Peer reviewed article

Mechanisms of alveolar epithelial repair in acute lung injury – a translational approach

Thomas Geiser

Division of Pulmonary Medicine and Department of Clinical Research, Inselspital, Bern

Summary

In patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), extensive damage to the alveolar epithelial and endothelial barrier is observed, resulting in the influx of protein-rich oedema fluid into the air spaces. Efficient alveolar epithelial repair is crucial to ALI/ARDS patients' recovery. Future therapeutic strategies may therefore include acceleration of the epithelial repair process in the injured lung. However, a better understanding of the cellular and molecular mechanisms that promote alveolar epithelial repair is needed if novel therapeutic strategies are to be developed. Pulmonary oedema fluid from patients with ALI/ARDS and from patients with hydrostatic oedema as control was obtained, and the effect on alveolar epithelial repair *in vitro* using our alveolar epithelial wound repair bioassay was studied. In contrast to the initial hypothesis, pulmonary oedema fluid from ALI/ARDS patients increased alveolar epithelial repair *in vitro* by an interleukin-1β (IL-1β)-dependent mechanism, demonstrating a novel, possibly beneficial role for IL-1β in patients with ALI/ARDS. Further studies using primary alveolar epithelial cells from rats revealed that IL-1β induced alveolar epithelial repair by an epidermal growth factor (EGF)/transforming growth factor-α (TGF-α)-dependent pathway. Besides EGF and TGF-α, keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) – both present in pulmonary oedema fluid obtained from patients with ALI/ARDS – stimulate alveolar epithelial repair *in vitro*. Further experimental and clinical studies will show whether acceleration of alveolar epithelial repair by modulating cytokines and growth factors in the injured lung represents a promising new therapeutic strategy in patients with ALI/ARDS.

Key words: epithelial repair; acute lung injury; ARDS

Introduction

The clinical course of patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is variable and influenced by different factors. One of the most important mechanisms that determines the severity of lung injury is the magnitude of injury to the alveolar epithelial barrier [1]. The possibility of repairing the epithelial injury at an early stage is a major determinant of recovery. Specific treatments to accelerate alveolar epithelial repair do not exist, although progress in studies with experimental models of ALI suggests that specific treatment may be possible in the future [2]. Most of the treatment modalities tested recently were based on diminution of the inflammatory response in the lung in order to minimise the initial injury. However, an alternative therapeutic approach is to accelerate the repair process in the alveolar epithelium in the early stages of ALI/ARDS, to enhance the resolution of pulmonary oedema and improve outcomes in these patients. Little is known at present about the cellular and molecular mechanisms of alveolar epithelial repair in ALI/ARDS. In particular, soluble mediators which play a key role in alveolar epithelial repair in ALI/ARDS patients must be identified and characterised if novel therapeutic strategies are to be developed. The effects on alveolar epithelial wound repair of undiluted pulmonary oedema fluid obtained from patients at different stages of ALI/ARDS were recently studied in a translational approach using an *in vitro* epithelial wound repair model with human alveolar epithelial-like A549 cells and primary alveolar type II epithelial cells from rat lungs [3–5]. The purpose of the present paper is to summarise the results of these studies.

Presentation at the annual meeting of the Swiss Respiratory Society 2003, St. Gallen (Young Swiss Investigators in Pulmonary Medicine).

Schweizerische Stiftung für Medizinisch-Biologische Stipendien.

Injury to the alveolar epithelium in acute lung injury

The normal alveolar barrier is composed of three different structures: (1) the capillary endothelium, (2) the interstitial space including the basement membrane and the extracellular matrix, and (3) the alveolar epithelium. The alveolar epithelium consists of alveolar type I and alveolar type II cells. The flat alveolar type I cells line more than 90% of the alveolar surface area. The attenuated cytoplasm provides for close approximation of the alveolar lumen and the bloodstream, optimising the exchange of respiratory gases. The cuboidal alveolar type II cells are multifunctional cells. They produce surfactant, are important for active alveolar liquid clearance, and represent the progenitor cells which regenerate the alveolar epithelium after injury [6]. Under normal conditions the epithelial barrier is much less permeable than the endothelial barrier and prevents cells and plasma from flooding the air spaces, thereby maintaining normal gas exchange [7]. Several studies have demonstrated the critical importance of the alveolar epithelium in the pathogenesis of and recovery from severe ALI/ARDS. Efficient alveolar epithelial repair is therefore crucial for ALI/ARDS patients' recovery.

