Eur Respir J 2011; 38: 959–970
DOI: 10.1183/09031936.00032111

SERIES "MATRIX METALLOPROTEINASES IN LUNG HEALTH AND DISEASE"
Edited by J. Müller-Quernheim and O. Eickelberg
Number 4 in this Series

Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair

A. Davey*, D.F. McAuley# and C.M. O'Kane#

ABSTRACT: Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) comprise a spectrum of acute inflammatory pulmonary oedema resulting in refractory hypoxaemia in the absence of an underlying cardiogenic cause. There are multiple pulmonary and extrapulmonary causes and ALI/ARDS patients are a clinically heterogeneous group with associated high morbidity and mortality.

Inflammatory injury to the alveolar epithelial and endothelial capillary membrane is a central event in the pathogenesis of ALI/ARDS, and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs) have been implicated in a wide variety of pulmonary pathologies and are capable of degrading all components of the extracellular matrix including the basement membrane and key non-matrix mediators of lung injury such as chemokines and cell surface receptors.

While many studies implicate MMPs in the injurious process, there are significant gaps in our knowledge of the role of specific proteases at different phases of injury and repair. This article examines the role of MMPs in injury and repair of the alveolar epithelial–endothelial capillary barrier and discusses the potential for MMP modulation in the prevention and treatment of ALI. The need for further mechanistic and *in vivo* studies to inform appropriate subsequent clinical trials of MMP modulation will be highlighted.

KEYWORDS: Acute lung injury, acute respiratory distress syndrome, extracellular matrix, inflammation, matrix metalloproteinases, repair

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are part of a disease spectrum associated with high mortality and considerable morbidity. The most recent diagnostic criteria for ALI/ARDS, developed by the 1994 American–European Consensus Conference Committee [1], are based on the gross clinico-radiological findings; acute onset, bilateral infiltrates on chest radiography, absence of left ventricular failure and the ratio of arterial oxygen tension (P_{a,O_2}, expressed in mmHg) to inspired oxygen fraction (F_{I,O_2}) measuring <200 (ARDS) or <300 (ALI) [2–4]. However, ALI/ARDS patients are clinically heterogeneous and this definition does not take into account the underlying aetiology or severity of illness reflected by failure of other organ systems [2]. Irrespective of aetiology, the pathogenesis of ALI/ARDS consists of an excessive and inappropriate inflammatory response to a range of pulmonary or extrapulmonary insults, resulting in damage to the alveolar epithelial–endothelial capillary barrier [3, 4]. Clinically, this manifests as refractory hypoxaemia and decreased pulmonary compliance. Pathologically, three phases are recognised: an initial acute inflammatory or exudative phase characterised by

AFFILIATIONS
*Anaesthetics Dept, Raigmore Hospital, Inverness, and
#Centre for Infection and Immunity, Queen's University Belfast, Belfast, UK.

CORRESPONDENCE
C.M. O'Kane
Centre for Infection and Immunity
Queen's University Belfast
Room 1.14 HSB
97 Lisburn Road
Belfast
BT9 7BL
UK
E-mail: c.okane@qub.ac.uk

Received:
Feb 20 2011
Accepted after revision:
April 30 2011
First published online:
May 12 2011

Previous articles in this Series: No. 1: Löffek S, Schilling O, Franzke C-W. Biological role of matrix metalloproteinases: a critical balance. *Eur Respir J* 2011: 38: 191–208; **No. 2:** Elkington PT, Ugarte-Gil CA, Friedland JS. Matrix metalloproteinases in tuberculosis. *Eur Respir J* 2011; 38: 456–464; **No. 3:** Gaggar A, Hector A, Bratcher PE, *et al.* The role of matrix metalloproteinases in cystic fibrosis lung disease. *Eur Respir J* 2011; 38: 721–727.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

proteinaceous oedema and neutrophil invasion of alveoli; subsequent hyaline membrane formation and interstitial fibroproliferation; followed by a variable degree of resolution [2–5]. These events are co-ordinated by a cascade of inflammatory mediators that ultimately results in the generation of reactive oxygen and nitrogen species along with proteolytic enzyme release, with resultant tissue destruction and respiratory failure. Histopathologically, there is disruption of the alveolar epithelial–endothelial capillary barrier [4, 5]. Also known as the blood–air barrier, this consists of alveolar epithelium, capillary endothelium, extracellular matrix (ECM) and other cells such as alveolar macrophages and fibroblasts. These elements are configured to optimise both surface area and thickness of the blood–air barrier for efficient gas exchange while maintaining an ECM framework with favourable mechanical properties to facilitate ventilation. Structurally, the blood–air barrier has either a thick or a thin configuration in relation to the type I alveolar epithelium, underlying capillary endothelium and ECM [6, 7]. Gas exchange occurs across the thinnest part of the blood–air barrier, where the type I alveolar cell is closely applied to the endothelium by fused basal lamina. For the remaining portion of the blood–air barrier, a layer of ECM is located between the epithelium and endothelium; this thick portion of the barrier is responsible for liquid and solute exchange [7]. ECM is a generic term that encompasses both the basement membrane (BM) and interstitial connective tissue. ECM is an important functional component of all tissues [8]. In the lung, ECM provides a mechanical framework that permits cyclical volume change in the terminal respiratory units. It consists of type I collagen, type III collagen, elastin and proteoglycans. In the thin segment of the blood–air barrier, alveolar epithelium overlies a BM (composed of type IV collagen, laminin, type V collagen and proteoglycans [8]), of which type IV collagen is the principal component. Recently, however, it has become evident that ECM is more than just an inert scaffold with dynamic mechanical properties. A range of processes, such as cell survival, proliferation and migration, are influenced by cell–matrix interactions and matrix turnover, making matrix biology in disease an expanding field of interest [8].

FUNCTION AND REGULATION OF MATRIX METALLOPROTEINASE ACTIVITY

Matrix metalloproteinases (MMPs) are a diverse family of extracellular proteinases, of which 24, loosely classified according to numerical sequence of discovery, have been identified in humans [9]. They are also commonly classified, using a combination of domain organisation, sequence homology and substrate specificity, as ''collagenases'' (MMP-1, -8 and -13), ''gelatinases'' (MMP-2 and -9), ''stromelysins'' (MMP-3 and -10), ''matrilysins'' (MMP-7 and -26), ''membrane-bound'' (MMP-14, -15, -16, -17, -24 and -25) and others (MMP-11, -12, -19, -20, -21, -22, -23, -27 and -28), as outlined in table 1 [9–11]. MMPs are produced by a variety of stromal, epithelial and inflammatory cells and together are capable of degrading all known components of the ECM with significant substrate overlap, suggesting some degree of functional redundancy between MMPs. It has also been demonstrated that MMPs act on several non-ECM substrates such as pro-tumour necrosis factor (TNF)-α, pro-interleukin (IL)-1β, pro-transforming growth factor-β2, chemokines, antiproteases and pro-proteases, with effects on processes such as cell growth, proliferation, survival and migration (table 1) [9]. Regulation of activity occurs both pre- and post-translationally. With the exception of neutrophils and to a lesser extent macrophages, cells do not store MMPs, and expression is induced by a variety of transcription factors, growth factors, cytokines/chemokines, reactive oxygen species and other exogenous environmental or pathogen-derived agents [9]. Secretion of a latent pro-enzyme form that requires activation by cleavage of the pro-domain, and the presence of natural inhibitors of MMPs such as tissue inhibitors of metalloproteinases (TIMPs), are important mechanisms that constrain the action of secreted MMPs. Furthermore, cellular and tissue localisation or compartmentalisation is also important in control of MMP activity [12].

MMPs are known to be involved in a variety of physiological and pathological processes and MMP involvement in embryological development [13], inflammation [14], innate immunity [15], tissue remodelling [16] and ECM biology [17] has been reviewed elsewhere. Reports implicating MMPs in ALI/ARDS are found in the literature from the early 1990s; however, research efforts have failed to translate into effective pharmacotherapies. Furthermore, recently published reviews tend to focus on the role of MMPs under the broad spectrum of destructive pulmonary pathologies [18, 19], without recognising their role in mediating tissue repair as demonstrated in more recent investigations [20]. This article will review the evidence implicating MMPs in aspects of ALI/ARDS most relevant to translational research. The potential for discovery of novel therapeutic targets by investigating the role of MMPs in mediating alveolar epithelial–endothelial barrier injury during the acute phase of ALI/ARDS will be discussed first, followed by similar consideration of MMPs during lung regeneration and repair following ALI/ARDS.

MMPs IN ALVEOLAR INJURY

Degradation of protein components in the alveolar epithelial–endothelial unit, including intercellular junction proteins, BM itself and proteins anchoring cells to the BM, is considered a central process in the pathogenesis of ALI/ARDS.

