Lack of matrix metalloproteinase-9 worsens ventilator-induced lung injury

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Albaiceta GM, Gutiérrez-Fernández A, Parra D, Astudillo A, García-Prieto E, Taboada F, Fuego A. Lack of matrix metalloproteinase-9 worsens ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol 294: L535–L543, 2008. First published January 25, 2008; doi:10.1152/ajplung.00334.2007.—Matrix metalloproteinase-9 (MMP-9) is released by neutrophils at the sites of acute inflammation. This enzyme modulates matrix turnover and inflammatory response, and its activity has been found to be increased after ventilator-induced lung injury. To clarify the role of MMP-9, mice lacking this enzyme and their wild-type counterparts were ventilated for 2 h with high- or low-peak inspiratory pressures (25 and 15 cmH2O, respectively). Lung injury was evaluated by gas exchange, respiratory mechanics, wet-to-dry weight ratio, and histological analysis. The activity of MMP-9 and levels of IL-1β, IL-4, and macrophage inflammatory protein (MIP-2) were measured in lung tissue and bronchoalveolar lavage fluid (BALF). Cell count and myeloperoxidase activity were measured in BALF. There were no differences between wild-type and Mmp9−/− animals after low-pressure ventilation. After high-pressure ventilation, wild-type mice exhibited an increase in MMP-9 in tissue and BALF. Mice lacking MMP-9 developed more severe lung injury than wild-type mice, in terms of impaired oxygenation and lung mechanics, and higher damage in the histological study. These effects correlated with an increase in both cell count and myeloperoxidase activity in the BALF, suggesting an increased neutrophilic influx in response to ventilation. An increase in IL-1β and IL-4 in the BALF only in knockout mice could be responsible for the differences. There were no differences between genotypes in MMP-2, MMP-8, or tissue inhibitors of metalloproteinases. These results show that MMP-9 protects against ventilator-induced lung injury by decreasing alveolar neutrophilic infiltration, probably by modulation of the cytokine response in the air spaces.

MECHANICAL VENTILATION is a supportive therapy that can be lifesaving. However, it is known that ventilation using high pressures or volumes can damage the lungs or worsen a previous lung injury, causing the so-called ventilator-induced lung injury (VILI) (11). The knowledge of this pathogenic mechanism led to the application of protective ventilatory strategies aimed at minimizing VILI (7), and ultimately, to a reduction in the mortality of the acute respiratory distress syndrome (1). However, there are patients at risk of VILI in spite of adequate ventilatory settings (44). This injury is initiated by a mechanical stimulus to the alveolar structures, including pneumocytes, capillaries, and the interstitium (15). This physical insult induces a cellular response by activation of different biochemical pathways and finally an inflammatory response. Released inflammatory mediators lead to chemotaxis of inflammatory cells, mainly neutrophils (4), from the circulation. The net result is an acute inflammation within the lungs that can also spread to distal organs (22).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that can cleave a variety of substrates, ranging from virtually all components of the extracellular matrix to a number of cytokines and chemokines (38). Currently, MMPs are viewed as modulators of cell-cell and cell-matrix interactions (37). Among them, MMP-9 (gelatinase B) has gained some attention in different models of lung injury (39, 42) including VILI (14, 25). The levels of MMP-9 have also been found to be raised in patients with acute lung injury (13, 40, 50). MMP-9 is stored in neutrophil granules, being rapidly released after cellular activation (36). This enzyme can cleave gelatin (denatured collagen), but also a variety of cytokines and chemokines (45). Because of its ability to target these substrates, MMP-9 has been implicated in extracellular matrix remodeling and cell migration during acute inflammation (30, 48).

On the basis of these findings, it has been suggested that MMP-9 inhibition could be useful in models of sepsis (42), acute lung injury (8), and VILI (14, 25) by decreasing the inflammatory response and tissue damage. However, nonselective inhibitors can modify the activity of other enzymes, precluding any firm conclusion on the specific role of MMP-9. Moreover, studies with mice lacking MMP-9 have shown that this enzyme is essential for an adequate inflammatory response and tissue repair, as these animals have an increased lung damage after the induction of peritonitis (39) and a poor outcome after an experimental brain hemorrhage (43).

So, MMP-9 could be involved in both inflammation and repair within the lungs. To clarify the specific role of MMP-9 in VILI, mice lacking MMP-9 (Mmp9−/−) were subjected to injurious ventilation, and the occurrence of putative differences in lung injury with their wild-type counterparts was evaluated.