Pathology of ALI/ARDS

Diffuse alveolar damage is a hallmark of patients with ALI/ARDS, whether the latter is caused directly (e.g. pneumonia or acid aspiration) or indirectly (e.g. sepsis or severe trauma). In histological sections from patients dying of ALI/ARDS, the first lesions appear to be interstitial oedema, followed by severe alveolar epithelial damage [8].

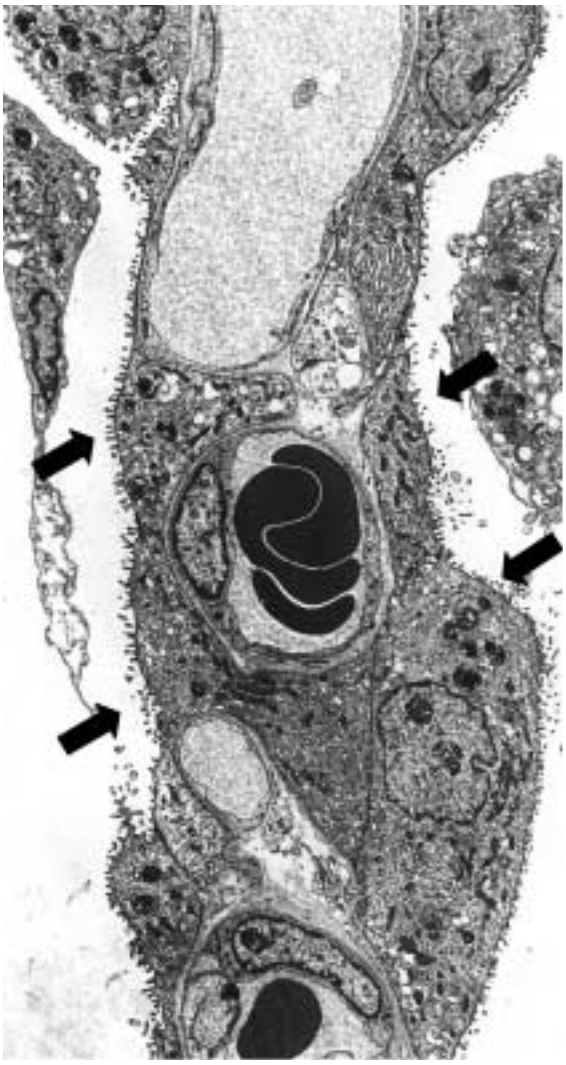


Figure 1

Electron microscopy of an alveolar septum of a patient who died of ALI/ARDS in the subacute, proliferative phase. There is evidence of re-epithelialisation of the damaged epithelial barrier with hyperplastic alveolar type II cells, resulting in coverage of the denuded basement membrane and re-establishment of the continuity of the alveolar epithelium. The arrows indicate typical alveolar type II cells with microvilli and lamellar bodies containing surfactant. Capillaries containing erythrocytes can be seen in the interstitial space (by courtesy of M. + H. Bachofen).

The alveolar epithelium usually exhibits extensive necrosis of alveolar type I cells, leaving a denuded, but mainly intact, basement membrane with overlying hyaline membranes. The type I alveolar epithelial cell is highly vulnerable to injury, whereas the alveolar type II cell is more resistant and can therefore function as a progenitor cell for regeneration of the alveolar epithelium after injury. The loss of the alveolar epithelium's integrity has several pathological and functional consequences: there is an influx of protein-rich oedema fluid into the air spaces, with deposition of hyaline membranes on the denuded basement membranes.

Hyperplastic alveolar type II cells typify the proliferative phase of ALI/ARDS. Alveolar type II cells migrate and begin to proliferate along the alveolar septa, in an attempt to cover the denuded basement membrane and re-establish the alveolar epithelium's continuity (Figure 1). Within the alveolar wall, fibroblasts proliferate and migrate through the basement membrane into the fibrinous intra-alveolar exudate. If the fibrinous exudates can be resolved, restoration of normal lung architecture may be achieved. However, if alveolar type II cells migrate over the surface of the organizing granulation tissue and thereby transform the intra-alveolar exudate into interstitial tissue, interstitial fibrosis of the lung may develop.

