation of Y-27632 at 30 min prevents the development of airway hyperresponsiveness both after the early and late airway reaction. Y-27632 reduces also reduces the cholinergic nerve-mediated contractions in the tracheal preparations of guinea pigs and mice in a dose-dependent manner (110). Witzenrath et al. (111) verified that the use of Y-27632 attenuated the methacholine-provoked airway response in the sensitized lungs.

Some studies suggested that the RhoA/ROCK system plays a role in eosinophil recruitment and Th-1 and Th-2 cytokine secretion (105, 112). In this regard, Henry et al. (112) demonstrated that pretreatment with Y-27632 reduced the number of eosinophils recovered from the bronchoalveolar lavage (BAL) fluid of OVA-sensitized mice.

Taki et al. (105) showed that another Rho kinase inhibitor, fasudil, reduced the presence of eosinophils in the BAL fluid, airways and blood vessels. In the BAL fluid, this Rho kinase inhibitor also diminished the augmented levels of IL-5, IL-13 and eotaxin. Aihara et al. (113) showed that Y-27632 suppressed the release of Th-1 cytokines and partially suppressed the release of Th-2 cytokines in healthy persons but reduced the release of IL-2 and IL-5 and weakly reduced the release of IL-4 and IFN-gamma in asthmatic patients.

Recently, we showed the chronic inhibition of Rho kinase reduced the airway and distal lung mechanical responses to an antigenic challenge with an associated reduction in NO_{EX}, eosinophilic infiltration, IL-2-, IL-4-, IL-5- and IL-13-positive cells, extracellular matrix remodeling and NF-κB-positive cells in the airways and distal lung. In addition, there was a significant reduction in the activation of the oxidative stress pathway, which was correlated with the attenuation of the maximal mechanical responses after antigen challenge (data not published).

These data suggest that treatment with an inhaled Rho kinase inhibitor contributes to the attenuation of the distal lung functional and structural changes induced by chronic allergic inflammation, both in the airways and distal lung. Taken together, this evidence suggests that Rho kinase inhibitors may be potential pharmacological tools to control distal lung asthmatic functional and histopathological alterations.

4. Modulators involved in airways and distal lung parenchyma contractile responses

4.1. Modulation of the lung contractile responses by physical exercises

The role of physical exercise in asthma is somewhat controversial. Exercise can induce bronchoconstriction in humans (114). Recently, however, various studies have shown that physical training, particularly at a moderate intensity, can improve lung function and is related to a reduction in asthma symptoms and AHR. Fanelli et al. (115) associated physical training improvements in the physiological variables at peak and submaximal exercise, and these authors also showed that trained patients have a reduction in the daily doses of inhaled steroids.

Studying adults, Mendes et al. (116) showed that 3 months after supervised training, patients presented a reduction in inflammation and asthma exacerbation and an increase in asthma symptom-free days. Although the authors did not directly measure the AHR, the reduction in symptoms and exacerbations indirectly reflects a reduction in the airway responsiveness. These authors clearly suggest that aerobic training might be useful as an adjuvant therapy in asthmatic patients under optimized medical care. In addition, physical training reduced the anxiety and depression levels with a significant correlation between improvements in the aerobic capacity and days without asthma symptoms (117).

Considering the experimental studies, Silva et al. (118) showed that aerobic training in mice with allergic chronic inflammation reduced both tissue elastance and resistance. These effects of aerobic training on lung mechanics could be at least partly mediated by the epithelium (119).

Based on these data, although AHR was frequently found among competitive athletes (120, 121), physical training may be beneficial to asthmatics, particularly when performed with supervision and at a moderate intensity.

4.2. Modulation of the lung contractile responses by stress

The stress response, which can be defined as the psychological reaction of the body to a variety of emotional or physical stimuli that threaten homeostasis (122), results in the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic and adrenomedullary systems. Although acute stress was shown to have anti-inflammatory effects, some studies have demonstrated that stressful situations and emotional states are triggers of asthmatic symptoms (123-125) and can influence the course and treatment of atopic diseases (126, 127). Chronic stress may induce a down-regulation of the expression and/or function of glucocorticoid receptors, leading to glucocorticoid resistance and contributing to the worsening of lung inflammation and pulmonary hyperreactivity.