In vivo *models*

Several studies have specifically investigated the activity of MMPs in experimental lung injury. In hyperoxia-treated pigs, animals developed features of lung injury such as increased wet-to-dry ratio, decreased P_{a,O_2}:F_{I,O_2} ratio and increased bronchoalveolar lavage (BAL) fluid cellularity after 72 h of exposure to 80% oxygen [21]. This was associated with elevated MMP-9 and -2 in BAL after 72 h; parameters of lung injury correlated dose-dependently with BAL MMP-9 concentrations. The investigators noted that these results were not reflected in the analysis of lung homogenates from representative animals. However, no mention is made of an attempt to standardise the assay by measuring total protein content in each of the different samples, which limits any conclusion regarding the difference between MMP activities in BAL or lung tissue in this study. It was also noted that elevated MMP-2 levels were detected earlier than MMP-9, with a peak at 72 h in contrast to 96 h for MMP-9. Immunohistochemistry localised MMP-9 to neutrophils, macrophages and epithelial cells, whereas MMP-2 mainly localised to alveolar macrophages [21]. Notably, the dominant form of both MMP-2 and-9 detected was the high

TABLE 1 The 24 identified human matrix metalloproteinases (MMPs), with examples of both matrix and non-matrix substrates

MMP	Other name(s)	*In vitro* ECM substrates	Other substrate example(s)
MMP-1	Interstitial collagenase	Type I, II, III, VII, VIII, X and XI collagen, gelatin, fibronectin, vitronectin, laminin	Perlecan, IGFBP-2 and -3, pro-TNF-α, PAR-1
MMP-2	Gelatinase A	Gelatin, type I, II, III, IV, V, VII, X and XI collagen, elastin, fibronectin, vitronectin, laminin	Pro-IL-1β, pro-TGF-β2, pro-TNF-α
MMP-3	Stromeolysin 1	Laminin, fibronectin, vitronectin, gelatin, elastin, aggrecan, fibrin, type III, IV, V, VII, IX, X and XI collagen	Pro-IL-1β, pro MMP-1, pro-TNF-α, α_1-proteinase inhibitor
MMP-7	Matrilysin, pump-1	Type I collagen, gelatin, elastin, vitronectin, laminin	Plasminogen, pro-TNF-α
MMP-8	Neutrophil collagenase	Type I, II and III collagen, aggrecan	α_1-proteinase inhibitor
MMP-9	Gelatinase B	Gelatin, type IV, V, XI and XIV collagen, elastin, vitronectin, laminin	Pro-TGF-β2, pro-TNF-α, pro-IL-1β, α_1-proteinase inhibitor, CXCL8
MMP-10	Stromeolysin 2	Gelatin, elastin, fibronectin, aggrecan, type II and IV collagen	Pro-MMP-1, -8 and -10
MMP-11	Stromeolysin 3	Gelatin, fibronectin, laminin	IGFBP-1, α_1-proteinase inhibitor
MMP-12	Metalloelastase	Elastin, type I and V collagen, fibronectin, vitronectin, laminin	Plasminogen
MMP-13	Collagenase 3	Type I, II, III, IV, VI, IX and X collagen, gelatin, fibronectin, aggrecan	α_1-antichymotrypsin
MMP-14	MT1-MMP	Type I, II and III collagen, gelatin, fibronectin, vitronectin, laminin, aggrecan	Pro-MMP-2 and -13, cell surface CD44
MMP-15	MT2-MMP	Fibronectin, tenascin, entacin, laminin, aggrecan	Cell surface tTG
MMP-16	MT3-MMP	Type III collagen, gelatin, fibronectin, vitronectin, laminin	Pro-MMP-2
MMP-17	MT4-MMP	Gelatin	Pro-MMP-2
MMP-19	RASI-1	Gelatin, laminin, entactin, fibronectin, aggrecan	None identified
MMP-20	Enamelysin	Amelogenin	None identified
MMP-21	XMMP	Unknown	None identified
MMP-22	CAMMP	Unknown	None identified
MMP-23	Femalysin	Gelatin	None identified
MMP-24	MT5-MMP	Fibronectin, gelatin, chondroitin sulphate proteoglycan, dermatan sulphate proteoglycan	Pro-MMP-2
MMP-25	MT6-MMP	Gelatin, fibronectin, chondroitin sulphate proteoglycan, dermatan sulphate proteoglycan	Pro-MMP-2
MMP-26	Matrilysin-2	Gelatin, fibronectin, vitronectin	α_1-proteinase inhibitor
MMP-27	CMMP	Gelatin, casein	None identified
MMP-28	Epilysin	Casein	None identified

Non-matrix substrates consist of pro-proteases (including MMPs), inflammatory mediators and other cell surface proteins. Missing MMPs have been removed from the list because further investigation demonstrated duplication or nonexistence of MMPs predicted by described gene products [9–12]. ECM: extracellular matrix; IGFBP: insulin-like growth factor binding protein; TNF: tumour necrosis factor; PAR: protease activated receptor; IL: interleukin; TGF: transforming growth factor; MT: membrane type; tTG: tissue transglutaminase; RASI: rheumatoid arthritis synovium inflamed; XMMP: *Xenopus* MMP; CAMMP: cysteine array MMP; CMMP: chicken MMP.

molecular weight (MW) latent form. The investigators also reported detection of additional but less abundant variant MW bands corresponding to the active forms of MMP-2 and -9.

Another model of hyperoxia in rats investigated MMP-2, -8 and -9 in BAL and lung parenchyma by zymography and immunohistochemistry [22]. Lung injury was assessed by protein levels in BAL and myeloperoxidase (MPO) activity in lung samples to reflect capillary permeability and neutrophil accumulation, respectively. Investigators demonstrated increased latent forms of MMP-2 and -9 in BAL by zymography after 48 h of hyperoxia but also identified different MW forms of MMP-8 in BAL by western blotting [22]. The authors reported that these different forms corresponded to neutrophil- and mesenchymal-derived MMP-8. Mesenchymal-cell derived MMP-8 was identified as the dominant form by western blotting and immunohistochemistry revealed that the mesenchymal MMP-8 was localised to cells with large cytoplasm, considered on this basis by the authors to be recruited macrophages [22].

Bleomycin injury in rats has also been used to investigate the profile of MMP-2 and -9 in early and late phases of lung injury [23]. Histology and BAL analysis demonstrated an acute injury pattern on day 4 following intratracheal instillation of 1.5 units·kg^{-1} bleomycin. Neutrophils were the dominant source of MMP-9 detected in BAL and macrophages were the main source of MMP-2 [23].

A pig model of lung injury after cardiopulmonary bypass (CPB) also demonstrated a rise of both MMP-2 and -9 in BAL that correlated with the alveolar–arterial oxygen gradient ($P_{A–a,O_2}$) [24]. However, no other markers of lung injury were used in this study to rule out increased $P_{A–a,O_2}$ caused by a high ventilation/perfusion ratio or physiological dead space secondary to suboptimal perfusion on CPB. Neutropenic septic lung injury induced by cyclophosphamide and caecal ligation and puncture (CLP) in mice is also associated with increased MMP-9 in lung tissue 4–7 days post-CLP [25]. In this study, investigators measured MMP activity by zymography in lung homogenates

of equal protein content in immunosuppresed or normal mice following CLP or sham operation.

Human data

Both adult and paediatric clinical studies provide useful information on the activity of MMPs in ALI/ARDS in patient cohorts with a spectrum of underlying aetiologies. Although neonatal respiratory distress syndrome (RDS) with progression to bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD) is distinct from ALI/ARDS in adults, similar inflammatory mechanisms may be involved in the pathogenesis of both conditions. Therefore, studies on both neonatal RDS and ALI/ARDS in the paediatric age group are included in the following section of this review.

