Asthma is a chronic inflammatory disease of the airways. The term “airway remodeling” has been applied to structural changes observed in asthmatic airways. Among other effects, this includes alterations in the extracellular matrix due to connective tissue cell dysfunction. Matrix metalloproteinases (MMPs) are key regulators of extracellular matrix composition. MMPs are a group of enzymes (endopeptidases) which participate in the degradation of almost all extracellular matrix protein components. Tissue inhibitors of metalloproteinases (TIMPs) regulate the synthesis, secretion, and activity of MMPs. It is not clear which mechanisms are involved in the maintenance of the equilibrium between MMPs and TIMPs in the asthmatic airways. An excess of TIMPs could favor airways fibrosis and thus lead to airway remodeling. Further studies are needed to develop novel asthma therapies directed against appropriate targets within the extracellular matrix, as the currently available corticosteroid treatment have little impact on extracellular matrix homeostasis and airway remodeling (Adv Clin Exp Med 2007, 16, 3, 417–423).

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tribute to the thickening of all layers of the asthmatic airway wall. The degree of airway wall thickening can oscillate between 10 and 100%, with a proportional predominance of the smooth muscle layer (25–150%). The majority of studies seem to indicate that the structural changes termed airway remodeling are more distinct in patients with severe asthma. Correlation between thickening of the bronchial wall and airway obstruction or duration of the disease have been observed [2].

Our attention was attracted in particular to alterations within the extracellular matrix (ECM) evoked by connective tissue cell dysfunctions. The ECM consists of fibrous proteins, structural proteins, and binding proteins which are embedded in a polysaccharide structure containing glycosamines and proteoglycans. Extracellular matrix components play a role in determining the mechanical properties of the lungs. In asthmatic airways, increased deposition of ECM proteins (laminin, tenasin, collagen I, III, and V, fibronectin, hialuronians, and other glycoproteins) is observed in opposition to decreased deposition of elastin and abnormal composition and thickness of collagen fibers. The ECM is a dynamic structure, and maintaining a balance between the synthesis and controlled degradation of its components is of great importance [2, 3]. Matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) play an important role in maintaining this homeostasis. In asthmatic airways, a major source of metalloproteinases are inflammatory cells. MMPs selectively degrade the components of the extracellular matrix. They are involved in neovascularization and smooth muscle cell hyperplasia. MMP-9 (gelatinase B), which cleaves collagen IV fibers and degrades abnormal collagen, is a main proteinase found in the BALf (bronchoalveolar lavage fluid) and bronchial biopsies of patients with asthma. The synthesis and secretion of MMP-9 depends on inflammatory activity in the airways. TIMP-1 is the endogenous factor which specifically inhibits MMP-9.

The ECM forms a dynamic environment in which small changes in concentrations of MMPs and other enzymes could alter the balance of bioactive proteins. There is evidence that an altered MMP-9/TIMP-1 ratio can influence the thickness of the airway wall in patients with asthma [3]. ECM damage probably directly precedes fibrosis, which can lead to airway remodeling. It is hypothesized that airway remodeling, besides its detrimental influence on the airways, might also be beneficial and may even protect against excessive airway narrowing. The increased ECM mass and thickening of all layers of the asthmatic airway wall could contribute to increased rigidity and stabilization of the airways and therefore to decreased airway distensibility and lung compliance, which could be a protective mechanism against excessive smooth muscle contraction or loss of elastic recoil [4].

Matrix Metalloproteinases

Matrix metalloproteinases are a numerous group of calcium- and zinc-dependent endopeptidases with structural and functional similarity. They are composed of three main domains: a propeptide domain, a catalytic domain, and a hemopexin-like C-terminal domain. A common structural feature of MMPs is a zinc ion in a catalytic center. Most of the MMPs are produced and stored in cells as latent pro-enzymes and are activated by proteolytic removal of the pro-peptide. MMPs can be activated by other MMPs or in vitro by chemical agents (such as HgCl₂), low pH, or high temperature [5]. The C-terminal domain and additional domains are responsible for substrate specificity. The MMP family consists of at least 28 endoproteinases with a broad spectrum of action which are produced by various cells. Twenty-four MMPs have been identified in vertebrates. They are currently classified into five groups according to substrate specificity and structural homology: the collagenases, the gelatinases, the stromelysins, the membrane-type MMPs, and other MMPs not classified in the other categories [6].