Capelozzi et al. (128) showed that swimming-induced stress amplified mononuclear cell recruitment to the lungs in guinea pigs that performed 31 days of the stress protocol. These authors also showed that the amount of these cells was reduced when the animals were

treated with fluoxetine. Recently, Leick et al. (129), studying the effects of stress induced by forced swimming in bronchoconstriction, observed that stress amplified the airway response to ovalbumin in guinea pigs. In addition, Marques et al. (130) showed that the malefic effects of stress in asthma are related not only to the airways but to the lung distal parenchyma. In sensitized animals, they showed that repeated stress increased the distal lung constriction associated with an augmentation of actin content, which is indirect evidence of the alveolar smooth muscle content. The authors also showed that iNOS inhibition attenuated the effects of stress in the lung parenchyma response in this animal model.

Considering humans, Ritz and Steptoe (125) observed a negative association between mood states and a reduction in the forced expiratory volume in the first second in asthmatic patients. Höglund et al. (131) studied 41 undergraduate students 22 with allergies, 16 asthmatics and 19 controls in a low-stress period and in a period associated with a large exam. The values of the forced expiratory volume in the first second of the control group differed significantly from that of the group of asthmatics only during the exam stress phase. These results collectively reinforced the idea that stress is an important modulator of the AHR present in asthma.

Collectively, these studies showed that chronic stress is harmful to asthmatic individuals and is involved in the AHR.

4.3. Oral tolerance

Immunotherapy has been considered a possible therapeutic strategy for asthma. Oral tolerance has been recognized as an alternative treatment to autoimmune and allergic diseases (132-134). Oral tolerance has classically been defined as the specific suppression of the cellular and/or humoral immune response to an antigen by the prior administration of the antigen by the oral route (135). There are two primary effector mechanisms of oral tolerance: the induction of regulatory T cells that mediate the active suppression and the induction of clonal anergy or deletion (135-137). In atopic patients, the oral, sublingual, or inhaled administration of antigens leads to a reduction in symptoms and local inflammation as well as a reduction in dyspnea and airway hyperresponsiveness. Some meta-analyses found that sublingual immunotherapy is beneficial for asthma treatment, although the magnitude of the effect is not very large (138-140).

Some authors (141-143) have previously evaluated the effects of oral tolerance in experimental models of airway disease. In an animal model, oral tolerance induced an attenuation of airway eosinophilic recruitment, bronchial hyperresponsiveness, and mucous secretion (143, 144). Russo et al. (141, 142) observed that animals submitted to an oral antigen administration protocol presented low levels of Th2 cytokines in the bronchoalveolar lavage fluid and a reduction in the production of ovalbumin-specific antibodies. The tolerance process is known to attenuate B-cell responses. Hasegawa et al. (145) demonstrated that B-cells have been implicated in myofibroblast activation mainly by secreting IL-6, IL-9, and fibroblast growth factor. Thus, considering that myofibroblasts are one of the contractile elements that modulate lung parenchyma responses is important (146, 147).