Adult studies

An early but small prospective case–control study investigated the presence of MMP-2 and -9 in BAL fluid from patients admitted to the intensive care unit (ICU) with ARDS compared with patients ventilated for other indications such as stroke, encephalitis and sepsis or pancreatitis without ARDS [26]. Zymography demonstrated the presence of activated and latent forms of MMP-2 and -9, but only MMP-2 levels were significantly elevated in the ARDS patients. In contrast to this, another small clinical study demonstrated a significant elevation in MMP-9 but not MMP-2 in BAL from patients with permeability pulmonary oedema compared with ventilated patients diagnosed with hydrostatic pulmonary oedema [27]. However, this study has some limitations in that patients were recruited over an 8-yr period and the criteria for selection/recruitment are unclear. Furthermore, the control group was much smaller than the study group (n=8 *versus* n=23). In contrast to the above studies where the control groups comprised patients with hydrostatic oedema or patients ventilated with systemic illness but no pulmonary involvement, a 2-yr prospective clinical study investigated concentration and activity of MMP-2 and -9 and TIMP-1 and -2 in BAL from ALI/ARDS patients and compared these with levels and activity in BAL from patients with hospital-acquired pneumonia [28]. Levels of MMP-2 and -9 were elevated in ALI/ARDS but less so than in the hospital-acquired pneumonia group. There were two groups of ALI/ARDS patients in this study: group 1 with illness duration $\leqslant$4 days; and group 2 with illness duration $>$8 days. The authors found high levels of MMP-9 and a MMP-9:TIMP-1 ratio $>$1 at day 0 in group 1 and a marked reduction in MMP-9 and the MMP-9:TIMP-1 ratio at day 4 in a group of ARDS patients with duration of illness $>$8 days [28]. This suggests that MMP activity may also play a role in progression or persistence of the disease, although it is not clear from this study which MMP-9 and TIMP-1 profile reflects the true causal relationship. Assessment of both MMP and TIMP levels in a more recent prospective case–control study demonstrated elevated levels of MMP-2, -8 and -9 and TIMP-1 compared with 12 healthy volunteer controls [29]. This study recruited 28 patients with ALI/ARDS from a medical ICU and controls from the local medical centre. BAL MMP-2 and -9 levels were assessed by zymography with MMP-1, -3 and -8 and TIMP-1 and -2 levels in BAL assessed by ELISA. Increased levels of MMP-2, -8 and -9 were not significantly correlated with prolonged ICU admission, duration of ventilation, Acute Physiology and Chronic Health Evaluation (APACHE) III score or P_{a,O_2}:F_{I,O_2} ratio at BAL. Although MMP-1 and -3 levels were not elevated in most patients, the investigators noted that a subset of patients with high mortality and more severe APACHE III scores also had detectable MMP-1 and -3 in BAL [29]. However, the timing of BAL was not reported and a wide timeframe of 48 h from ARDS diagnosis was permitted for sampling. It is therefore possible that this association could simply be temporal bias in the MMP profile from delayed BAL in patients with more severe illness involving other organ failure.

A very recent clinical study utilised a multianalyte array to profile MMP-1, -2, -3, -7, -8, -9, -12 and -13 in BAL from ALI/ARDS patients in the control arm of a phase-II randomised controlled trial of intravenous β_2-agonists in ALI (the BALTI trial) [20]. Compared with BAL from seven healthy controls, patients with ALI/ARDS had increased levels of all eight MMPs analysed within 48 h of fulfilling the criteria for ALI. This study was the first to describe the temporal changes in MMP profile over the course of lung injury. MMP-1 and -3, both present at baseline, fell by approximately one-third by day 4, while MMP-2 showed a nonsignificant declining trend over the same time-course. MMP-7, -8, -12 and -13 were all significantly elevated in ARDS BAL compared with BAL from normal volunteers, but the concentrations did not change appreciably between baseline and day 4. In contrast, MMP-9 was the only MMP measured that showed a trend to elevation (two-fold increase in median concentration) over the first 4 days, although this was not statistically significant. Further analysis of MMP-9 concentration and activity in the treatment arm of this study will be considered along with lung repair in ALI/ARDS later in this review. Inhalation of low-dose lipopolysaccharide (LPS) by healthy volunteers was used in another recent clinical study to mimic the neutrophilic alveolar inflammation that occurs in ALI [30]. BAL was performed 6 h after LPS inhalation, and significantly increased levels of MMP-2, -7, -8 and -9 were detectable (but not of MMP-1, -3, -12 and -13).

Paediatric studies

An early study on neonates with respiratory distress investigated the activity of type I collagenases (MMP-1 and -8) in babies born before 33 weeks requiring mechanical ventilation within the first 6 days of life [31]. Measurement of MMP-1 and -8 in 100 BAL samples from 45 children by ELISA did not detect MMP-1. MMP-8 was detected in 68 samples and tended to rise over the first 6 days of life. Higher MMP-8 concentrations were found in BAL during this period from patients who proceeded to develop CLD than from those who did not. In another study in pre-term infants with RDS, investigators measured MMP-2, -8 and -9 and TIMP-2 by western blot analysis in tracheal aspirates of 16 babies intubated at birth. Both latent and active forms of MMP-2 and -9 and two MW forms of latent and active MMP-8 were detected [32]. There was no association between MMP-2 or -9 and the development of BPD. The authors reported the higher MW form of MMP-8 to be polymorphonuclear (PMN) in origin and the lower MW form to be mesenchymal cell in origin, although there are no data in this study to confirm the source of either form detected. When MMP-8 was compared between babies who developed BPD, an association between BPD and significantly higher PMN MMP-8 was found. Analysis of TIMP-2 demonstrated elevated levels in 15 babies and a significant correlation

between TIMP-2 and both surfactant treatment and prolonged mechanical ventilation [32]. In contrast to this, a larger prospective study found MMP-2 and -9 in 73% and 79%, respectively, of BAL samples from premature babies requiring ventilation within the first 48 h of life [33]. Analysis by degree of prematurity and subsequent development of CLD demonstrated a significantly elevated MMP-9:TIMP-1 ratio in very premature babies who developed CLD.

More recently, MMP and TIMP activity in children with ALI/ARDS were investigated in a case–control study comparing serial lung secretions obtained by endotracheal suctioning within 24 h of diagnosis to samples from otherwise healthy patients ventilated for minor elective surgery [34]. The authors demonstrated detectable MMP-8, -9 and TIMP-1 in the majority of ALI/ARDS samples by western blotting. ELISA-based activity assays demonstrated significantly increased activity of MMP-8 and -9 in children with ALI/ARDS. When the authors looked at MMP profile and disease progression, they used ALI/ARDS lasting $\geqslant$10 days to define a prolonged course. Analysis of the MMP-8 profile demonstrated the predominance of a lower MW form after day 10 that was presumed, but not confirmed, to be of mesenchymal cell origin. Also, in cases of ALI/ARDS lasting $<$10 days, the MMP-9:TIMP-1 ratio was significantly elevated compared with that in patients with ALI/ARDS lasting $>$10 days [34].

From the studies discussed so far, MMP-1, -2, -3, -7, -8, -9, -12 and -13 have been implicated in the pathogenesis of ALI/ARDS, with most attention focused on collagenolytic (MMP-1 and -8) and gelatinolytic (MMP-2 and -9) MMPs and stromeolysin (MMP-3). However, significant detail is still lacking on the basic profile of *in vivo* MMP activity and potential substrates in ALI/ARDS. This is important, as differences in MMP form, source, site and presence of inhibitors would have significant implications for both net MMP activity and potential substrates *in vivo*, which are obviously important for both determination of an underlying causal role, and understanding of the potential mechanisms of action for MMPs in the pathogenesis of ALI/ARDS.

In most studies reported, the dominant form of MMPs detected was a higher MW latent form with the inhibitory domain still attached. However, net MMP activity depends on the balance between active protease concentration and the presence of endogenous inhibitors. Therefore, if MMPs have a pathogenetic role in ALI/ARDS it must be demonstrated that net MMP activity is increased. Attempts to explain detection of MMP in the latent form have been limited, but some authors did postulate that activation of the latent form *in vivo* by reactive oxygen or nitrate species without proteolytic cleavage could account for the large proportion of latent form detected. Other studies, but not all, have also analysed the presence of TIMP in samples collected, which gives a better indication of the likely net MMP activity. Therefore, further studies investigating a possible role for a given MMP should consider both its form and the presence of any endogenous inhibitors in addition to a simple semiquantitative description of a particular MMP.

Accepting that MMP activity is elevated in lung injury, the next step is to consider how these proteinases may be involved in the pathogenesis of ALI/ARDS. The early structural degradation concept outlined above is likely to be an oversimplification, as the ECM mediates a wide variety of cellular functions important in acute inflammation and tissue injury, in addition to providing structural integrity to the alveolar epithelial–endothelial unit. General mechanisms by which proteolysis of ECM/BM and non-ECM substrates by MMPs may affect inflammatory injury have been reviewed extensively elsewhere and were briefly outlined earlier in this article [13–17]. However, it is not possible to determine the exact role of MMPs in ALI/ARDS from the above data and several possible conclusions can be drawn from observations that a range of MMPs are elevated in ALI/ARDS and that they are produced by a variety of cells. Implication of so many MMPs may reflect MMP interaction or amplification in a proteolytic cascade. Alternatively, each MMP may have a specific and separate role mediating degradation of ECM/BM and non-ECM substrates with various subsequent biological effects, at discrete time-points in the evolution of the illness. Other explanations include functional redundancy, based on the wide range of overlapping substrates, between MMPs currently implicated in ALI/ARDS; or that simple variation between species and insults in experimental models used accounts for the range of MMPs implicated. In terms of overlapping substrate specificity and potential functional redundancy, it is important to understand that *in vivo* substrate specificity is determined by many factors, as outlined above, in addition to *in vitro* biochemical properties of stereospecificity and reaction constants for a given enzyme–substrate interaction. Obviously, use of a wide range of animal species and various injurious insults does limit comparison between studies, but such studies are useful for hypothesis generation and should not be ignored.

Taken together, this information suggests that MMPs may have multiple pathophysiological roles as both the mediators and effectors of tissue injury in ALI/ARDS. Although significant and specific details are still lacking on MMP profiles, detection and profiling of MMPs in fluids and tissues of animals with experimental ALI/ARDS cannot establish details of a causal role and specific mechanisms of action. More sophisticated techniques and models are required and manipulation of MMP activity either pharmacologically or genetically, in experimental lung injury, offers important information on both the role and mechanisms of MMP involvement in alveolar injury in ALI/ARDS.