METHODS

Animals. The generation of Mmp9−/− mice in a C57BL/6J background has been previously described (47). All experiments were performed with 8- to 12-wk-old male mice, and wild-type littermates were used as controls. Mean weight was 26.0 ± 2.8 g. All mice were kept under specific pathogen-free conditions, with free access to food and water. The experiments were approved by and conducted in accordance with the guidelines of the Committee on Animal Experimentation of the Universidad de Oviedo, Oviedo, Spain.

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Experimental protocol. Mice were anesthetized with ketamine/xylazine that was administered intraperitoneally. A tracheotomy was performed, and the airway was cannulated with a 20G angiocatheter, tightly tied to avoid leaks. Then, the animals were connected to a mechanical ventilator (Evita 2 Dura with NeoFlow, Drager, Germany) in pressure-controlled mode. They were assigned to one out of two ventilatory settings: low pressure (peak inspiratory pressure 15 cmH2O, PEEP 2 cmH2O, rate 100 breaths/min) or high pressure (peak inspiratory pressure 25, PEEP 0, rate 50 breaths/min). FiO2 was 0.5 and inspiratory to expiratory ratio was 1. Respiratory rates were selected to achieve normocapnia according to preliminary experiments. Absence of leaks was assessed during a prolonged inspiratory pause, and air trapping was discarded by absence of intrinsic PEEPi during a prolonged expiratory pause.

Mice were ventilated for 2 h. Then, a laparotomy was performed, and the aorta was dissected and punctured to obtain an arterial blood sample for gas analysis (NPT 7; Radiometer, Denmark). The animal was killed by exsanguination, disconnected from the ventilator, and a thoracotomy was performed. With the thorax opened, a pressure-volume curve was traced (see below). The heart-lung bloc was removed, and the right bronchus was ligated. The upper lobe of the right lung was weighed before and after drying in an oven (50°C, 72 h) to calculate the wet-to-dry weight ratio. The remaining right lung was frozen immediately at −80°C. The left lung was fixed with intratracheal formaldehyde at a pressure of 20 cmH2O and immersed in the same fixative for 24 h. After fixation, it was included in paraffin and processed for standard hematoxylin-eosin staining.

Pressure-volume curve. Using a three-way stopcock, an air-filled syringe and a differential pressure transducer (Honeywell) were connected to the tracheal catheter. Volume history was standardized by inflating the lungs with 0.7 ml of air and allowing them to deflate before measurement. Lungs were then inflated with 0.1 ml aliquots of air until reaching 0.7 ml and deflated in the same steps. The airway pressure in static conditions (with an equilibrium time of 4−5 s after each volume step) was recorded in a computer using an analog-to-digital converter (National Instruments). In four animals (2 Mmp9−/− after high-pressure ventilation, 1 Mmp9+/− after low-pressure ventilation, and 1 Mmp9−/− after high-pressure ventilation), the curves were not recorded due to air leaks.

Bronchoalveolar lavage. In additional animals of both genotypes, subjected to the same ventilatory strategies, a bronchoalveolar lavage (BAL) was performed after 2 h of ventilation. Four aliquots (1 ml) of saline were injected through the tracheotomy and recovered. Cell count in BAL fluid was measured using a Neubauer chamber. The remnant BAL fluid was immediately frozen at −80°C. No other samples were collected from these animals.

Histological analysis. One pathologist, blinded to the genotype and experimental conditions, evaluated three slices of the left lung. A quantitative scale scoring congestion and edema, hemorrhage, inflammatory cells, and septal thickening (scored from 0 to 4 each) was used (4).

Gelatin zymography. Standard gelatin zymography was done to evaluate the activity of MMP-2 and -9 in lung tissue and BAL fluid. Lung tissue was homogenized in a lysis buffer containing 20 mM Tris, 300 mM sucrose, 1% Triton X-100, and complete protease inhibitor cocktail without EDTA (Roche). The protein content of the lysate was quantified (in arbitrary density units) using ImageJ software (National Institutes of Health). The protein content of the lysate was quantified (in arbitrary density units) using ImageJ software (National Institutes of Health). The protein of lung homogenate corresponding to 95 µg of protein was loaded in an 8% SDS-polyacrylamide gel containing 0.2% gelatin. An electrophoresis was performed, and the gel was washed three times in 2.5% Triton X-100 and incubated overnight at 37°C in a buffer containing 20 mM Tris-HCl, 5 mM CaCl2, pH 7.4. Afterwards, it was stained using Coomassie blue and destained with a mixture of acetic acid and methanol. Gels were scanned, and the intensity of the bands was quantified (in arbitrary density units) using ImageJ software (National Institutes of Health). To overcome differences between gels, two samples of all experimental groups were loaded in each gel. The same procedure was done in BAL fluid samples, with a fixed volume of 12 µl.