It is not known what factors determine whether pulmonary fibrosis or restoration of the normal pulmonary architecture occurs after ALI/ARDS. Efficient alveolar epithelial repair may reduce the development of fibrosis in animal models, since the presence of an intact alveolar epithelial layer suppresses fibroblast proliferation and matrix deposition [9]. Efficient restoration of the alveolar epithelium in the early phase of ALI/ARDS may therefore be a means not only of speeding recovery by enhancing alveolar liquid clearance, but also of preventing the development of pulmonary fibrosis [2].

Repair of the alveolar epithelium in acute lung injury

The epithelial repair process includes cell-cell interactions and interactions between the alveolar type II cell and the extracellular matrix which are coordinated by a variety of soluble mediators released into the alveolar space during ALI/ARDS. Many of them have been detected in elevated concentrations in bronchoalveolar lavage fluid from ALI/ARDS patients [10]. However, although bronchoalveolar lavage procedure is standardised, the dilution factor may differ from patient to patient, rendering direct comparisons between patient groups difficult. Recently, to minimise the risks involved in bronchoalveolar lavage in critically ill patients with ALI/ARDS, undiluted pulmonary oedema fluid was obtained by direct suction through a wedged endotracheal catheter [11]. This safe procedure allows repeated drawing of several mL of undiluted pulmonary oedema fluid from the distal air space which can be used for further evaluation in the laboratory. Cytokine concentrations are usually determined by means of enzyme-linked immunosorbent assays (ELISA) or radioimmunoassays. These assays determine the total immunoreactive content of the cytokine, without providing any information regarding its biological activity. Since naturally occurring specific inhibitors may also be increased in patients with ALI/ARDS [12], the net biological activity of a cytokine may well turn out to be less relevant than was first thought. It is therefore crucial to supplement immunoreactivity assays with standardised bioassays serving to determine the biological activity of clinical samples. We therefore further developed an *in vitro* alveolar epithelial wound repair assay that makes it possible to quantify the epithelial repair activity of pulmonary oedema fluid and plasma from patients with ALI/ARDS [4, 14]. As a control, pulmonary oedema fluid and plasma from ventilated patients with hydrostatic pulmonary oedema was used [4].

Pulmonary oedema fluid from patients with ALI/ARDS increases alveolar epithelial repair *in vitro* by an IL-1β-dependent mechanism

Because of the extensive damage to the alveolar epithelium in patients with ALI/ARDS, we initially formed the hypothesis that pulmonary oedema fluid inhibits alveolar epithelial repair *in vitro*. To test this, pulmonary oedema fluid or plasma was added to a mechanically wounded monolayer of alveolar epithelial cells and the rate of wound closure over time was determined by means of a digital imaging system connected to the microscope and an appropiate image analysis software (Figure 2). Surprisingly, alveolar epithelial repair activity induced by pulmonary oedema fluid from patients with ALI/ARDS was markedly increased compared to plasma obtained from the same patients or pulmonary oedema fluid from patients with hydrostatic oedema [4] (Figure 3). These results indicate that biologically active mediators capable of enhancing alveolar epithelial repair *in vitro* are released into the alveolar space in patients with ALI/ARDS. The epithelial repair process probably begins in the early phase of ALI/ARDS, since pulmonary oedema fluid obtained during the first 12 hours after intubation enhanced epithelial repair activity to a greater extent than pulmonary oedema fluid obtained more than 12 hours after intubation.

