Elevated Levels of the IGF-Binding Protein Protease MMP-1 in Asthmatic Airway Smooth Muscle

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We have previously demonstrated that the asthma-associated proinflammatory eicosanoid leukotriene D₄ (LTD₄) is co-mitogenic with insulin-like growth factors (IGFs) in airway smooth-muscle (ASM) cells in vitro. This synergistic effect of LTD₄ and IGF on ASM cell growth involves proteolysis of ASM-produced IGF binding proteins (IGFBPs), which are cell growth-inhibitory proteins. We also identified this IGFBP protease to be the matrix metalloproteinase-1 (MMP-1), and showed that this enzyme had a significant role in modulating IGF action in ASM cells. In the present study, we tested the hypothesis that ASM hyperplasia in vivo involves induction of MMP-1 leading to IGFBP proteolysis. We detected the presence of MMP-1 and measured its levels in human airway tissue sections prepared from nonasthmatic and asthmatic subjects. Six nonasthmatic and six asthmatic airway tissue samples were analyzed for immunoreactive MMP-1 through an immunohistochemical detection method. Both the bronchial and tracheal smooth-muscle cells from different regions of the same sample were examined and documented. The immunostaining for MMP-1 was significantly elevated in both the bronchial and tracheal smooth-muscle cells of the airway sections from asthmatic samples relative to that of the nonasthmatic samples. The differences in levels of MMP-1, IGFBP-2, IGFBP-3, and IGFBP proteolytic activity were quantified using densitometric analyses of the ASM tissue extracts that were separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The MMP-1 levels in the asthmatic airway tissue extracts were 12-fold higher than those found in control samples. In addition, IGFBP-2 and IGFBP-3, which we have previously demonstrated to be proteolytic substrates of MMP-1, were found to be cleaved in asthmatic airway tissue extracts. Furthermore, the asthmatic airway extracts contained IGFBP proteolytic activity that was shown by immunodepletion studies to be due to MMP-1. These observations demonstrate that MMP-1 may play a significant role in inducing ASM hyperplasia and airway obstruction in asthma by modulating the IGF axis.


The insulin-like growth factor (IGF) axis is a multicomponent network of molecules that is ubiquitously involved in the regulation of growth, proliferation, and differentiation of a variety of cell types (1). This axis includes two major ligands, IGF-I and IGF-II (2), the type 1 and type 2 IGF receptors (3, 4), a family of at least six high-affinity IGF binding proteins (IGFBPs) that determine the bioavailability of IGFs (5–8), and a group of IGFBP proteases that cleave IGFBPs and hence modulate the bioavailability of IGFs (9–17). Several groups of proteases capable of cleaving specific IGFBPs have been identified, including kallikreins (9–12), cathepsins (13–15), and matrix metalloproteinases (MMPs) (16, 17). The MMPs are peptide hydrolases, active at neutral pH, and require a metal ion for their catalytic activity. The MMPs have been recognized as IGFBP proteases in the serum of pregnant rodents (16) and in fibroblast-conditioned medium (17).
The IGFs have been found to participate in the regulation of cell proliferation, migration, and differentiation (18–31). Although several studies demonstrate the importance of IGFs in the regulation of vascular smooth muscle (VSM) growth (24–31), limited information is available on the pathophysiology of IGFs and related molecules in airway tissues (32). An airway smooth muscle (ASM) hyperplasia is an important histopathologic feature of chronic asthma (33) that contributes to airway obstruction and exaggerated bronchoconstriction. Asthma is also associated with increased levels of locally expressed inflammatory agents such as eicosanoids and cytokines (34–38). In this regard, we have previously demonstrated that the IGFs and IGFBPs are important regulators of ASM cell growth, and that the IGF axis exerts a potent regulatory action on the proliferation of ASM cells (39–41). Accordingly, we have shown that ASM cells express IGF-II, IGF receptors, IGFBP-3, and IGFBP-2 (39). We have further demonstrated that the proinflammatory eicosanoid leukotriene D\(_4\) (LTD\(_4\)) is co-mitogenic with IGFs in ASM cells in vitro, and that this synergistic effect of LTD\(_4\) and IGF on ASM cell growth involves proteolysis of ASM-produced inhibitory IGFBPs such as IGFBP-2 and IGFBP-3. Furthermore, we have demonstrated that the IGFBP-2 that is secreted into the conditioned medium by ASM cells blocks the IGF-induced ASM proliferation (40). In light of these observations, we hypothesized that key regulators of the asthmatic reaction in the airway act by modulating the IGF axis in ASM. Recently, we identified the IGFBP protease secreted by LTD\(_4\)-treated human ASM cells as MMP-1 and determined the role of MMP-1 as a co-mitogenic agent linking the synergism between IGF-I and LTD\(_4\) on the growth of these cells (41). In this study, we further demonstrate the pathophysiologic significance of MMP-1 in asthmatic airways in situ, implicating its role as a proliferation-associated molecule that proteolyzes IGFBPs and hence modulates the IGF axis in the asthmatic airway.