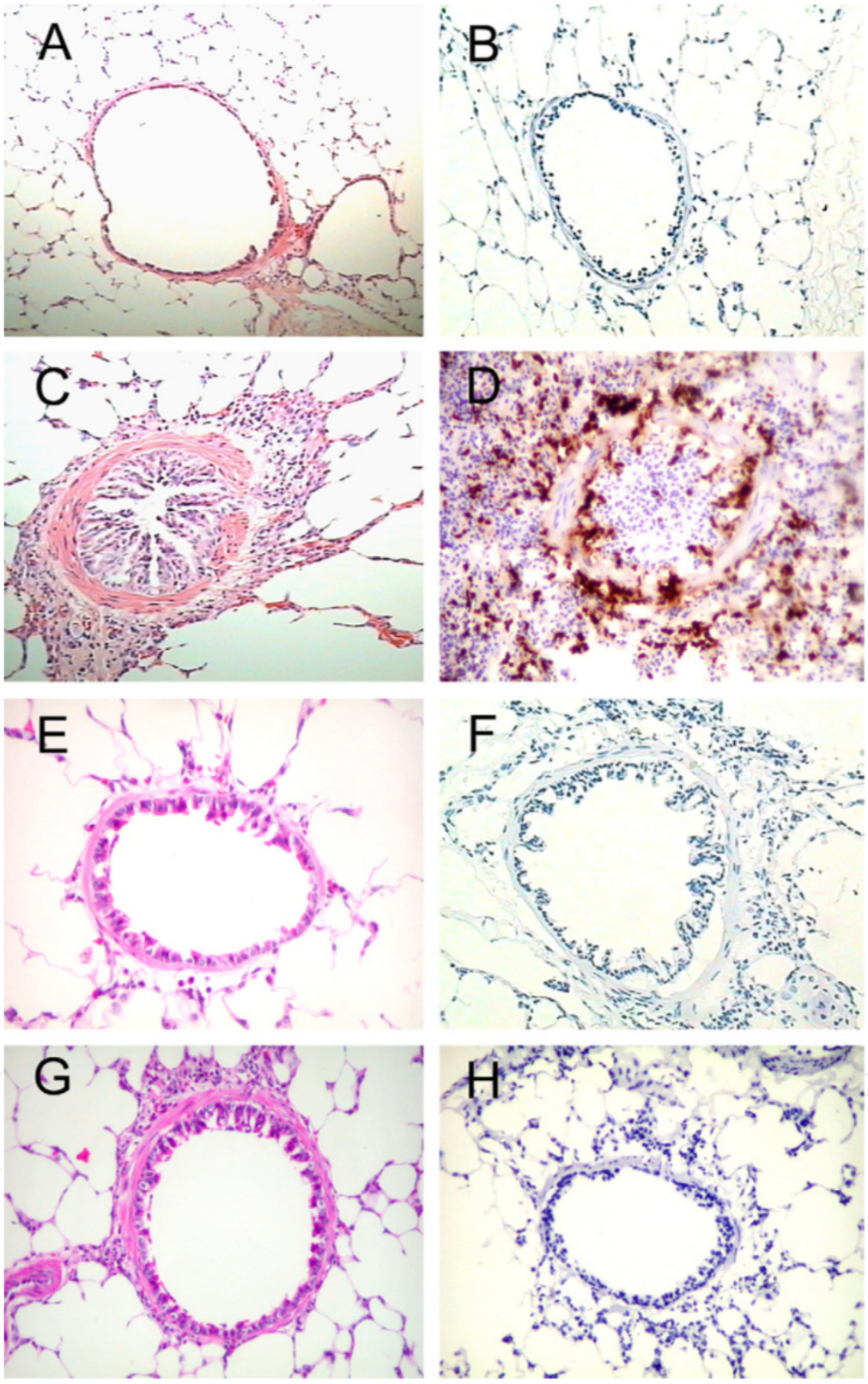

Figure 2. Photomicrographs of distal airways from the guinea pig (×200), stained with haematoxylin-eosin (left panels) and EPO+ eosinophils (right panels). Panels A and B: NS group. Panels C and D: OVA group. Panels E and F: OT1 group. Panels G and H: OT2 group. Reproduced with permission. *Published in Ruiz Schtüz et al. (143).*

Our group evaluated the airway responses in two different models of oral tolerance (ovalbumin-exposed and treat with oral tolerance beginning together with the 1st inhalation (OT1 group) and ovalbumin-exposed and treated with oral tolerance beginning after the 4th inhalation (OT2 group), and showed that both models counteract the bronchoconstriction induced by a specific antigen (ovalbumin) and by a nonspecific challenge using methacholine (143) (Figure 2). These data suggested that oral tolerance is an effective treatment to induce the relaxation of airway smooth muscle in asthma.

Although previous investigations showed that oral tolerance attenuated the airway responses, few studies have provided evidence of the effects of oral tolerance in lung periphery responses in an experimental model of chronic lung inflammation. In this regard, Nakashima et al. (43) showed that inducing oral tolerance attenuates peripheral lung tissue responsiveness, eosinophilic inflammation and extracellular matrix remodeling in an experimental model of chronic allergic pulmonary inflammation (Figure 3), suggesting that this approach could attenuate or prevent the distal lung functional and structural changes induced by chronic allergic inflammation.