Use of chemical inhibitors to study the role of MMPs in ALI

Regulation of MMP activity *in vivo* occurs at multiple levels and there are several agents available that act at both pre- and post-transcriptional levels to reduce MMP activity. Doxycycline and other chemically modified tetracyclines such as COL-3 and CMT-3 are the most commonly used nonspecific MMP inhibitors. Tetracyclines directly inhibit both MMP activity and transcription [35]. The hydroxamates, prinomastat (AG-3340) and batimastat (BB-94), are chemicals that chelate metal ions, including zinc, which is found in the MMP active site [36]. Although these agents are reported to be relatively specific, the hydroxamates are not zinc-specific chelators and therefore have the potential to inhibit other closely related protease families [36]. Both prinomastat and batimastat have been used in clinical trials in cancer and rheumatological diseases to inhibit MMPs, and have been used in animal models to study their role in ALI.

Tetracyclines

Rats ventilated with high tidal volumes (30 mL·kg^{-1}) for 2 h developed ventilator-induced lung injury (VILI) as determined by wet-to-dry ratio, histopathological scoring and neutrophil MPO activity and count. Investigators demonstrated that injury was associated with increased MMP-9 expression and activity, but when pre-treated with CMT-3, histological indices of injury were reduced [37]. More recently, the effect of tetracycline-based MMP inhibition on the pulmonary proteome was investigated in a rat model of lung injury [38]. Oral pre-treatment with doxycycline improved oxygenation and compliance in rats undergoing high-volume ventilation compared with placebo treatment and low-volume ventilation. Proteomic analysis of lung homogenates identified elevated levels of nine proteins in the treatment group, including soluble receptor for advanced glycation endproduct (sRAGE), apolipoprotein A-1, peroxiredoxin II, four molecular forms of albumin and two unnamed proteins [38]. sRAGE has been proposed to act as a decoy receptor that might attenuate inflammation induced by RAGE ligands in lung injury, but the pathogenic significance of the other proteins in ALI/ARDS is uncertain. However, this does highlight some potentially novel non-ECM MMP substrates that may modulate alveolar injury in ALI/ARDS. Pigs pre-treated with COL-3 12 h prior to *i.v.* LPS (100 μg·kg^{-1})-induced lung injury demonstrated less histological injury and less hypoxia and were easier to ventilate than control groups [39]. In an indirect model of ALI in rats subjected to CLP, treatment with COL-3 at time of injury showed reduced lung water, improved histological grading for lung injury and increased survival [40]. Importantly, the effect on survival was noted to be more pronounced with further dosing post lung injury [39]. This has significant clinical importance as the first evidence in animals that treatment post lung injury with a nonspecific MMP inhibitor can both reduce severity of lung injury and improve survival. However, intervention groups were small and there were no physiological measures of hypoxia or compliance that would have made this model potentially more comparable with the human disease.

In a CPB pig model of lung injury, CMT-3 was used to assess the response to MMP inhibition [41]. 30 min after CPB was initiated, either low-dose LPS (1 μg·kg^{-1}), a control or simultaneous LPS and CMT-3 were given. Animals receiving CPB and LPS developed ARDS-like pathology with 60% survival. Clinical features of ARDS were prevented by CMT-3 treatment, with a 100% survival rate compared with 60% during the 270 min of experimentation [41]. It is also worth noting that all these animals were subject to tidal volumes of 12 mL·kg^{-1} during this experiment, which essentially provides another injurious stimulus to the lungs in all groups.

Subcutaneous administration of doxycycline in a pancreatitis model of lung injury reduces the histological grade of ALI and amount of MMP-9 in lung as detected by an enzyme activity fluorescence assay [42]. In a separate *in vitro* experiment, transmigration of neutrophils across matrigel and MMP-9 levels in chamber supernatants were reduced by addition of doxycycline in a concentration to match that achieved in the plasma after subcutaneous administration in the animal experiment [42].

Hydroxamates

Prinomastat has been shown to reduce lung injury in rat model of VILI [43]. Animals in this study were exposed to high-volume ventilation for 4 h and severity of lung injury was assessed by wet-to-dry weight ratio and BAL protein content. Increased latent and active MMP-2 and -9 in untreated groups were detected by zymography and immunoblotting of BAL after high tidal volume ventilation [43]. Levels of TNF-α and extracellular matrix metalloproteinase inducer (EMMPRIN) were also elevated in VILI as determined by *in situ* hybridisation and ELISA, respectively. Intraperitoneal administration of prinomastat for 2 days and 2 h before ventilation reduced MMP-2 and -9 expression in lung tissues and activity in BAL. Wet-to-dry weight ratio and BAL protein content were also reduced. The investigators noted that prinomastat reduced levels of TNF-α, most likely due to the inhibitory effect of prinomastat on TNF-α converting enzyme, which cleaves and activates TNF-α.

Use of batimastat has also been shown to reduce lung injury following acute pancreatitis in rats. Investigators demonstrated reduced proteinaceous exudate and neutrophil accumulation in alveoli during acute pancreatitis with *i.p.* injection of batimastat 48 h prior to initiation of injury [44]. Histological grading of lung injury also improved with pre-administration of batimastat. In another study, investigators assessed the *in vivo* and *in vitro* roles of MMP-9 in pancreatitis-associated lung injury using batimastat. The investigators demonstrated that pancreatitis was associated with lung injury as determined by albumin leak in BAL and increased levels of MMP-9 in lung homogenates [45]. Neutrophils isolated from animals with pancreatitis-associated lung injury demonstrated increased levels of MMP-9 in the cell culture supernatants. Stimulation of human neutrophils by IL-1β and TNF-α in this study also demonstrated increased migration across a matrigel chamber that was abrogated by the administration of batimastat [45].

Anti-proteases

Manipulation of MMP activity has also been demonstrated by the administration of recombinant TIMPs or a specific anti-TIMP immunoglobulin. Such studies help demonstrate that endogenous protease inhibitors are important contributors to the overall activity of a given protease *in vivo*. An early study on the role of neutrophil proteases in the pathogenesis of ALI/ARDS demonstrated that activated neutrophil secretions possessed both serine protease and gelatinase activity. Inhibition of these enzymes by addition of recombinant human protease inhibitors, secretory leukocyte protease inhibitor (SLPI) and TIMP-2, suppressed immune complex-mediated lung injury in rats [46]. The activity of a given protease can also be augmented by inhibiting its natural inhibitor using specific immunoglobulins. Administration of anti-TIMP-2 and anti-SLPI immunoglobulin G in a rat model of immune complex-mediated lung injury demonstrated intensified lung injury with increased neutrophil recruitment to the alveolar space [47].

Other inhibitors

Nonspecific anti-inflammatory agents that affect MMP gene transcription, such as tyrosine kinase inhibitors or pentoxyfiline (PTX), have also been shown to reduce lung injury by mechanisms involving decreased MMP-2 and -9 levels in models of lung injury secondary to sepsis and resuscitation from haemorrhagic shock. In a rat model of *i.v.* LPS-induced lung injury, administration of 5 mg·kg^{-1} LPS *via* internal jugular vein caused histologically confirmed lung injury with increased

levels of MMP-2 in BAL and MMP-9 in plasma at 4 h [48]. Co-administration of 25 $mg \cdot kg^{-1}$ PTX, a nonspecific phosphodiesterase inhibitor, reduced severity of lung injury and MMP-2 levels in BAL. There was no reduction of plasma MMP-9 and the authors did not comment on their reasons for omitting results for plasma MMP-2 levels. The authors analysed nuclear factor (NF)-κB activation using electrophoretic mobility shift assay and found increased activation in the LPS group, suggesting that these effects were mediated *via* NF-κB-dependent transcription of MMPs [48]. The lack of reduction in MMP-9 in this study may represent the release of stored MMP-9 from neutrophils in the treated group. In another study, intratracheal administration of 6 $mg \cdot kg^{-1}$ LPS in rats was used to induce lung injury defined by increased BAL protein and lactate dehydrogenase content [49]. The investigators measured MMP-9 activity by zymography in BAL and cell cultures of alveolar macrophages retrieved from BAL in the same animals. In the treatment arm of the study, animals were pre-treated with intratracheal administration of the tyrosine kinase inhibitor genistein 2 h prior to injury. Both BAL and supernatant from alveolar macrophage cultures had increased levels of MMP-9 but treatment with genistein reduced MMP-9 levels in BAL associated with reduced NF-κB activation in lung tissue and alveolar macrophages [49]. Use of PTX along with hypertonic saline resuscitation from haemorrhagic shock in rats has also been shown to reduce lung injury and MMP-2 and -9 levels in BAL fluid and lung homogenates, compared with animals with standard Ringer's lactate resuscitation strategy [50].