**Materials and Methods**

**Materials**

Recombinant IGFBP-2 was a generous gift from Sandoz (Geneva, Switzerland), and recombinant IGFBP-3 was a gift from Celtrix Pharmaceuticals, Inc. (Santa Clara, CA). IGF-I, IGFBP-3 and affinity-purified antibodies to IGFBP-3 were purchased from Diagnostic Systems Laboratories (Webster, TX). Polyclonal antibodies to IGFBP-2 were purchased from Upstate Technology, Inc. (Lake Placid, NY). Both control and monoclonal antibodies to MMP-1, MMP-2, MMP-3, and MMP-9 were purchased from Oncogene Sciences (San Diego, CA). Polyclonal antibodies to collagen type IV were purchased from ICN Immunobiochemicals (Costa Mesa, CA). Monoclonal anti-pan cytokeratin and the conjugation system for immunocytochemistry were purchased from Vector Laboratories (Burlingame, CA). Diaminobenzidine used in the detection system, phenylmethylsulfonyl fluoride (PM SF), aprotinin, sodium orthovanadate, and lupeptin were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) reagents were purchased from Bio-Rad (Richmond, CA).

**Airway Tissue Samples**

Formaldehyde-fixed, paraffin-embedded lung tissue sections were obtained through the Pathology Department at Children’s Hospital of Philadelphia (CHOP) from nonasthmatic control subjects (n = 6) and from patients who died from asthma (n = 6). Sections were selected to examine the trachea and large bronchi. Two asthmatic and two control samples were obtained twice at the time of organ harvest and organ donation. These four control samples and four asthmatic samples were used for preparing ASM tissue extracts. Studies were approved by the CHOP Institutional Review Boards.

**Immunohistochemistry**

Staining was carried out by the indirect immunoperoxidase method (42). The tissue sections were deparaffinized, hydrated, and buffered in phosphate-buffered saline (PBS), then placed in absolute methanol with 1% H\(_2\)O\(_2\) to ablate endogenous peroxidase activity. Following this, the tissue sections were hydrated, treated with protease K, and permeabilized with 0.1% Tween-20 in PBS, then placed in PBS. To block nonspecific binding and facilitate specific binding of the antibody, cells were sequentially incubated with the following reaction mixtures: (1) normal horse serum at room temperature and 1% bovine serum albumin, (2) primary monoclonal antibodies for MMP-1 (1:250) or pre-immune control serum and 10% normal serum (donkey) in PBS for 18 to 20 h at 4°C, and (3) secondary antibody (1:500) dilution of biotinylated antimouse immunoglobulin G (made in horse) in PBS for 45 min at room temperature. After each incubation, cells were washed three times with PBS. Immunodetection was performed using the avidin–biotin complex (Vector stain A BC kit; Vector Laboratories) for 60 min at room temperature. Finally, the labeling was detected by incubating with 3,3’-diaminobenzidine (0.01% in 0.05 M Tris buffer, pH 7.6, containing 0.03% H\(_2\)O\(_2\)) reaction solution (Sigma) for 5 min. The slides were then counterstained with methyl green (pH 4.2) at 37°C for 15 min, dehydrated, cleared in xylene, mounted permanently in Permaslip mounting medium (Alban Scientific, St. Louis, MO), and covered with a glass coverslip. Slides were photographed, scanned into A dobe Photoshop, and printed using a Tektronix Phaser 440-color printer. The difference in the MMP-1 staining between the asthmatic and control samples was visually estimated in a semiquantitative manner, rating the samples from insignificant staining (+), to light staining (++), to darkest staining (++++)