MMPs are involved in many physiological processes, such as pregnancy and delivery, angiogenesis, wound healing, and blood platelet function [5, 7, 8]. Increased MMP activity has also been noted in pathological processes, including carcinogenesis, metastasis, atherosclerosis, skin diseases, rheumatoid arthritis, endometriosis, multiple sclerosis, and others [9–12]. The expressions of MMP-9 and MMP-2 in tumour cells and metastases arouse interest in synthetic MMP inhibitors and medications which inhibit MMP activity. Some of these (batimastat, marimastat, prinomastat, tanomastat, neovastat) have been evaluated in clinical trials as options in the treatment of cancer; however, the results have not been satisfactory [13, 14].

In asthma, MMPs are believed to take part in angiogenesis and airway smooth muscle cell hyperplasia by the secretion and activation of growth factors such as TGF-β (transforming growth factor β) and VEGF (vascular endothelial growth factor) [15–17]. MMPs also play a role in inflammatory cell migration to the airway lumen and influence airway hyperresponsiveness and
remodeling [1, 7, 18]. MMP-2 (gelatinase A), MMP-8 (collagenase-2), MMP-9 (gelatinase B) and MMP-3 (stromelysin) have been detected in the airways of asthmatic subjects [5, 19]. MMP-3 is involved in extracellular matrix component degradation and the activation of numerous pro-MMPs [5]. MMP-9 is predominant and one of the best-known endopeptidases in asthma. MMP-9 is produced and released by a wide variety of cells, including neutrophils (which are believed to be the main source of MMP-9 in asthma [20–23]), macrophages, eosinophils, epithelial cells, fibroblasts, lymphocytes, dendritic cells, mast cells, and airway smooth muscle cells [24, 25]. MMP-9 degrades collagen IV, V, VII, X, and XV, denatured collagen, fibronectin, elastin, proteoglycans, growth factors, and cytokines, including VEGF, IL-8, pro-TNF-α, IL-1β, and pro-IL-1β [6, 7, 13].

MMP-9 appears in various forms: a latent pro-form (92 kDa), an active form (88 kDa), a homodimer (220 kDa), and a high-molecular-weight (HMW) form (125 kDa). The HMW form consists of MMP-9 and a neutrophil gelatinase-associated lipocalin (NGAL), which supports the thesis of its neutrophil source [20]. Neutrophils are believed to produce MMP-9 during their maturation in the bone marrow. MMPs are stored in neutrophil granules and, after stimulation by IL-8, for example, are secreted as monomers or HMW forms [24].

The production, secretion, and activation of MMP-9 are tightly regulated by gene transcription and activation and inhibition of the latent form by endogenous inhibitors. MMP-9 gene transcription is controlled by proinflammatory cytokines, growth factors, and matrix-cell and cell-cell interactions. The MMP-9 gene is localized on chromosome 20q11.2-13.1. Activation of the MMP latent form (zymogen) is achieved by removal of the pro-peptide domain that blocks the catalytic center by proteinases such as trypsin, elastase, plasminogen activators, or other MMPs, including MMP-2 and MMP-3. Tissue inhibitors of matrix metalloproteinases together with endogenous inhibitors such as tissue factor pathway inhibitor-2, thrombospondin-1, and α2-macroglobulin, which is believed to be the main MMPs serum inhibitor, play key roles in the MMP inactivation process [26, 27].

### Tissue Inhibitors of MMPs

Four types of tissue inhibitors of matrix metalloproteinases have been identified: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. TIMP-1 controls almost all MMPs, although it is less effective in inhibiting the membrane-type matrix metalloproteinases, which include MT1-MMP, MT2-MMP, MT3-MMP, MT5-MMP, and MMP-19 [27, 28]. TIMP-1 binds MMP-9 in a 1:1 stoichiometry and inhibits MMP-9 enzymatic activity. However, TIMP function is not limited only to MMP inhibition. TIMPs are also necessary for MMP activation. For example, the TIMP-2-MT1-MMP complex promotes the activation of pro-MMP-2 [5]. In animal models of asthma, TIMPs have been demonstrated to regulate inflammatory cell migration by inhibition of ICAM-1 and VCAM-2 adhesive molecule expressions [29].