Use of knockout mice to study the role of MMPs in ALI

Chemical inhibition of MMPs is relatively nonspecific and is likely to have multiple effects on other cellular processes and mediators involved in lung injury. Therefore, data from such studies have significant limitations in determining details of the roles of, or interactions between, MMPs as either mediators or terminal effectors of lung injury. Use of knockout mice lacking expression of MMPs offers a more selective method of investigating the role of an individual MMP in ALI/ARDS. Recent experiments using a range of knockout mice models have provided further information on the potential role of MMP-3, -7, -8 and -9 in the pathogenesis of injury in ALI/ARDS.

Results from studies using MMP-9 knockout mice in several different models of lung injury contradict each other and conclusions from experiments using nonspecific chemical inhibition of MMPs. Investigation of hyperoxic injury in the developing lung using MMP-9 knockout mice demonstrated that deficient MMP-9 seems to abrogate the BPD-like features generated by hyperoxia in wild-type controls [51]. Lung injury induced by intratracheal instillation of immunoglobulin G in mice was shown to be reduced in MMP-9 knockouts compared with wild-type controls [52]. However, a recent investigation using an IL-1β-induced model of BPD in MMP-$9^{-/-}$ mice demonstrated worse lung injury in mutants compared with wild-type controls [53]. In another study using MMP-9 knockout mice, investigators demonstrated that lack of MMP-9 resulted in a more severe lung injury following VILI [54], suggesting a protective role for MMP-9 in this model of injury.

Other MMPs, such as MMP-3 and MMP-7, have also been shown to have a pathogenic role in development of experimental lung injury in knockout models. In mice lacking MMP-3, intratracheal administration of immunoglobulin G resulted in less lung injury and neutrophil influx [55]. In another study, investigators reported a significant difference in neutrophil recruitment between MMP-3 knockout mice and MMP-9 knockout animals with the same injury, suggesting that MMP-3 plays an essential role in migration of neutrophils from the pulmonary circulation to the alveolus [52].

In the bleomycin model of lung injury, MMP-7 contributes to neutrophil migration by shedding of syndecan-1 [56]. Instillation of bleomycin in MMP-7 null mice resulted in decreased migration of neutrophils into the alveolus. The authors showed that MMP-7 cleaved syndecan-1 from the mucosal surface of the epithelium: absence of shed syndecan-1 prevented the formation of a KC (murine orthologue of CXCL8)–syndecan gradient that directed neutrophils to the alveolar space [56].

Regulation of MMP activity *in vivo* has also been demonstrated by TIMP-1 knockout mice. Deficiency of this endogenous inhibitor *in vivo* resulted in significantly greater lung injury as measured by pulmonary neutrophilia, permeability and haemorrhage in TIMP-1 null mice compared with wild-type controls [57]. The investigators assessed gelatinase activity by zymography and demonstrated increased activity of MMP-9, emphasising the importance of a balance between proteases and antiproteases in determining overall proteolytic activity in ALI/ARDS. However, the absence of TIMP-1 would leave functionally unopposed activity of other proteases in addition to MMP-9, and this study did not investigate other MMPs. Therefore, it is not possible to conclude that increased lung injury in this study was due to the functionally unopposed MMP-9 activity.

Investigation of MMP-8 involvement in lung injury using knockout mice in a variety of models has yielded conflicting results. In other animal models and clinical studies, MMP-8 has been detected in BAL, possibly produced by both neutrophils and mesenchymal cells. In these studies involving MMP-8 knockout mice, the investigators report that activity of MMP-8 is derived from neutrophils and has been demonstrated to be largely membrane bound and highly resistant to TIMP. Mice lacking MMP-8 had greater lung injury in an intratracheal LPS model, suggesting an anti-inflammatory role for MMP-8 [58]. Furthermore, MMP-8 deletion was shown to increase severity of injury and inflammation in LPS-, bleomycin- and hyperoxia-induced lung injury [59]. The investigators demonstrated increased neutrophil and macrophage recruitment due to increased macrophage inflammatory protein (MIP)-1α, but not other traditional PMN chemoattractants, in BAL fluid of MMP-$8^{-/-}$ mice [59]. In contrast to an anti-inflammatory effect, MMP-8 has been shown to play an important role in mediating VILI, as mice lacking MMP-8 have reduced lung injury when subjected to injurious ventilatory strategies [60].

From the above evidence, it is clear that MMPs are more than just terminal effectors of ECM/BM destruction in an inappropriate and/or excessive innate response to tissue injury or infection. MMP activity is elevated in ALI/ARDS, but individual roles in pathogenesis are probably determined by the source of secretion, the substrates available at the site of activity and a local balance between latent or active form and any inhibitors present. Figure 1 highlights the key MMPs and cells

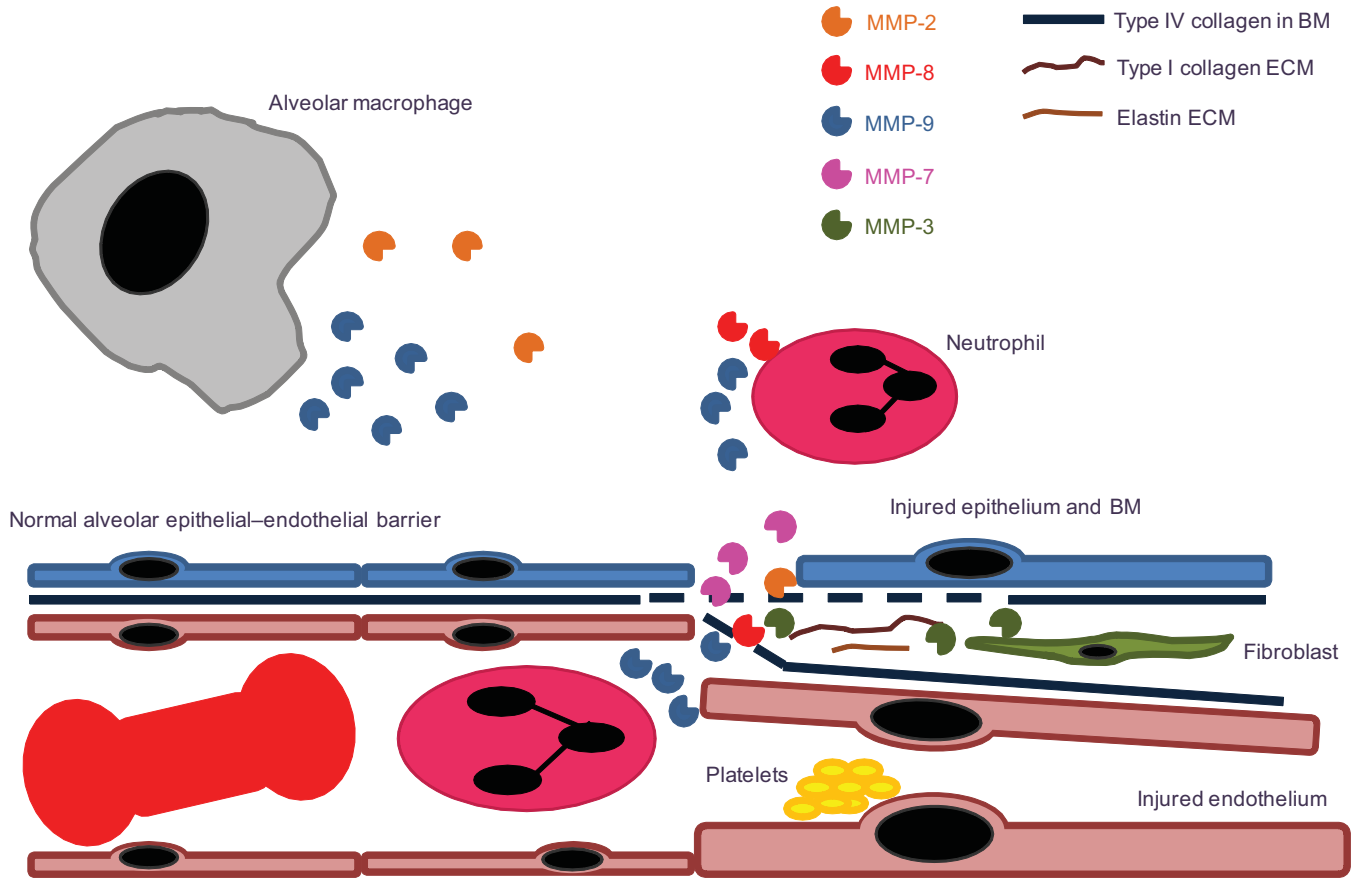

FIGURE 1. Matrix metalloproteinase (MMP) involvement in pathogenesis of acute lung injury/acute respiratory distress syndrome. MMP-9 and MMP-8 are produced by neutrophils. Alveolar macrophages and epithelial cells secrete MMP-2 and -9 and MMP-7, respectively, while MMP-3 is secreted by activated fibroblasts. Likely key substrates include extracellular matrix (ECM) proteins and inflammatory mediators such as chemotaxins. MMP-dependent shedding of syndecan-1 seems to be important in maintaining a chemotactic gradient. BM: basement membrane.

involved in the pathogenesis of ALI/ARDS. Furthermore, MMPs may have a role in repair such that after established injury, a deficiency in one or more proteases may inhibit the epithelial reparative process. This highlights the need for more work on these basic aspects of MMP biology to better characterise the pathogenic role of individual MMPs at any given stage of the ALI process. Nonspecific inhibition of MMPs has demonstrated reduced lung injury in pre-treatment models, but in one study a treatment effect was noted after lung injury had been caused. Importantly, this suggests that a possible therapeutic window exists for the treatment of early ALI/ARDS in humans with agents that are nonspecific MMP inhibitors. Gene targeting of individual MMPs offers a potentially selective method of controlling individual MMP activity. Results, however, have been conflicting. MMP-8 and -9 knockout mouse models of lung injury demonstrate both exacerbation and reduction of lung injury from a variety of insults. MMP-7-mediated shedding of syndecan-1 seems to be consistently important *in vivo*, but MMP-7 has also been implicated in epithelial repair, while MMP-3 also seems to have an important role in mediating experimental lung injury but no specific substrate has been identified. This gap in knowledge between the lung injury phenotype of knockout mice and lung injury in other methods of MMP inhibition highlights the importance of assessing both the molecular and cellular consequences of MMP manipulation *in vivo*.