5. Contribution of the airway and distal parenchyma structural changes to the pulmonary contractile responses.

The underlying persistent component of AHR, by contrast, is likely related to the structural (and/or physiological) airway changes often collectively referred to as airway remodeling. Structural changes in the airways and in the distal lung parenchyma, which were recently addressed, are involved in the remodeling process and include the epithelium basal membrane thickness, subepithelial fibrosis, mucous gland and goblet cell hypertrophy and hyperplasia, neoangiogenesis, increased ASM mass (hypertrophy of the smooth muscle cell and wall thickening), increased amount of actin and changes in the extracellular matrix (ECM), such as the deposition of fibronectin, laminin, and collagen fiber, alterations in the airway elastic fibers, and the increased expression of several metalloproteinases (MMP-1, MMP-2 and MMP-9) (45, 54). Such airway structural alterations or airway remodeling is associated with airway hyperresponsiveness to diverse triggers and with a decrease in the lung function of asthmatic patients.

In addition, an important structural change of the airways is related to the smooth muscle. One of the pathological consequences of remodeling is airway hyperresponsiveness. Myocyte hypertrophy and hyperplasia and myofibroblast hyperplasia are known to contribute to this hyperresponsiveness and the worsening of lung function in these patients (148). Throughout breathing, airway stiffening is a feasible contributor to airway hyperresponsiveness through the attenuation of the transmission of a potently bronchodilating cyclical stress to the ASM (37). ASM hyperplasia is characterized by a proliferation of cells, a reduction in the apoptosis of the ASM cells and migration of myofibroblasts within the ASM layer (19). Hence, alterations in the smooth muscle, either in the airways or in regions that are associated with perturbed alveolar attachments, may be factors that affect airway-parenchyma uncoupling and alterations in the mechanical properties of the distal lung that lead to constriction.