**ASM Tissue Extracts**

A airway tissue samples were cleared of cartilage, epithelial lining, loose connective tissue, and alveoli under the dissecting microscope. The tissues (~50 mg wet weight) were then minced in 0.5 ml ice-cold Tris buffer (50 mM Tris, 150 mM sodium chloride, pH 7.4) containing protease inhibitors (aprotinin, 30 mg/ml; PM SF, 10 mg/ml; sodium orthovanadate, 10 mg/ml; lupeptin, 5 μg/ml). Subsequently, the tissue samples were homogenized in a 3-ml volume of...
buffer containing protease inhibitors. The homogenates were centrifuged at 1,000 rpm for 5 min and the supernatant was used as tissue extract. Samples were frozen in aliquots at −70°C until further use.

Western Immunoblots
The Western immunoblot analysis was performed as previously described (4). Airway tissue samples from control subjects and asthmatics were used. Samples of 50 μl (50 μg) were subjected to electrophoresis through nonreducing SDS-PAGE (10%) overnight at constant voltage, electroblotted onto nitrocellulose, blocked with 5% nonfat dry milk in Tris-buffered saline, and probed with specific antibodies for MMP-1, IGFBP-2, or IGFBP-3. The proteins were detected using a peroxidase-linked enhanced chemiluminescence (ECL) detection system (Pierce, Rockford, IL).

Identification of IGFBP Proteolytic Activity
The assays for IGFBP-2 and IGFBP-3 proteolysis in the control and asthmatic tissue extracts were carried out as detailed previously (43), with slight modifications. Digestion reactions for IGFBP-3 were carried out in a total volume of 50 μl with 25 μl of tissue extract and 30,000 cpm 125I-recombinant human (rh)-IGFBP-3 in Dulbecco’s PBS, and incubated for 3 h at 37°C. Digestion reactions for IGFBP-2 were carried out in a total volume of 50 μl with 25 μl of tissue extract and 50 ng of unlabeled rh-IGFBP-2 in Dulbecco’s PBS, and incubated for 3 h at 37°C. At the completion of the experiment, proteolysis was terminated by adding a stop solution that contained protease inhibitors including pepstatin, aprotinin, ethylene diaminetetraacetic acid, and sodium orthovanadate, along with SDS, which will arrest most proteases, including MMPs and PMSF (1 mM), at 4°C. Samples were then subjected to 10% SDS-PAGE under nonreducing conditions at 60 V overnight. After electrophoresis, the gels were subjected to electrophoresis through nonreducing SDS–PAGE (10%) overnight at constant voltage, electroblotted onto nitrocellulose membrane and probed with antihuman IGFBP-2 antibodies. The levels of intact and fragmented IGFBPs were analyzed densitometrically.

Densitometric and Statistical Analysis
Densitometric measurement of protease assay autoradiographs and Western immunoblots was performed using a Bio-Rad GS-670 imaging densitometer (Bio-Rad, Melville, NY). Protease activity was expressed as the change in band intensity and calculated as percent of optical density of proteolytic cleavage bands relative to total optical density of the lane. All experiments were repeated at least three times. When applicable, means ± SEM are shown. Student’s t tests were used for statistical analysis.