Since IL-1β was shown to be biologically active in pulmonary oedema fluid in early ALI/ARDS [13] and to be upregulated in skin wounds, it seemed likely that this early response cytokine mediates epithelial repair activity in pulmonary oedema fluid from patients with ALI/ARDS. Specific inhibition of IL-1β by IL-1 receptor antagonist (IL-1ra) significantly reduced alveolar epithelial repair *in vitro*, whereas blocking of TNF-α, another inflammatory early response cytokine, had no effect, indicating that IL-1β mediated a major fraction of the enhanced *in vitro*

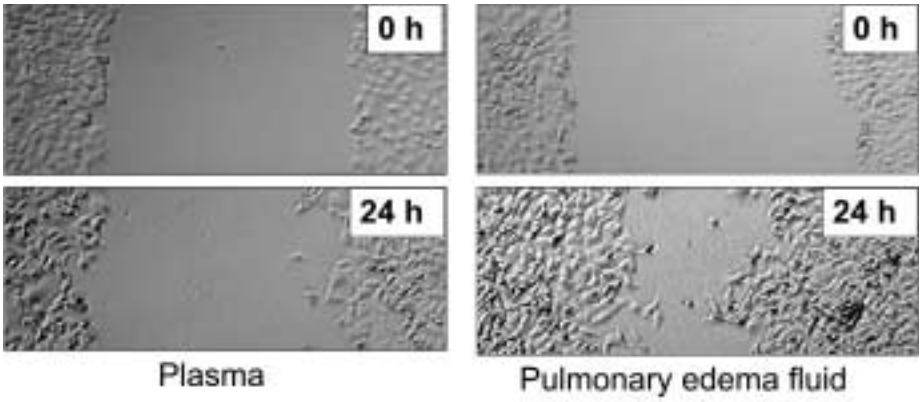


Figure 2

An *in vitro* alveolar epithelial wound repair model was established to determine the epithelial repair activity of pulmonary oedema fluid and plasma from patients with ALI/ARDS. Human alveolar epithelial-like cells were grown to a monolayer and after mechanical wounding the cells were incubated with pulmonary oedema fluid from patients. As an example, the effect on alveolar epithelial repair *in vitro* of plasma (left panels) and pulmonary oedema fluid (right panels) from a patient with ALI/ARDS is shown at 0 and 24 hours after incubation.

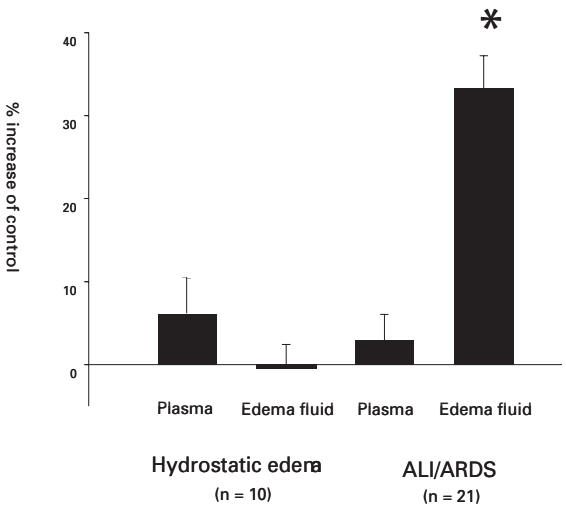


Figure 3

Effect of pulmonary oedema fluid and plasma from patients with ALI/ARDS or hydrostatic pulmonary oedema on alveolar epithelial repair *in vitro*. The epithelial repair activity of pulmonary oedema fluid or plasma was determined for each sample using an *in vitro* epithelial wound repair assay with human A549 alveolar epithelial cells and expressed as the percentage increase of alveolar epithelial repair compared to pooled plasma from healthy donors (control). Results are reported as mean ± SEM, *p <0.01 (compared to control). From [4].

alveolar epithelial repair. This conclusion was supported by experiments with recombinant IL-1β that showed a concentration-dependent increase in alveolar epithelial repair in our *in vitro* wound repair model using the human alveolar A549 epithelial cell line or freshly isolated rat alveolar type II epithelial cells. Concentrations of IL-1β showing significant alveolar epithelial repair *in vitro* were in a similar range to the concentrations of IL-1β found in pulmonary oedema fluid from patients with ALI/ARDS, indicating that IL-1β may contribute to repair of the alveolar epithelium in early ALI/ARDS.