Reverse zymography. Reverse zymographies were done to study the activity of tissue inhibitors of metalloproteinases (TIMPs). Homogenized lung tissue (25 µg of protein) was loaded in a 15% SDS-polyacrylamide gel containing 0.2% gelatin and 50 ng/ml purified murine MMP-9. A standard of TIMP-1 was loaded on each gel as positive control. Standard 15% SDS-polyacrylamide gels without gelatin or MMP-9 were run in parallel to verify that bands were due to TIMP activity and not to other proteins. Gels were run, processed, and stained as in gelatin zymographies.

Western blot. Quantification of MMP-8 in lung tissue and BAL fluid was done by Western blotting. Samples containing 50 µg of protein or 12 µl of BAL fluid were loaded in an 8% SDS-polyacrylamide gel. Gels were run and proteins transferred to a nitrocellulose membrane. These membranes were blocked using 3% milk and incubated with a polyclonal antibody against murine MMP-8 raised in rabbits (3). A secondary peroxidase-linked antibody (Anti-Rabbit IgG; Cell Signaling) was added, and bands of MMP-8 were detected by chemiluminescence (Immobilon, Millipore). Membranes were scanned in a LAS-3000 mini camera (Fujifilm), and intensity of bands was measured using ImageJ software (National Institutes of Health).

Myeloperoxidase assay. Myeloperoxidase was quantified as a marker of neutrophil infiltration. Samples of BAL fluid (a fixed volume of 50 µl) were incubated with O-dianisidine and H2O2 in phosphate buffer as previously described (6). Light absorbance at 460 nm was measured.

Enzyme-linked immunoassays. Levels of IL-1β, IL-4, and macrophage inflammatory protein-2 (MIP-2) were measured in lung homogenates and BAL fluid using standard ELISA kits purchased from R&D and BD Biosciences. These mediators were chosen as representative Th1 and Th2 cytokines and a chemokine that have shown differences in other studies dealing with inflammation and MMP-9 knockout mice (32, 33, 28). Limits of detection were 15 pg/ml (IL-1β and MIP-2) and 31 pg/ml (IL-4).

Statistical analysis. All data are expressed as means ± SE. Variables were compared using a two-way ANOVA, with genotype and ventilatory strategy as factors. Post hoc tests were done when appropriate using Bonferroni’s correction. Pressure-volume curves were compared using a repeated measurement ANOVA, adding lung volume as a within-group factor. A P value lower than 0.05 is considered significant.

RESULTS

Thirty-two animals were included in the main experiment: eight mice lacking MMP-9 and eight wild-type counterparts were assigned to each ventilatory strategy. BALs were done in 14 additional mice (see below). All animals survived the experiment.

Gas exchange. Lung function was studied by analysis of arterial blood gases at the end of the experiment (Table 1). Oxygenation significantly worsened after 2 h of injurious

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<td>433±46</td>
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*a* P < 0.05 vs. the same genotype after low-pressure ventilation. #P < 0.05 vs. Mmp9+/+ with the same ventilatory strategy. PIP, peak inspiratory pressure.
ventilation compared with low-pressure ventilation. This change was more pronounced in Mmp9−/− animals, which were more hypoxic than their wild-type counterparts subjected to the same ventilatory strategy. Reflecting the more severe lung injury, Mmp9−/− mice showed a raise in PaCO2 levels after injurious ventilation, although it did not reach statistical significance (P = 0.18). This increase in PaCO2 was also associated with the development of respiratory acidosis in this subgroup of animals (P = 0.053). No animal developed metabolic acidosis, thus discarding severe circulatory impairment. There were no differences in arterial blood gases in animals from both genotypes subjected to low-pressure ventilation.