TIMP-1 regulates the proliferation, apoptosis, and angiogenesis of malignant cells [27]. TIMP-1 has been found in stable asthma and diseases connected with excessive fibrosis. In scleroderma, increased TIMP-1 level correlated with illness severity and lung fibrosis. TIMP-1 was involved in skin fibroblasts proliferation, which could contribute to fibrosis in this disease [30]. Increased TIMP-1 level has also been found in viral C hepatitis and correlated with liver fibrosis [30]. Recently, an association between asthma and polymorphism of the TIMP-1 gene, which codes the region responsible for MMP-9 binding and inhibition, has been described [26]. Alteration of TIMP-1 expression may lead to MMP-9 over-activity in asthma and contribute to imbalance in extracellular matrix homeostasis [31]. Further investigations on this subject are required.

### MMPs and Their Inhibitors in Asthma

The role of MMPs and TIMPs in inflammatory processes of the airways in mild and severe asthma, during asthma exacerbations, and after allergen challenge have been investigated. Increased levels of MMP-9 and TIMP-1 in induced sputum and significant correlation between low MMP-9/TIMP-1 ratio and decreased FEV-1 have been observed in asthmatic subjects, unlike the control group [23]. However, Culpitt et al. did not notice increased MMPs and TIMP-1 levels in induced sputum of subjects with mild asthma [32]. There is extensive evidence that altered MMP-9 levels in induced sputum and BALf correlate with disease severity [16, 23, 28, 31].

In severe asthma, neutrophils are believed to be the main source of MMP-9 and a significant correlation between disease severity and neutrophil population in the airways of asthmatics was confirmed. Increased MMP-9 activity in serum is also present in asthma exacerbations [33]. Some observations revealed that increased MMP-9 level...
Corticosteroids and MMPs

Increased TIMP-1 levels have been detected in the BALf of patients with mild asthma, particularly those treated with corticosteroids [28]. According to Bosse et al. [30], a low serum MMP-9/TIMP-1 ratio before treatment is characteristic of the steroid-unresponsive group of patients. These observations suggest that in this population, fibrosis is the main pathological process; thus there is a lower effectiveness of corticosteroids, which mainly inhibit inflammatory processes. Decreased MMP-9 level accompanied by an increased TIMP-1 level in airway submucosal membrane has been observed in asthmatics treated with inhaled corticosteroids [7]. Profita et al. [17] showed an influence of flunizolide on decreased expressions of MMP-9 and TIMP-1 with an altered MMP-9/TIMP-1 ratio in cells isolated from induced sputum. However, another study did not confirm changes in the levels and activity of MMP-9 and TIMP-1 in the BALf of subjects with mild asthma treated with inhaled corticosteroid for four weeks [31]. Hoshino et al. [39] demonstrated decreased collagen III deposition, decreased epithelial and submucosal MMP-9 expression, and increased submucosal TIMP-1 level accompanied by decreased numbers of inflammatory cells and myofibroblasts after six months of treatment with inhaled corticosteroid (BDP, beclomethasone dipropionate, 800 µg daily).

Corticosteroids, which are especially involved in anti-inflammatory process by inhibiting the activity of various proinflammatory cytokines, decrease MMP production in macrophages and inhibit the process of excessive fibrosis [17, 40]. In vitro studies have shown that corticosteroids inhibit MMP and TIMP gene transcription in macrophages and fibroblasts [40, 41]. Furthermore, Vanacker et al. [42] demonstrated in animal models of asthma that fluticasone inhibited bronchial hyperresponsiveness and airway remodeling, but did not influence previously formed structural changes. An early outset of corticosteroid therapy in asthma can probably delay or diminish the progression of airway remodeling in some asthmatics. In a group of patients in whom remodeling and irreversible airflow obstruction already exist, there is no significant improvement after corticosteroid treatment.