Results
Patient History Relevant to the Samples
Lungs of asthmatic patients showed the bronchial changes characteristic of chronic asthma, including smooth-muscle cell hyperplasia, eosinophilic infiltration, thickened epithelial basement membrane (basal lamina), and epithelial goblet cell hyperplasia. These changes were not seen in the airways of nonasthmatic patients. Relevant clinical data are presented in Table 1. All asthmatic patients had clinically apparent asthma for several years before death, and most had been hospitalized for treatment of asthma before their final admission or demise.

Histologic Differences between the Control and Asthmatic Airway Tissues
The histology of all of the sections collected (six control and six asthmatic subjects) were characterized by using the basic stain hematoxylin and eosin to determine the cellular features of these samples. To differentiate the stromal compartments containing the smooth-muscle cells from the epithelial compartments, the sections were stained with antibodies to cytokeratin (Figure 1, C1 and A1) and collagen type IV (Figure 1, Cii and Aii). Figure 1 shows the immunolocalization of epithelial cell marker cytokeratin (C1 and A1) and the basal lamina marker collagen type IV (Cii and Aii) that delineate the epithelial and stromal compartments in the airway tissue sections. Of note is that the cytokeratin-stained epithelium (E) was denuded in the majority of tissue samples derived from asthmatic patients (A1 and Aii). Moreover, relative to the controls (C1ii), the distribution of immunoreactive collagen type IV demonstrated significant extension of the stromal compartment containing the hyperplastic smooth-muscle (SM) cells in asthmatic airway sections (A1ii)

MMP-1 in Airway Tissue Sections
To detect the in situ presence and expression of MMP-1 in the airway tissue sections, we examined the immunoreactive MMP-1 levels by immunohistochemistry (Figure 2). The MMP-1 antibodies have been previously characterized (44), and are commercially available. MMP-1 stain was observed primarily in the stromal compartment. Most of the stained cells were identified as smooth-muscle cells by morphologic criteria. MMP-1 staining of epithelial cells and macrophages was observed in tissue sections from both nonasthmatics and asthmatics. Although light staining for MMP-1 was observed in the A SM cells of nonasth-

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<td><strong>Nonasthmatic airway tissue samples</strong></td>
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Definition of abbreviation: MVA = motor vehicle accident.
matic tissues, the staining for MMP-1 in the ASM cells of the asthmatic subjects was strikingly higher than in control subjects (Table 2).

Detection and Quantitative Analysis of MMP-1 in Airway Tissue Extracts

Western immunoblots of cell extracts from control samples demonstrated faint staining for MMP-1, whereas the MMP-1 level was dramatically increased in the airway tissue extracts from the asthmatic subjects. Figure 3 shows MMP-1 staining in tissue samples (extracts from freshly frozen tissues) prepared from two nonasthmatic and two asthmatic subjects following SDS-PAGE separation and protein transfer onto a nitrocellulose membrane. The immunoblotting revealed the differences in the levels of MMP-1 under these two conditions, and densitometric analyses showed a 12-fold increase in MMP-1 levels in the asthmatic samples relative to that of the nonasthmatic samples.

Demonstration of IGFBP-2 and IGFBP-3 in Airway Tissue Extracts

Western immunoblots of cell extracts from control samples demonstrated staining for both IGFBP-2 and IGFBP-3. Figure 4 shows staining for IGFBP-3 (Figure 4A) and IGFBP-2 (Figure 4B) in the tissue extract samples of two nonasthmatic and two asthmatic subjects following SDS-PAGE separation and gel transfer on to a nitrocellulose membrane. The immunoblot revealed the differences in the levels of intact IGFBP-3 and IGFBP-2 under these two conditions. Densitometric analyses showed a 12-fold increase in MMP-1 levels in the asthmatic samples relative to that of the nonasthmatic samples.