**Lung injury.** The structural lung injury was assessed by comparing the lung mechanical properties, the degree of edema, and the histological alterations in wild-type and Mmp9−/− animals. We first analyzed lung mechanics by using pressure-volume curves. As can be seen in Fig. 1A, the curves in animals of both genotypes ventilated with low pressures were not different. These curves were shifted to the right after injurious ventilation, reflecting a decrease in lung compliance (P = 0.013 for the difference between ventilatory strategies in the ANOVA). Again, there was a difference between genotypes: mice lacking MMP-9 had a greater displacement of these curves than wild-type animals, reaching statistical significance (P = 0.031 for the difference between genotypes after high-pressure ventilation, Fig. 1A).

Lung edema was evaluated using the wet-to-dry weight ratio. There was an increase in lung edema after high-pressure ventilation in both genotypes, with no significant differences between them (Fig. 1B).

The degree of tissue injury was quantified in histological preparations (Fig. 1C). Animals ventilated using low pressures developed a mild lung injury, with no differences between genotypes. However, there was a significant increase in the injury score after high-pressure ventilation, with the development of alveolar wall thickening, pneumocyte hyperplasia, and inflammatory infiltrates, as shown in Fig. 2. These alterations yielded higher histological scores of lung injury (P < 0.01 in the ANOVA). In line with our previous results, there was a significant difference between Mmp9−/− and wild-type mice after injurious ventilation, with higher damage in the former (3.8 ± 1.3 vs. 2.5 ± 0.8, P = 0.02 in the post hoc test).

**Gelatinase activity.** The activity of MMP-2 and MMP-9 (gelatinases A and B, respectively) was assessed by means of gelatin zymography. High-pressure ventilation induced an increase in the gelatinolytic activity of MMP-9 in lungs from wild-type animals compared with those animals ventilated using low pressures (Fig. 3A). There were no significant changes in MMP-2 activity in tissue, regardless of the ventilatory strategy and the genotype (Fig. 3B). As expected, there was no signal of MMP-9 activity in any Mmp9−/− mice. A representative zymography is shown in Fig. 3C.

**BAL.** In additional animals (4 mice from each genotype assigned to high-pressure ventilation and 3 to each low-pressure group), a BAL was performed after 2 h of mechanical ventilation. The protein content of BAL significantly increased in animals assigned to high-pressure ventilation compared with low-pressure ventilation, respectively, (3.8 ± 0.8, 0.16 mg/ml for high- and low-pressure ventilation, respectively; Mmp9−/−: 1.6 ± 0.14 vs. 0.27 ± 0.16 mg/ml for high- and low-pressure ventilation respectively, P < 0.01 in both post hoc tests), reflecting the disruption of the alveolocapillary barrier.

The cellularity of BAL samples was also studied. There were no differences in cell content or myeloperoxidase activity in animals after low-pressure ventilation. In mice ventilated using high pressures, only those lacking MMP-9 showed a trend to an increase in cell count (P = 0.067 for the interaction between “genotype” and “ventilatory strategy” factors, Fig. 4A) and a significant increase in myeloperoxidase activity (P =
MIP-2 increased in both genotypes.  

0.05 for the interaction between genotype and ventilatory strategy factors and \( P = 0.007 \) in the post hoc test against \( Mmp9^{-/-} \) mice, Fig. 4B), suggesting an increase in neutrophils.

Gelatin zymography of BAL samples showed no MMP-9 activity in \( Mmp9^{-/-} \) mice (Fig. 4, C and E). However, ventilation with high pressures induced an increase in the activity of MMP-9 and MMP-2 in the BAL fluid (\( P = 0.01 \) in the ANOVA, Fig. 4, C–E). Moreover, this increase in the activity of MMP-2 was more pronounced in wild-type animals than in \( Mmp9^{-/-} \) mice (\( P = 0.043 \) for the interaction between genotype and ventilatory strategy, \( P = 0.008 \) in the post hoc test). Figure 4E shows a representative gelatin zymography of BAL samples.

Cytokines and chemokines. The levels of inflammatory mediators in lung tissue and BAL fluid were different between genotypes (Fig. 5). After low-pressure ventilation, levels of IL-1\( \beta \) and MIP-2 in tissue (\( P = 0.022 \) and \( P = 0.017 \), respectively) and BAL fluid (\( P = 0.021 \) and \( P = 0.003 \)) were lower in \( Mmp9^{-/-} \) mice, with no differences in IL-4. However, the response to injurious ventilation was more pronounced in knockout mice, which had significant increases of lung IL-4 (\( P = 0.016 \)) and MIP-2 (\( P = 0.017 \)). There was a trend to increase in IL-4 in wild-type mice (\( P = 0.096 \)). IL-1\( \beta \) increased in both genotypes (\( P = 0.043 \) and 0.033 for wild-type and knockout mice, respectively).