MMPS IN REPAIR OF THE ALVEOLAR EPITHELIAL–ENDOTHELIAL UNIT

By the time ALI is clinically recognised, an overwhelming inflammatory cascade is already established. MMPs are also known to be involved in physiological processes that may be important to tissue repair, such as development and morphogenesis, and there are some data indicating that they are important in alveolar epithelial repair. Further understanding of their role in repair may highlight potential new strategies to aid resolution of ALI.

Epithelial wound repair in the lung

Basic mechanisms of wound repair in the lung have been extensively reviewed. Current understanding focuses on cellular and molecular aspects of epithelial repair in the alveolar capillary membrane. Essentially, the alveolar epithelial defect (wound) is filled by spreading and migration of neighbouring type II alveolar epithelial cells along a provisional ECM followed by proliferation and differentiation to type I alveolar epithelial cells with restoration of epithelial integrity [61]. However, recent reviews [62–64] highlight that

current understanding of this process and what influences the time-course (onset of fibroproliferative response) and trajectory (balance between repair and fibroproliferation) of healing in the lung is limited, as there are obvious difficulties in modelling *in vivo* alveolar epithelial cell repair *in vitro*.

Time-course and trajectory of epithelial repair in the lung

Currently, the accepted paradigm of ALI/ARDS evolution is that of a linear progression through exudative, fibroproliferative and resolution phases. However, more recent investigation of repair and remodelling in lung injury demonstrates that fibroproliferation begins early in the development of ALI/ARDS. Using an *i.p.* injection of paraquat model of lung injury in rats, investigators have demonstrated that altered tissue mechanics associated with markers of ECM remodelling are detected as early as 24 h after injury [65]. In another animal study, pulmonary mechanics were assessed in mice with lung injury induced by administration of LPS (either 125 μg *i.p.* or 10 μg intratracheal) [66]. Collagen fibre deposition was increased and pulmonary compliance decreased at 24 h post injury. This finding is also supported in humans, with an autopsy study of ALI/ARDS patients demonstrating collagen deposition early in the course of the syndrome [67]. Clinical studies in patients with ALI/ARDS also demonstrate that fibroproliferation begins early in the course of disease. In one prospective trial, BAL from 77 patients with ALI/ARDS was assessed for mitogenic activity on human fetal lung fibroblasts and collagen synthesis by presence of procollagen peptide. Compared with a small number of controls, patients with ALI/ARDS had potent mitogenic activity and high levels of procollagen peptide III (PCP III) in BAL from day 1 [68]. Another study investigated the relationship between chord compliance calculated from the linear part of pressure–volume curves in ventilated patients, PCP III and MMP in patients with ALI/ARDS undergoing BAL within 4 days of diagnosis. The investigators found decreased chord compliance and evidence for increased collagen synthesis from PCP III levels in these early samples [69].

Some investigators believe the primary site or component of the alveolar epithelial–endothelial unit injured has important effects on the trajectory of healing and balance between fibroproliferation and repair after lung injury. It is intuitive that injury to either the epithelial or endothelial face of the alveolar unit would dominate in pulmonary or extrapulmonary ALI/ARDS, respectively. Therefore different causes of ALI/ARDS could be important in determination of the fibroproliferative response to injury.

Investigation of the degree of fibroproliferation in two models of extrapulmonary experimental lung injury in mice demonstrated that a greater degree of endothelial injury is associated with greater fibrosis following lung injury [70]. The researchers assessed mechanical properties and content of collagen and elastin by histology after 125 μg *i.p.* of either LPS or CLP. Mice with lung injury had a greater degree of endothelial injury determined by electron microscopy, associated with more collagen and elastin fibre deposition in the lung and reduced compliance [70]. However, it seems the primary hypothesis in this investigation was not formed *a priori* and these findings contradict other studies and current understanding that suggest epithelial damage is associated with a greater degree of fibroproliferation [62–64]. In contrast to this study, the importance of alveolar epithelial damage as a determinant of fibroproliferation has been demonstrated in a mouse model of ALI/ARDS using intratracheal or *i.p.* LPS already mentioned [66]. Animals with a dominant pulmonary insult provided by intratracheal LPS yielded fibroelastogenesis, but damage to the endothelium caused fibrosis that resolved early in the course of injury. In humans, autopsy studies already discussed also reveal a potential difference in the degree of collagen deposition depending on the aetiology of injury, classified as pulmonary or extrapulmonary [67].

MMPs and alveolar epithelial repair

As already discussed, MMPs have various biological effects by proteolytic action on cell–cell and cell–matrix interactions that ultimately manipulate cell and tissue processes likely to be important to repair. However, current understanding of MMP involvement in remodelling and repair following ALI/ARDS is limited. Epithelial repair in the lung has been shown to involve MMP-7 in the regulation of cell–cell adhesion and cell-matrix adhesion by shedding of E-cadherin and syndecan-1 from the cell surface [71]. In experiments using a variety of *in vitro* and *in vivo* methods, investigators assessed the rate of wound closure in A459 adenocarcinoma cell cultures and tracheal explants in wild-type or MMP-7$^{-/-}$ mice. Knockout mice were also used to assess shedding of E-cadherin in the BAL fluid of MMP-7$^{-/-}$ mice treated with intratracheal bleomycin. A459 cells transfected with an active MMP-7 gene had increased rates of epithelial wound closure, while E-cadherin shedding was reduced in culture supernatant of wounded tracheal explants and in BAL fluid from bleomycin-induced lung injury in mice lacking MMP-7. Furthermore, immunohistochemistry also demonstrated co-localisation of MMP-7 and E-cadherin in wild-type mouse trachea with a controlled wound. Modulation of $\alpha_2\beta_1$ integrin affinity for ECM by MMP-7-mediated syndecan-1 shedding may also play a role in epithelial repair. Syndecan-1 shedding by MMP-7 has already been discussed where this process facilitates neutrophil migration across the epithelium in lung injury [56]. Investigation using complex *in vitro* methods demonstrates that shedding of syndecan-1 mediated by MMP-7 also negatively influences $\alpha_2\beta_1$ integrin affinity for collagens in the ECM, thereby facilitating migration of epithelial cells at the wound edge [72]. Another study investigated the role of MMP-7 and TIMP-1 in airway epithelial wound repair using *in vitro* and knockout models of injury [73]. There was increased spreading and migration of airway epithelial cells *in vitro* from wild-type cultures compared with MMP-7 knockout cultures. Immunofluoresence in wild-type cultures demonstrated co-localisation of MMP-7 and TIMP-1 at cells proximal to the wound edge. Furthermore, wound repair in cell culture experiments from the TIMP-1 null mice demonstrated unrestrained *in vitro* re-epithelialisation by cell spreading and migration compared with wild-type controls [73]. These findings were also confirmed *in vivo* using a naphthalene lung injury model, suggesting that TIMP-1 plays a functionally important role in determining net MMP-7 activity *in vivo*.