Characterization of MMP-1 as IGFBP-2 and IGFBP-3 Protease in Airway Tissue Extracts

Performing the protease assay with MMP-1–immunodepleted tissue extracts demonstrated that the proteolytic effect of MMP-1 is present in ASM tissue extracts (Figures 6 and 7). Tissue extracts from asthmatic samples demonstrated significant proteolytic effect on IGFBP-3 and -2 (Figures 6A and 7A, lanes 4 and 5) relative to tissue extracts from nonasthmatic samples (Figures 6A and 7A, lanes 2 and 3). Tissue extracts immunodepleted with other MMP antibodies (MMP-2, MMP-3, and MMP-9) were

Figure 1. Demonstration of smooth-muscle hyperplasia in asthmatic airways. Large airway sections were prepared from fixed, embedded specimens from a normal 18-yr-old male trauma victim (Control) and a 12-yr-old male who died from asthma (Asthmatic). Tracheal epithelium (E) labeled with cytokeratin in control sections (Ci) is denuded in the asthmatic sections (Ai). An increase in smooth-muscle (SM) content is distinct in the asthmatic preparations (Aii). Immunohistochemical staining with collagen type IV antibody was performed in nonasthmatic (Cii) and asthmatic (Aii) airway sections. Staining for immunoreactive collagen type IV is visualized in the smooth-muscle cells of the asthmatic airway section (Aii), revealing its hyperplastic condition.
also examined for their proteolytic effect on IGFBP-3 and -4. The protease activity of ASM tissue extracts against IGFBP-3 (Figure 6A, lanes 6 and 7) and IGFBP-2 (Figure 7A, lanes 8 and 9) was effectively inhibited by MMP-1 immunodepletion, confirming this protease to be MMP-1. In addition, immunodepletion of the tissue extracts with antibodies against MMP-2, -3 and -9 did not show a significant inhibitory effect on IGFBP-3 (Figure 6A, lanes 8 and 9) or IGFBP-2 (Figure 7A, lanes 8 and 9) proteolysis. As an example for MMPs other than MMP-1, results from MMP-2-depleted samples are shown. These observations confirm that the IGFBP protease found in the ASM tissue extracts is indeed MMP-1.

**Discussion**

Growth induction in various cell types has been demonstrated to be tightly regulated by the maintenance of a homeostatic IGF axis. Cell growth and proliferation are affected by alterations in the levels of IGFs (2), IGFBPs (5–7), IGF receptors (3), or IGFBP proteases (9–17). The ontogeny and tissue-specific expression of these components of the IGF axis are further regulated by hormonal, metabolic, and genetic factors (45–51). An example of the

![Figure 2. In situ immunolocalization of MMP-1 in nonasthmatic and asthmatic airway tissue samples. Tissues were obtained from autopsies of asthmatic or control subjects as described in MATERIALS AND METHODS. The staining for MMP-1 was mainly seen in the smooth-muscle (SM) compartment. Darker staining for MMP-1 protein in the smooth muscle (SM) of the six asthmatic airway tissue sections (A1–A6, right panel) was invariably observed compared with control samples (C1–C6, left panel). See also Table 2.](image)

![Figure 3. Demonstration of elevated levels of MMP-1 in asthmatic airway tissue extracts. A total of 50 μl airway tissue extracts (50 μg total protein) from nonasthmatic (Control) and asthmatic subjects were electrophoresed on 10% SDS–PAGE and blotted on nitrocellulose membrane. MMP-1 was visualized using a monoclonal anti–MMP-1 antibody and the ECL detection system. Lanes 1 and 2 show the MMP-1 levels in the airway tissues of nonasthmatic subjects, and lanes 3 and 4 show the MMP-1 levels in the airway tissues of asthmatic subjects. Densitometric analyses of MMP-1 protein levels from asthmatic (n = 2) and nonasthmatic subjects (n = 2) demonstrated a 12-fold increase over MMP-1 levels in the asthmatic samples.](image)

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+ and ++: insignificant to light staining.
+++ to ++++: significant to strong staining.

*TABLE 2* Semiquantitative analyses of MMP-1 staining in nonasthmatic airway tissue samples

![Table 2](image)
indirect mechanism of cellular growth induction by IGFBP proteases that modulate the IGF axis status is the stimulatory action of follicle-stimulating hormone on granulosa cells, an event associated with induction of a protease for IGFBP-5 (51). Proteolysis of IGFBPs appears to be an important and widespread mechanism for the regulation of IGF bioavailability in tissues, on the basis of the fact that proteases cleave IGFBPs to form low-affinity fragments. This process allows for increased association of the IGFs with their receptors on effector cells (11, 18, 50).