The changes in BAL fluid were even more marked. There was a significant increase in IL-1\( \beta \) (\( P = 0.05 \)) and IL-4 (\( P = 0.006 \)) in \( Mmp9^{-/-} \) animals, but not in wild-type mice (\( P = 0.99 \) and \( P = 0.78 \) for IL-1\( \beta \) and IL-4, respectively), whereas MIP-2 increased in both genotypes.

Compensatory mechanisms. To discard other compensatory mechanisms in \( Mmp9^{-/-} \) mice, we measured levels of MMP-8, other metalloproteinase released by neutrophils, and TIMP-1, the main inhibitor of MMP-9, in lung tissue and BAL. Injurious ventilation significantly increased MMP-8 in tissue (wild-type mice: \( 1,655 \pm 504 \) vs. \( 9,376 \pm 2,256 \); knockout mice: \( 925 \pm 172 \) vs. \( 10,189 \pm 3,272 \) density units for low- and high-pressure ventilation, respectively, \( P < 0.01 \) in post hoc tests) and BAL fluid (wild-type mice: \( 2,764 \pm 301 \) vs. \( 12,206 \pm 2,256 \); knockout mice: \( 1,994 \pm 498 \) vs. \( 10,791 \pm 2,680 \) density units for low- and high-pressure ventilation, respectively, \( P < 0.01 \) in post hoc tests), with no differences between genotypes. In the same manner, TIMP-1 increased in lung tissue after injurious ventilation (699 ± 314 vs. 2,268 ± 299 and 648 ± 213 vs. 2,058 ± 386 arbitrary density units in wild-type and knockout mice, respectively, \( P < 0.05 \) in post hoc tests) with no differences between genotypes. No TIMP-1 activity was detected in BAL fluid.

DISCUSSION

Our results show that mice lacking MMP-9 develop more severe lung damage after high-pressure ventilation than their wild-type counterparts. These data suggest that, in this type of insult, MMP-9 activation is a protective rather than a pathogenetic mechanism.

MMP in lung injury. During VILI, high airway pressures and volumes lead to cell stretch, causing epithelial damage and an inflammatory response within the lungs. Increased levels of MMPs have been documented in different models of acute lung injury (8, 13, 27) and in the special case of VILI (14, 25). MMP-9 is one of the most studied MMPs. It has been reported that the activity of this enzyme markedly increases after experimental lung injury induced by endotoxin (41), pancreatitis (24), or mechanical ventilation (14). Likewise, MMP-9 levels have been found to be elevated in patients with acute respiratory distress syndrome (13, 40). Our results show an increase in MMP-9 activity and MMP-8 levels after high-pressure ventilation in lung homogenates and BAL fluid, whereas MMP-2 increases substantially only in the latter. This differential release is in line with the findings by Steinberg and
It should be noted that MMP-2 and MMP-9 were assessed by gelatin zymography. This technique, however, does not identify the specific effects of the enzyme. A similar difference is obtained when comparing the effect of COL-3, an MMP inhibitor, in a model of peritonitis (42) with a similar model using mice lacking MMP-9 (39). The benefit seen in the former article turns into higher injury in the latter.

However, we cannot discard other differences. The experiments cited above were done in rats instead of mice, so differences in the susceptibility to VILI, hemodynamic tolerance, or inflammatory response can occur. Different ventilatory strategies or timing could also be partially responsible.

Mechanisms of injury in MMP-9-deficient mice. Different mechanisms could be responsible for the increased injury observed in animals lacking MMP-9. The increased cell and myeloperoxidase activity in BAL suggests that neutrophils are actively recruited in the airway in this model of injury. In opposite, lack of MMP-2 is related to the sequestration of neutrophils inside the interstitium, without invasion of the air spaces (9). It is known that MMP-9 can cleave different cyto- and chemokines, like IL-1β (23). By these effects, MMPs can modulate cell migration (18). Unexpectedly, wild-type mice did not present an increase in BAL cells or myeloperoxidase activity, as it is known that VILI causes an increased alveolar cell infiltration. We hypothesize that the short course of the experiment (2 h of ventilation) could have dampened this effect, and only in cases of dysregulation of cell migration (like in Mmp9−/− mice) this phenomenon occurs early. In the same sense, there were no differences in lung edema or protein content in the BAL fluid between genotypes, suggesting that MMP-9 modulates cell migration but not lung edema. It has been observed previously that cell infiltration and edema have different regulatory mechanisms during lung injury (31, 51).