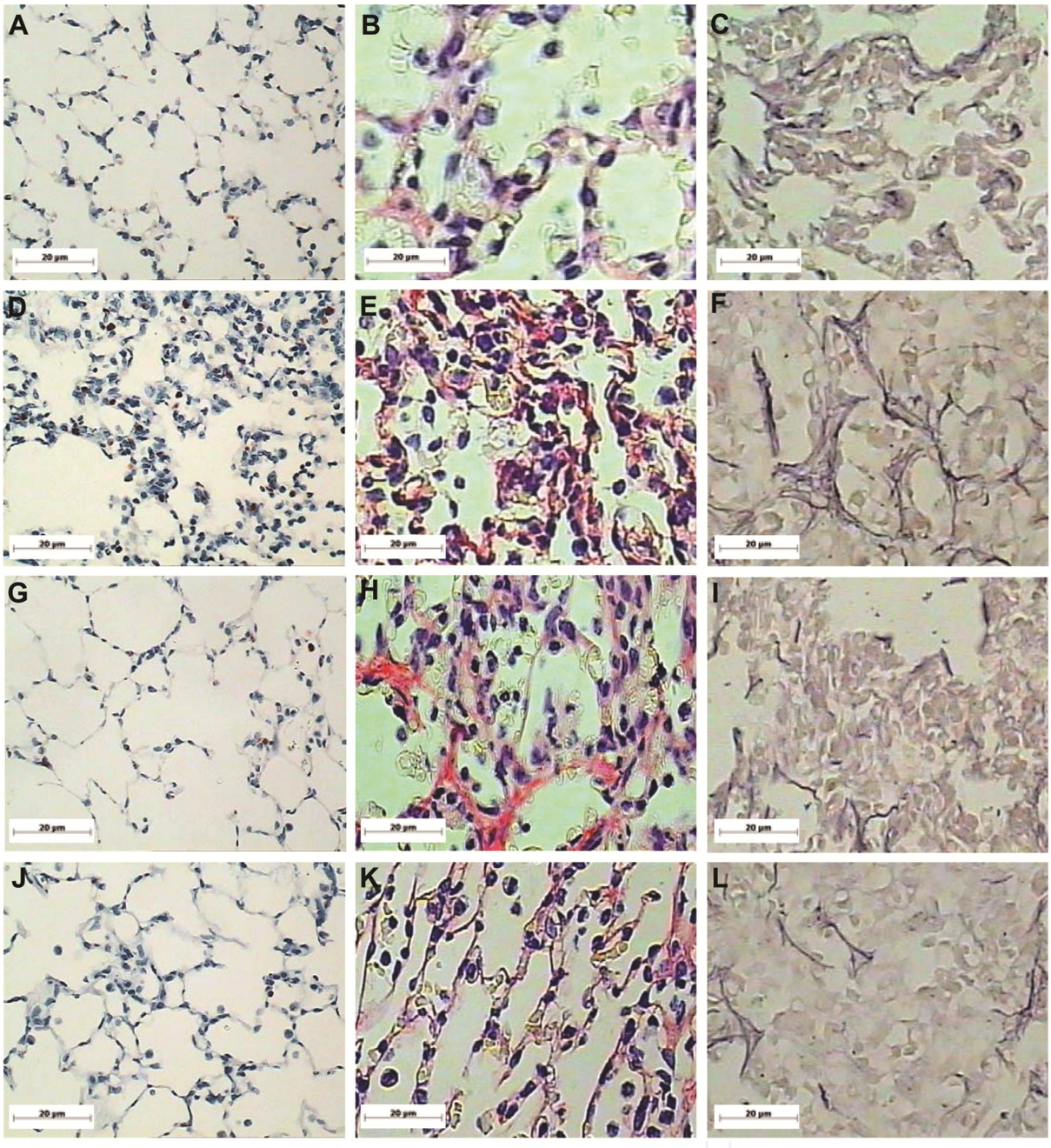

Figure 3. Photomicrographs of lung parenchymal strips eosinophilic infiltration (A, D, G, and J – x400), collagen density (B, E, H, and K – x1000) and elastic fibers (C, F, I, and L – x1000) in saline-exposed (NS group - panels A to C), ovalbumin-exposed (OVA group – panels D to F), ovalbumin-exposed and treat with oral tolerance beginning together with the 1st inhalation (OT1 group – panels G to I) and ovalbumin-exposed and treated with oral tolerance beginning after the 4th inhalation (OT2 group – panels J to L). Ovalbumin-exposed animals showed a significant increase in eosinophilic infiltration as well collagen and elastic density compared to saline-exposed ones. Both oral-induced tolerance protocols attenuated all these responses in ovalbumin-exposed animals. Reproduced with permission. *Published in Nakashima et al. (43).*

5.1. Mechanisms involved in lung remodeling

A chronic inflammatory process is almost invariably related to tissue damage and healing. The consequences of healing are repair and the replacement of injured cells by viable cells. Repair comprises regeneration (the replacement of damaged cells by cells of the same type) and replacement (by connective tissue). Chronic inflammatory processes have a wide variety of consequences leading from the complete or partial restoration of the affected structure to fibrotic processes. The mechanisms underlying remodeling move from the highly dynamic process of cell migration, differentiation, and maturation to changes in the connective tissue deposition and to the altered restitution of the structures (149).

The airway epithelium constitutes a continuous physical barrier, crucial to maintaining tissue homeostasis, which lines the airway lumen and separates the underlying tissue from environmental antigens (150, 151). Currently, the airway epithelium is acknowledged to also sense and react to antigens by regulating innate (through pattern-recognition receptors, including Toll-like receptors [(TLRs]) and adaptive immune mechanisms, driving both allergic sensitization and airway remodeling through the release of inflammatory cytokines and chemokines. In addition, direct physical interactions with immune cells protect the internal milieu of the lung (152) and therefore contribute to airway narrowing. Furthermore, the increased loss of epithelial barrier integrity is known to correlate with more severe airway hyperresponsiveness, which may lead to the augmented exposure of the ASM to inhaled contractile agonists (153). Therefore, epithelial cells participate in a wide range of repair mechanisms, including the epithelization of the nude luminal surface, the production of chemotactic factors, and the expression of some surface markers and a broad range of molecules that participate in the tissue repair, such as fibronectin, growth factors, cytokines and chemokines (149).