A role for IGFs and IGFBPs in regulating the proliferation and migration of VSM cells has been demonstrated (18–31). In this regard, the growth response of VSM cells following arterial injury has been well characterized (18–25). An IGFBP-2 protease has been detected in VSM cell-conditioned media, but this appears to be a serine protease, different from MMP-1 (20). Other proteases, including IGFBP-4 protease, may also be functional in VSM cells (21). A role for IGFs and IGFBPs in the regulation of VSM cell growth associated with the pathogenesis of asthmatic samples.

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Erosclerosis has been implicated (29–31). It is interesting to note that many of the chemical mediators responsible for the hyperplasia associated with atherosclerosis are also shown to be elaborated in asthmatic airways (34–38). These studies led us to investigate the consideration that the ASM cell hyperplasia associated with asthma may be a consequence of modulation of the IGF axis in ASM cells by cytokines and other chemical mediators that are elaborated in asthmatic airways. Accordingly, using rabbit tracheal smooth-muscle cells, we have demonstrated a mitogenic synergism between IGF-I and the inflammation-associated eicosanoid LTD4 in inducing cell growth in vitro (39, 40). These effects include primarily the reduction in intact IGFBP-2 in the extracellular environment, leading to enhanced IGF action secondary to attenuation of the inhibitory actions of IGFBP-2. The reduction in intact IGFBP-2 level is achieved by the induction of a protease that potently cleaves IGFBP-2 to lower molecular-weight, low-affinity fragments. This decrease in IGFBP-2 levels in the ASM conditioned media presumably leads to an increase in free IGF levels and thereby provides a mechanism for the mitogenic synergism between IGF-I and LTD4. Thus these findings implicate a role for LTD4 in regulating ASM growth, wherein the eicosanoid acts as a co-mitogenic agent by inducing an IGF protease that modulates the IGF axis by proteolyzing IGFBPs. Furthermore, we have clearly identified this IGF protease to be a matrix metalloproteinase, specifically MMP-1 (40).

In the present study, we systematically examined whether the ASM hyperplasia observed in asthmatic airways in vivo is associated with the changes in expression of the IGF protease MMP-1, and consequently with the changes in the levels of IGFBPs. The airway tissue sections from the lungs of deceased nonasthmatic individuals and patients who died from asthma were isolated from different regions in the airways, including the tracheal, bronchial, and alveolar regions. Our observations confirm that the expression of immunoreactive MMP-1 is dramatically increased in smooth-muscle cells from asthmatics, as quantified using both visual and densitometric analyses. Furthermore, our observation in this study that IGFBP-3 and -2 fail to undergo proteolysis in the absence of MMP-1 in situ supports our previously demonstrated in vitro study that MMP-1 in airway tissue extracts has a co-mitogenic effect with IGF secondary to the proteolysis of IGFBP-2 and IGFBP-3. Thus, our findings suggest that MMP-1 plays a significant role in mediating the increase in ASM cell mass that characterizes the airways of patients with asthma and other types of chronic airway inflammatory diseases. Accordingly, together with our earlier observations that LTD4 and/or other proinflammatory cytokines may be important mediators of ASM cell proliferation in vitro (40, 41), our findings suggest that these mediators act as modulators of the IGF axis by inducing MMP-1 to stimulate smooth-muscle cell proliferation in vivo. In this context, synthetic inhibitors of MMP-1 may...
emerge as potential therapeutic agents to attenuate the progression of chronic asthma associated with airway remodeling and obstruction.