Although there are no differences between genotypes in lung tissue cytokines, only Mmp9−/− mice have a significant increase in BAL IL-1β and IL-4. IL-1β can promote pulmonary injury (29), and IL-4 is a pleiotropic cytokine that has been related to cell recruitment in models of airway disease in Mmp9−/− (33) and Mnp8−/− (19) mice. Interestingly, both IL-1β and IL-4 can delay neutrophil apoptosis (10, 17), thus impairing the resolution of inflammation. However, we cannot discard other molecules as responsible for our results.

It is noteworthy that levels of IL-1β and MIP-2 were lower in knockout animals after low-pressure ventilation, suggesting a blunted basal inflammatory response. These differences,
which have been observed in other studies (28), could be responsible for the benefits of lacking MMP-9 in other models of injury (12). Together, these results suggest a translocation of the inflammatory response to the alveolar space in knockout animals ventilated with high pressure, in spite of decreased levels of cytokines in basal conditions (low pressure ventilation).

Other compensatory mechanisms related to differences in MMP-8, which is another MMP released mainly by neutrophils, or TIMP-1, the main tissular inhibitor of MMP-9, were discarded. However, we cannot discard other mechanisms of injury. It has been reported that expression of MMP-9 improves airway epithelial wound repair (5), so a direct effect of this metalloprotease in keeping the epithelial integrity or in the extracellular matrix could be considered.

In a very different experiment, lack of MMP-9 was associated with a worse outcome and more brain injury after collagenase-induced brain hemorrhage (43). Vascular injury during mechanical ventilation is another mechanism of VILI, so the role of MMP-9 in the maintenance of the vascular wall should be taken into account. Moreover, it has been reported that these knockout mice have an increased vascular permeability after inflammation, which is mediated by prostaglandins (26). However, lack of differences in lung edema (wet-to-dry weight, protein concentration in BAL) between genotypes suggest that the protective effects are more related to the modulation of the inflammatory response than to a direct effect on vascular permeability.

Our results suggest that MMP-9 modulates inflammation, neutrophilic influx, and epithelial and endothelial integrity in response to a variety of stimuli in the respiratory system and that isolated MMP-9 inhibition is not responsible for the benefits observed using nonselective drugs against MMPs.

Clinical implications. In a heterogeneously injured lung, it is impossible to gain aeration of injured alveoli without causing some degree of alveolar overdistension (2). Although ventilatory strategies aimed to minimize VILI have decreased mortality in acute lung injury (1), there are patients in which even a low pressure/volume strategy can promote alveolar overstretching (34, 44).
There are no therapies against VILI other than optimization of mechanical ventilation. In this setting, MMP inhibitors are a promising therapeutical option. However, different MMPs may have opposite functions in inflammation and tissue repair. We have shown that MMP-9 is protective against VILI, whereas others have described beneficial effects of inhibition in different models of inflammation (14, 25). To cite another example, lack of MMP-8 is protective in a model of acute liver injury (46) but can delay the resolution of inflammation in skin and lungs (3, 20). These examples illustrate how nonselective inhibitors of MMPs can induce opposite effects, thus decreasing the probability of showing some clinical benefit. A similar explanation has been proposed for the poor clinical results obtained after treating cancer patients with the first generation of wide-spectrum MMP inhibitors (35).

In the case of VILI, our results demonstrate that MMP-9 function should be preserved. We must recognize, however, that ventilatory strategies used in the study were chosen to increase the signal-to-noise ratio of the experiments, and they are not directly comparable to those used in clinical practice.

Therefore, other MMPs might be investigated as therapeutic targets. Lack of MMP-12 has some protective effect against IL-13-induced emphysema (49), and MMP inhibitors decrease MMP-2 activation in a cellular stretch model (21). The functional implication of different enzymes must be clarified to use selective inhibitors that could improve the chance of beneficial results minimizing side effects. Without this knowledge, use of nonselective drugs against MMPs to prevent VILI cannot be recommended.

Our results show that there is a significant increase of MMP activity after VILI. In this setting, absence of MMP-9 leads to a more severe injury with an increase in neutrophil activity in the alveolar spaces. Abnormal gradients of cyto- and chemokines could be responsible for this alteration. Therefore, the use of drugs with activity against MMP-9 to prevent VILI should be viewed with caution.
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