MMP-9 may also play a role in alveolar epithelial repair: evidence from an early study using cultured alveolar epithelial cells from hyperoxia-treated rats also demonstrated an increased migratory phenotype, correlating with increased MMP-9 activity

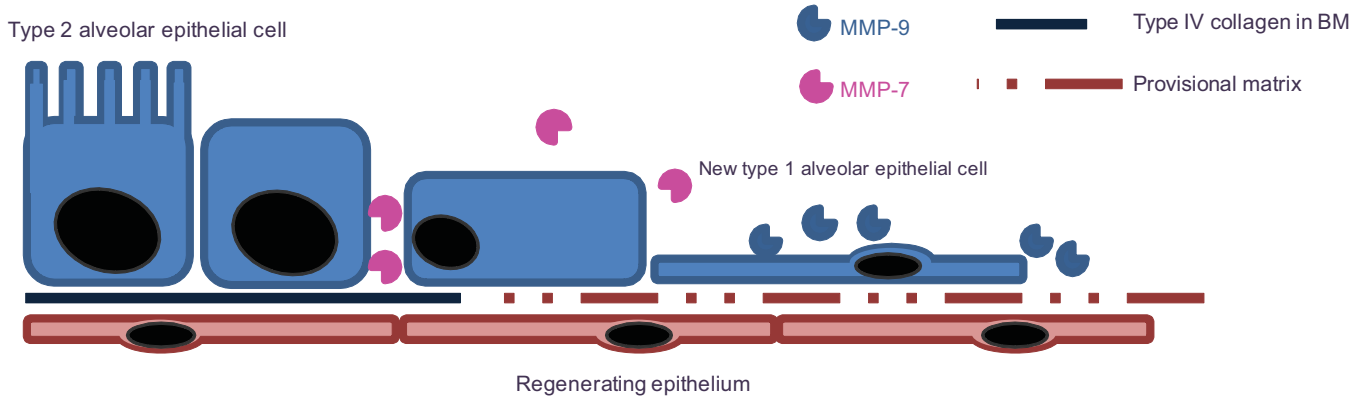

FIGURE 2. Matrix metalloproteinase (MMP) involvement in alveolar epithelial repair. MMP-7 and -9 from distal alveolar epithelial cells are important in epithelial repair. Facilitation of cell migration by shedding of syndecan-1 and probable proteolysis of provisional matrix and abnormal collagens are important in epithelial restitution following acute lung injury/acute respiratory distress syndrome. BM: basement membrane.

that was reduced by inhibition using doxycycline [74]. Unexpectedly, in humans, a recent study using BAL from ALI/ARDS patients receiving *i.v.* salbutamol in the BALTI trial demonstrated a possible role for MMP-9 in alveolar epithelial repair [20]. This study showed a significant fold change in MMP-9 at day 4 in the salbutamol group, which was associated with reduced extravascular lung water. Salbutamol specifically increased secretion of MMP-9 from distal lung epithelial cells (DLECs), as a model of alveolar epithelial cells, but not macrophages or neutrophils. Inhibition of MMP-9 in wounded alveolar epithelial cell cultures using dihydrolipoic acid caused a dose-dependent reduction in wound repair. This study suggested that MMP-9 was required for lung epithelial function *in vivo* and wound repair *in vitro* and highlights the point that broad-spectrum inhibition of MMPs also has the potential to inhibit the repair phase of ALI/ARDS and therefore potentially worsen outcomes for patients. The potential role of MMPs in repair of the alveolar capillary membrane is outlined in figure 2.

CONCLUSION

The studies discussed in this review demonstrate that MMPs play a central role as both mediators and effectors of alveolar capillary membrane injury and repair in ALI/ARDS. Recent animal work and human studies demonstrate the role of inflammatory, mesenchymal and possibly epithelial cells that produce MMP-2, -3, -7, -8 and -9, in development of injury to the alveolar capillary membrane. Nonstructural ECM components important in chemotaxis such as syndecan-1/KC and MIP-1α have been shown to be important in mediating injury in experimental lung injury. However, significant details on cellular source, particularly for MMP-3 and -7, and other key substrates, remain to be established.

Understanding of the time-course and trajectory of alveolar epithelial–endothelial repair in ALI/ARDS suggests that this process starts early, but factors determining the development of fibrosis are poorly understood. However, rapid restitution of the epithelial barrier is likely to be important in limiting fibroproliferation. MMP-7 production by airway epithelium and degradation of syndecan-1 and E-cadherin has been shown to be important in facilitating cell migration in epithelial repair and MMP-9 production by DLECs has, more recently, also been demonstrated to be important in epithelial wound repair.

From a translational perspective, targeting of MMP activity using broad-spectrum inhibitors has been shown to limit injury when used as a pre-injury treatment strategy and may also be of benefit when given early in the course of experimental lung injury. However, involvement of MMPs in both injury and repair has important implications for potential pharmacotherapeutic strategies as indiscriminate inhibition of MMPs may have deleterious effects on repair.

In conclusion, while there is much evidence implicating MMPs in the injury process in ALI/ARDS, significant work is required to fully understand the role of MMPs in the pathogenesis and progression of ALI/ARDS. More consistent detail on MMP profile in clinical studies over the time-course of ALI/ARDS and relevant animal models is required. Further work on knockout models of lung injury phenotypes to elucidate the roles of individual MMPs and associated alterations of other important mediators of lung injury should provide more detail on the mechanism of MMP involvement in alveolar injury and repair. These data will be necessary to provide information about which MMPs should be inhibited at which specific stages in the course of ALI, to allow well-designed clinical trials of inhibitors and also to inform studies of when supplemented or unopposed protease activity may be appropriate to enable epithelial repair and resorption of abnormal collagens.

STATEMENT OF INTEREST

A statement of interest for D.F. McAuley can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

REFERENCES

1 Bernard GR, Artigas A, Brigham KL, *et al.* The American–European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994; 149: 818–824.
2 Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000; 342: 1334–1349.
3 Tsushima K, King LS, Aggarwal NR, *et al.* Acute lung injury review. *Intern Med* 2009; 48: 621–630.

4 Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol* 2011; 6: 147–163.
5 Matthay MA, Zimmerman GA. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am J Respir Cell Mol Biol* 2005; 33: 319–327.
6 Empey DW. Diseases of the respiratory system. Introduction: structure and function of the lungs. *Br Med J* 1978; 1: 631–633.
7 Murray JF. The structure and function of the lung. *Int J Tuberc Lung Dis* 2010; 14: 391–396.
8 Dunsmore SE, Rannels DE. Extracellular matrix biology in the lung. *Am J Physiol* 1996; 270: L3–L27.
9 Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol Rev* 2007; 87: 69–98.
10 Lemaitre V, D'Armiento J. Matrix metalloproteinases in development and disease. *Birth Defects Res C Embryo Today* 2006; 78: 1–10.
11 O'Kane CM, Frank J, McAuley DF. Matrix metalloproteinases: a potential role in the pathogenesis of ARDS, *In:* Vincent JL, ed. Yearbook of Intensive Care and Emergency Medicine. 1st Edn. Heidelberg, Springer, 2004; pp. 287–300.
12 McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 2001; 13: 534–540.
13 Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000; 14: 2123–2133.
14 Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. *Semin Cell Dev Biol* 2008; 19: 34–41.
15 Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 2004; 4: 617–629.
16 Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol* 2007; 8: 221–233.
17 Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 2004; 16: 558–564.
18 Elkington PT, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. *Thorax* 2006; 61: 259–266.
19 Oikonomidi S, Kostikas K, Tsilioni I, *et al.* Matrix metalloproteinases in respiratory diseases: from pathogenesis to potential clinical implications. *Curr Med Chem* 2009; 16: 1214–1228.
20 O'Kane CM, McKeown SW, Perkins GD, *et al.* Salbutamol up-regulates matrix metalloproteinase-9 in the alveolar space in the acute respiratory distress syndrome. *Crit Care Med* 2009; 37: 2242–2249.
21 Gushima Y, Ichikado K, Suga M, *et al.* Expression of matrix metalloproteinases in pigs with hyperoxia-induced acute lung injury. *Eur Respir J* 2001; 18: 827–837.
22 Cederqvist K, Janer J, Tervahartiala T, *et al.* Up-regulation of trypsin and mesenchymal MMP-8 during development of hyperoxic lung injury in the rat. *Pediatr Res* 2006; 60: 395–400.
23 Kim JY, Choeng HC, Ahn C, *et al.* Early and late changes of MMP-2 and MMP-9 in bleomycin-induced pulmonary fibrosis. *Yonsei Med J* 2009; 50: 68–77.
24 Eichler W, Bechtel JF, Schumacher J, *et al.* A rise of MMP-2 and MMP-9 in bronchoalveolar lavage fluid is associated with acute lung injury after cardiopulmonary bypass in a swine model. *Perfusion* 2003; 18: 107–113.
25 Hirsh M, Carmel J, Kaplan V, *et al.* Activity of lung neutrophils and matrix metalloproteinases in cyclophosphamide-treated mice with experimental sepsis. *Int J Exp Pathol* 2004; 85: 147–157.
26 Delclaux C, d'Ortho MP, Delacourt C, *et al.* Gelatinases in epithelial lining fluid of patients with adult respiratory distress syndrome. *Am J Physiol* 1997; 272: L442–L451.
27 Pugin J, Verghese G, Widmer MC, *et al.* The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit Care Med* 1999; 27: 304–312.
28 Lanchou J, Corbel M, Tanguy M, *et al.* Imbalance between matrix metalloproteinases (MMP-9 and MMP-2) and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) in acute respiratory distress syndrome patients. *Crit Care Med* 2003; 31: 536–542.
29 Fligiel SE, Standiford T, Fligiel HM, *et al.* Matrix metalloproteinases and matrix metalloproteinase inhibitors in acute lung injury. *Hum Pathol* 2006; 37: 422–430.
30 Shyamsundar M, McKeown ST, O'Kane CM, *et al.* Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. *Am J Respir Crit Care Med* 2009; 179: 1107–1114.
31 Sweet DG, McMahon KJ, Curley AE, *et al.* Type I collagenases in bronchoalveolar lavage fluid from preterm babies at risk of developing chronic lung disease. *Arch Dis Child Fetal Neonatal Ed* 2001; 84: F168–F171.
32 Cederqvist K, Sorsa T, Tervahartiala T, *et al.* Matrix metalloproteinases-2, -8, and -9 and TIMP-2 in tracheal aspirates from preterm infants with respiratory distress. *Pediatrics* 2001; 108: 686–692.
33 Sweet DG, Curley AE, Chesshyre E, *et al.* The role of matrix metalloproteinases -9 and -2 in development of neonatal chronic lung disease. *Acta Paediatr* 2004; 93: 791–796.
34 Kong MY, Gaggar A, Li Y, *et al.* Matrix metalloproteinase activity in paediatric acute lung injury. *Int J Med Sci* 2009; 6: 9–17.
35 Griffin MO, Fricovsky E, Ceballos G, *et al.* Tetracyclines: a pleitropic family of compounds with promising therapeutic properties. Review of the literature. *Am J Physiol Cell Physiol* 2010; 299: C539–C548.
36 Fingleton B. Matrix metalloproteinases as valid clinical targets. *Curr Pharm Des* 2007; 13: 333–346.
37 Kim JH, Suk MH, Yoon DW, *et al.* Inhibition of matrix metalloproteinase-9 prevents neutrophilic inflammation in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2006; 291: L580–L587.
38 Doroszko A, Hurst TS, Polewicz D, *et al.* Effects of MMP-9 inhibition by doxycycline on proteome of lungs in high tidal volume mechanical ventilation-induced acute lung injury. *Proteome Sci* 2010; 8: 3.
39 Carney DE, McCann UG, Schiller HJ, *et al.* Metalloproteinase inhibition prevents acute respiratory distress syndrome. *J Surg Res* 2001; 99: 245–252.
40 Steinberg J, Halter J, Schiller HJ, *et al.* Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. *J Surg Res* 2003; 111: 185–195.
41 Carney DE, Lutz CJ, Picone AL, *et al.* Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* 1999; 100: 400–406.
42 Sochor M, Richter S, Schmidt A, *et al.* Inhibition of matrix metalloproteinase-9 with doxycycline reduces pancreatitis-associated lung injury. *Digestion* 2009; 80: 65–73.
43 Foda HD, Rollo EE, Drews M, *et al.* Ventilator-induced lung injury upregulates and activates gelatinases and EMMPRIN: attenuation by the synthetic matrix metalloproteinase inhibitor, Prinomastat (AG3340). *Am J Respir Cell Mol Biol* 2001; 25: 717–724.
44 Muhs BE, Patel S, Yee H, *et al.* Inhibition of matrix metalloproteinases reduces local and distant organ injury following experimental acute pancreatitis. *J Surg Res* 2003; 109: 110–117.
45 Keck T, Balcom JH 4th, Fernandez-del Castillo C, *et al.* Matrix metalloproteinase-9 promotes neutrophil migration and alveolar capillary leakage in pancreatitis-associated lung injury in the rat. *Gastroenterology* 2002; 122: 188–201.
46 Mulligan MS, Desrochers PE, Chinnaiyan AM, *et al. In vivo* suppression of immune complex-induced alveolitis by secretory leukoproteinase inhibitor and tissue inhibitor of metalloproteinases 2. *Proc Natl Acad Sci USA* 1993; 90: 11523–11527.