Because of the association between MMPs and airway remodeling, there have been approaches to asthma treatment with a specific MMPs inhibitor, marimastat. However, three weeks of therapy did not influence airway hyperresponsiveness, parameters of pulmonary ventilation, NO exhalatory concentration, and cell formation in the

in induced sputum correlated with increased number of neutrophils and decreased FEV₁ and FVC after allergen challenge. In another study, increased MMP-9 activity and a high MMP-9/TIMP-1 ratio was noted 24 hours after allergen challenge [31]. Kelly et al. [21] described not only increased levels of MMP-9 in BALf 48 hours after allergen challenge, but also of TIMP-1, which correlated with altered neutrophil number. The differences among the mentioned studies can be ascribed to the different time periods from allergen exposure and indicate delayed TIMP-1 growth as a reaction to increased MMP-9 level. Increased production and release of TIMP-1 can be beneficial to altered MMP-9 activity in asthma and might promote the healing process, but can also contribute to excessive fibrosis and airway remodeling. The results of the cited studies suggest that the inflammatory process induced by allergen exposure can lead to airway remodeling caused by the production of TIMP-1.

Considering a possible correlation between MMPs and airway structural changes, Wenzel et al. [16] designed a study to search for the presence of MMP-9 in the subepithelial basement membrane (SBM) and BALf of subjects with mild, moderate, and severe asthma and compare it with a healthy population. They demonstrated that the expression of MMP-9 in SBM and BALf was characteristic of patients with severe asthma. These data showed that FEV₁ and FVC were significantly lower in the MMP-9-positive group than in the MMP-9-negative subjects. Another study confirmed MMP-9 presence in airway mucus epithelium and ECM of asthmatics, but did not find it in controls [35]. In contrast, Dahlen et al. [36] did not observe a significant difference between MMP-9 in the ECM and MMP-3 in the reticular membrane and airway epithelium of asthmatics and a control group. Hoshino et al. [37] demonstrated correlation between MMP-9 and TIMP-1 in airway epithelium and submucosal membrane and the amount of collagen III and V and tenascin in the reticular basement membrane of asthmatics. Matsumoto et al. [38] demonstrated a positive correlation between TIMP-1 level and airway wall thickness and a negative correlation between the MMP-9/TIMP-1 ratio and irreversible airway obstruction. They also found a positive correlation between TIMP-1 and MMP-9 levels and the number of neutrophils in the sputum.

These data suggest that MMPs play a role in airway remodeling by influencing ECM homeostasis, angiogenesis, and smooth muscle cell hyperplasia. It has been demonstrated in a murine asthma model that MMP-9 contributes to TGF-β1 activation, which leads to subepithelial fibrosis.
sputum [43]. There are some indications which may suggest a beneficial action of MMP inhibitors in asthma; however, a study by Corry et al. is worth mentioning [44]. They demonstrated that in mice exposed to allergen after MMP−2 knock-out, the migration of inflammatory cells towards the airway lumen was inhibited, which led to their accumulation in the parenchyma of the lungs and increased mortality due to asphyxiation. We need to remember that MMPs and their inhibitors play an important role in physiological processes. In one study it was shown that mutation of the human MMP-2 gene, contributing to the lack of an active enzyme form, leads to multi-focal osteolysis [45].

**Conclusions**

Asthma is a chronic disease in which an inflammatory process is accompanied by airway remodeling. Increased MMP expression in severe asthma and asthma exacerbations is followed by elevated TIMP levels, which are responsible for repair mechanisms and play a protective role. The MMP-9/TIMP-1 ratio reflexts this process and the balance between MMPs and their tissue inhibitors. In severe asthma and during asthma exacerbation the MMP-9/TIMP-1 ratio is high, whereas in stable asthma, TIMP-1 elevation is predominant and the MMP-9/TIMP-1 ratio is low. If there is an imbalance between MMPs and TIMPs favoring TIMPs, the protective and beneficial action of TIMPs might provoke the development of fibrosis, and in consequence may lead to airway remodeling. The mechanisms responsible for extracellular matrix homeostasis and the balance between MMPs and TIMPs are complex and need further investigation. Their explanation can contribute to discovering new potential targets for asthma therapy. Corticosteroids exert only a small influence on airway remodeling and homeostasis of the extracellular matrix components.

**References**


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