We have shown here that asthmatic airways harbor not only increased levels of MMP-1 but also increased levels of MMP-1 activity. We have also demonstrated that both IGFBP-2 and IGFBP-3 (the main targets of MMP-1) are secreted by ASM cells in vivo. Using both IGFBP-2 and IGFBP-3 immunoblots and protease assays, we have shown that asthmatic airway tissue extracts have a potent effect on cleaving these IGF-binding proteins. We also have shown that IGFBP-2 and IGFBP-3, which are normally secreted by ASM cells, are cleaved in asthmatic airway tissues, and that no intact IGFBPs are found in the airway tissues of asthmatics (Figure 4).

This is the first in vivo demonstration of an IGFBP protease in human airways showing a link between the elevated levels of this IGFBP protease, the presence of cleaved IGF-binding proteins, and ASM cell hyperplasia in asthma. In this context, it is relevant to note that many factors (cytokines, chemicals, growth factors, and cell aging) modulate MMP expression at the transcriptional level (44, 52, 53). Indeed, it is postulated that MMPs play a critical role in tissue remodeling, such as during wound healing and aging-associated changes in tissue architecture. Abnormal expressions of MMPs have also been associated

Figure 7. Inhibition of the IGFBP-2 protease activity in airway tissue extracts. Mean values of IGFBP-2 protease activity were derived using densitometric analysis of bands representing intact rh-IGFBP-2 on immunoblots. Values are expressed as percentage of control. Samples of tissue extracts from nonasthmatics (NA) and asthmatics (AS) were used. Both intact and immunodepleted samples for MMP-1, -2, -3, and -9 (only MMP-2 shown in the top panel) were examined for IGFBP-2 protease activity. Samples were incubated with rh-IGFBP-2 for 3 h at 37°C. (Top panel) Western immunoblot showing the intact rh-IGFBP-2 in control with buffer alone (lane 1), in nonasthmatic samples (lanes 2 and 3), in asthmatic samples (lanes 4 and 5), in MMP-1-immunodepleted asthmatic samples (lanes 6 and 7), and in MMP-2-immunodepleted asthmatic samples (lanes 8 and 9). (Bottom panel) The effect on IGFBP-3 proteolysis, when calculated as percentage of intact IGFBP-3, demonstrated significant (**P < 0.001) inhibitory effect on IGFBP-3 in proteolysis in MMP-1-depleted tissue extracts. An anti-MMP-1 antibody eliminated almost 90% of the protease activity in tissue extract from asthmatic subjects. Immunodepletion of the tissue extracts from asthmatics with anti-MMP-2, -3, and -9 antibodies did not have any effect on the proteolysis of IGFBP-2.
with neoplastic traits such as loss of negative growth regulation and high invasive potential (53). However, the growth-regulatory role of MMPs, based on their proteolytic effect on IGF binding proteins (IGFBPs), has been postulated only recently, wherein MMPs have been shown to act as IGFBP-3 proteases in fibroblast-conditioned media and in the serum of late-gestation pregnant rats (16). Our recent demonstration confirms that MMP-1 is an LTIA-induced IGFBP protease in human ASM cells (19). Finally, our present study once again provides evidence of a growth-regulatory role for MMP-1 by demonstrating its potent IGFBP protease activity, in the synergistic growth effect of LTDA and IGF-I in ASM cells in situ. All these observations provide strong evidence for the implication of IGFBP proteolysis by MMP-1 in the pathophysiology of altered tissue histioarchitecture, such as in asthma.

In light of our present observations, and given that chronic asthma may involve the progression from an initial reversible airway obstruction to a subsequent irreversible airway obstruction secondary to airway remodeling, therapeutic intervention involving modulation of the IGF axis may be of potential clinical importance. A cohering, specific inhibition of the IGFBP proteolysis by MMP-1 in the ASM may serve to arrest or attenuate ASM hyperplasia, as MMP-1 has been shown to assist in cell invasion or movement by facilitating degradation of extracellular matrix components. In this respect, it appears that inhibition of MMP-1 action may serve a dual purpose of both slowing down or arresting cell growth and proliferation as well as limiting cell invasion and movement that is induced by MMP-1. Thus, this enzyme could constitute a target for future drug development.

References