47 Gipson TS, Bless NM, Shanley TP, *et al.* Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. *J Immunol* 1999; 162: 3653–3662.
48 Coimbra R, Melbostad H, Loomis W, *et al.* LPS-induced acute lung injury is attenuated by phosphodiesterase inhibition: effects on proinflammatory mediators, metalloproteinases, NF-κB, and ICAM-1 expression. *J Trauma* 2006; 60: 115–125.
49 Kang JL, Lee HW, Lee HS, *et al.* Genistein prevents nuclear factor-κB activation and acute lung injury induced by lipopolysaccharide. *Am J Respir Crit Care Med* 2001; 164: 2206–2212.
50 Deree J, Martins JO, Leedom A, *et al.* Hypertonic saline and pentoxifylline reduces hemorrhagic shock resuscitation-induced pulmonary inflammation through attenuation of neutrophil degranulation and proinflammatory mediator synthesis. *J Trauma* 2007; 62: 104–111.
51 Chetty A, Cao GJ, Severgnini M, *et al.* Role of matrix metalloprotease-9 in hyperoxic injury in developing lung. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L584–L592.
52 Warner RL, Beltran L, Younkin EM, *et al.* Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol* 2001; 24: 537–544.
53 Lukkarinen H, Hogmalm A, Lappalainen U, *et al.* Matrix metalloproteinase-9 deficiency worsens lung injury in a model of bronchopulmonary dysplasia. *Am J Respir Cell Mol Biol* 2009; 41: 59–68.
54 Albaiceta GM, Gutierrez-Fernandez A, Parra D, *et al.* Lack of matrix metalloproteinase-9 worsens ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2008; 294: L535–L543.
55 Nerusu KC, Warner RL, Bhagavathula N, *et al.* Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. *Exp Mol Pathol* 2007; 83: 169–176.
56 Li Q, Park PW, Wilson CL, *et al.* Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* 2002; 111: 635–646.
57 Kim KH, Burkhart K, Chen P, *et al.* Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. *Am J Respir Cell Mol Biol* 2005; 33: 271–279.
58 Owen CA, Hu Z, Lopez-Otin C, *et al.* Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* 2004; 172: 7791–7803.
59 Quintero PA, Knolle MD, Cala LF, *et al.* Matrix metalloproteinase-8 inactivates macrophage inflammatory protein-1α to reduce acute lung inflammation and injury in mice. *J Immunol* 2010; 184: 1575–1588.
60 Albaiceta GM, Gutierrez-Fernández A, García-Prieto E, *et al.* Absence or inhibition of matrix metalloproteinase-8 decreases ventilator-induced lung injury. *Am J Respir Cell Mol Biol* 2010; 43: 555–563.
61 Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. *Am J Physiol Lung Cell Mol Physiol* 2010; 298: L715–L731.
62 Rocco PR, Dos Santos C, Pelosi P. Lung parenchyma remodeling in acute respiratory distress syndrome. *Minerva Anestesiol* 2009; 75: 730–740.
63 Chapman HA. Disorders of lung matrix remodeling. *J Clin Invest* 2004; 113: 148–157.
64 Dos Santos CC. Advances in mechanisms of repair and remodelling in acute lung injury. *Intensive Care Med* 2008; 34: 619–630.
65 Rocco PR, Negri EM, Kurtz PM, *et al.* Lung tissue mechanics and extracellular matrix remodeling in acute lung injury. *Am J Respir Crit Care Med* 2001; 164: 1067–1071.
66 Santos FB, Nagato LK, Boechem NM, *et al.* Time course of lung parenchyma remodeling in pulmonary and extrapulmonary acute lung injury. *J Appl Physiol* 2006; 100: 98–106.
67 Negri EM, Hoelz C, Barbas CS, *et al.* Acute remodeling of parenchyma in pulmonary and extrapulmonary ARDS. An autopsy study of collagen-elastic system fibers. *Pathol Res Pract* 2002; 198: 355–361.
68 Marshall RP, Bellingan G, Webb S, *et al.* Fibroproliferation occurs early in the acute respiratory distress syndrome and impacts on outcome. *Am J Respir Crit Care Med* 2000; 162: 1783–1788.
69 Demoule A, Decailliot F, Jonson B, *et al.* Relationship between pressure–volume curve and markers for collagen turn-over in early acute respiratory distress syndrome. *Intensive Care Med* 2006; 32: 413–420.
70 Chao MC, Garcia CS, de Oliveira MB, *et al.* Degree of endothelium injury promotes fibroelastogenesis in experimental acute lung injury. *Respir Physiol Neurobiol* 2010; 173: 179–188.
71 McGuire JK, Li Q, Parks WC. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am J Pathol* 2003; 162: 1831–1843.
72 Chen P, Abacherli LE, Nadler ST, *et al.* MMP7 shedding of syndecan-1 facilitates re-epithelialization by affecting $\alpha_2\beta_1$ integrin activation. *PLoS One* 2009; 4: e6565.
73 Chen P, McGuire JK, Hackman RC, *et al.* Tissue inhibitor of metalloproteinase-1 moderates airway re-epithelialization by regulating matrilysin activity. *Am J Pathol* 2008; 172: 1256–1270.
74 Buckley S, Driscoll B, Shi W, *et al.* Migration and gelatinases in cultured fetal, adult, and hyperoxic alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L427–L434.