One of the mechanisms that may account for ASM hyperplasia is the migration of myofibroblasts within the ASM layer, which differentiate into ASM-like cells (154). Fibroblasts differentiate into the highly synthetic and contractile myofibroblast phenotype when exposed to substrates with an elastic modulus corresponding to pathologically stiff fibrotic tissue. Myofibroblasts, which are cells that display features intermediate between fibroblasts and smooth muscle cells, are involved in this process and are able to synthesize several extracellular matrix substances and contract the lung parenchyma (155).

Although the hypertrophy in ASM has been described in studies with tissue specimens from intermittent, mild, severe (156) and fatal (45) asthma, which have been characterized as having an increase in the ASM cell size, there are conflicting findings (157) that suggest that the ASM cell hypertrophy could be a hallmark of severe asthma because it can be used to differentiate between patients with severe asthma and patients with milder disease (156). In asthmatics, ASM cell proliferation occurs faster than in nonasthmatics (27), and it can be explained by alterations in the calcium homeostasis in these cells and a subsequent increase in mitochondrial biogenesis (158).

The main characteristics of myofibroblasts are the secretion of extracellular matrix components, the development of adhesion structures with the substrate by the incorporation

of de novo expressed α-smooth muscle actin (α-SMA), and the formation of contractile bundles composed of actin and myosin, which help the myofibroblasts to develop a high contractile activity. These cytoskeletal features enable the myofibroblast to not only remodel and contract the extracellular matrix but also adapt its activity to changes in the mechanical microenvironment. In addition, immunohistochemistry and electron microscopy studies demonstrated that airway myofibroblasts and the smooth muscle bundles lie in close physical proximity in asthma (159, 160). The myofibroblasts have an intermediate phenotype between that of a fibroblast and that of a smooth muscle cell, which raises the possibility that these cells contribute to the increased smooth muscle mass because of their plasticity.

The arrangement and modification of the ECM involve dynamic processes of the production and degradation of matrix proteins, which are related to the ASM and parenchyma remodeling that are present and enhanced in asthma (161). The deposition of ECM proteins is increased by airway resident cells, such as epithelial cells, fibroblasts, myofibroblasts, and ASM cells. Some authors studying asthmatic bronchial samples demonstrated an increased deposition of ECM proteins in the bronchial wall, such as collagens I, III, and V, fibronectin, tenascin, hyaluronan, versican, laminin, lumican, and biglycan (162, 163), and a decreased deposition of collagen IV and elastin (164). Enhancing the ECM may be due to a reduced production of matrix metalloproteinases (MMPs), which degrade ECM proteins, and/or the enhanced production of tissue inhibitors of MMPs (TIMPs). Moreover, fibronectin and collagens III and V have been shown to enhance ASM migration (165) in the ASM cell contact with membranes coated with ECM components.

Notably, the epithelium in asthmatic children (aged 5-15 years) is stressed or injured without significant submucosal eosinophilic inflammation. This observation emphasizes the concept that the early pathological changes in asthma are linked to changes in the local tissue microenvironment related to epithelial stress and injury. The lamina reticularis from asthmatic biopsy sections was thicker than normal, with an increased deposition of collagen III. This alteration in the epithelial phenotype is associated with an enhanced collagen deposition in the lamina reticularis, suggesting that the epithelial mesenchymal trophic unit is active early in the natural history of asthma and may contribute to the pathogenesis of asthma (166).

ASM cells and the lung parenchyma have a crucial importance in the pathophysiology of asthma, leading to pulmonary remodeling, which remains unresponsive to conventional treatments, such as bronchodilators and anti-inflammatory drugs (167). Therefore, the development of new therapeutic tools targeting pulmonary remodeling is desirable.

6. Conclusions

ASM cells have a critical role in AHR in asthma, considering that these cells are part of the inflammatory process, have altered contractile, proliferative and secretory functions and contribute to airway remodeling.

Considering that many patients with AHR respond fairly well to conventional therapies, such as anti-inflammatory and bronchodilator drugs, and that ASM remodeling is insensitive to these treatments, further studies are necessary to evaluate ways to prevent or reverse ASM remodeling.

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