The clinical outcome in the ALI/ARDS patients was analysed retrospectively and a there was a trend towards longer survival and shorter duration of mechanical ventilation in patients with increased alveolar epithelial wound repair activity induced by oedema fluid (L. Ware, unpublished observation). These data suggest that the alveolar epithelial repair activity of pulmonary oedema fluid *in vitro* may have prognostic value, although prospective studies of larger patient numbers are needed.

Several mechanisms play a major role in alveolar epithelial repair *in vivo* and *in vitro*. Proliferation of alveolar type II cells is the most obvious and easily measurable event in epithelial repair *in vivo*, but takes one or two days to be significant [2]. Other mechanisms may therefore contribute to early alveolar epithelial wound repair. *In vitro* studies in our alveolar epithelial wound repair model using primary alveolar type II epithelial cells isolated from rat lungs showed that cell spreading and migration are primarily responsible for efficient epithelial wound repair [3, 14]. Hence we may reasonably speculate that *in vivo* cell spreading and migration are the primary mechanisms during the early phase of alveolar epithelial repair, followed by cell proliferation leading to alveolar type II hyperplasia (Figure 1).

The EGF/TGF-α pathway is involved in IL-1β-induced alveolar epithelial repair

There is increasing evidence that epidermal growth factor (EGF), transforming growth factor-α TGF-α and their common receptor, epidermal growth factor receptor (EGFR), may regulate epithelial repair *in vivo* and *in vitro*. TGF-α is elevated in pulmonary oedema fluid from patients with ALI/ARDS and has been shown to induce alveolar epithelial repair *in vitro* [14]. We therefore formed the hypothesis that the EGF/TGF-α pathway may be involved in IL-1β-induced alveolar epithelial repair. Neutralising antibodies to EGF and TGF-α decreased IL-1β-induced alveolar epithelial repair and blocking of the EGFR or its intracellular signalling pathway by inhibitors of the mitogen-activated protein kinase (MAPK) pathway specifically inhibit the effect of IL-1β [3]. These data indicate that IL-1β enhances alveolar epithelial repair *in vitro* by activating the epithelial EGF/TGF-α pathway in an autocrine-paracrine fashion. The EGF/TGF-α pathway would therefore offer an attractive new therapeutic target and is being further studied in animal models with several types of lung injury. However, the potential value of TGF-α or EGF in patients with ALI/ARDS has not yet been evaluated.

Keratinocyte growth factor and hepatocyte growth factor

Recent animal experiments suggest that keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) may also play a significant role as new prophylactic or therapeutic agents in lung injury. KGF is chiefly produced by fibroblasts and has been shown to induce alveolar type II proliferation *in vitro* [15] and *in vivo* [16]. Studies in different lung injury models show a protective effect of KGF when given before inducing lung injury [17, 18]. Elevated levels of KGF were found to correlate with alveolar epithelial cell proliferation after bleomycin-induced lung injury in rats, suggesting that the elevated levels of KGF may induce efficient epithelial repair [19]. This hypothesis is supported by our *in vitro* epithelial wound healing studies showing that KGF increases alve-

olar epithelial repair [5]. Experiments using specific inhibitors of the EGF/TGF-α pathway suggest that the effects of KGF on alveolar epithelial repair are mediated, in part, by the EGFR pathway. These findings, coupled with our work with IL-1β and TGF-α, suggest that the EGFR may serve as a final common pathway in stimulating alveolar epithelial repair *in vitro*.

HGF is another potent mitogen for alveolar type II cells *in vivo* and *in vitro* which is upregulated by IL-1β and other inflammatory cytokines in lung fibroblasts [20]. HGF was found in elevated concentrations in pulmonary oedema fluid from patients with ALI/ARDS, signalling a potential role in alveolar epithelial repair [21]. Ongoing studies in our laboratory are focused on defining the role of HGF in alveolar epithelial repair *in vitro* and *in vivo*.

In conclusion, it must be emphasised that the alveolar epithelial repair process in the lung is very complex and modulated not only by several growth factors and cytokines, but also by other secreted products from inflammatory cells that are accumulated in the alveolar space during lung injury (e.g. proteases, reactive oxygen species) and by a variety of components of the extracellular matrix. One major goal is therefore to establish more sophisticated *in vitro* and *in vivo* models to improve our understanding of the mechanisms involved in the alveolar repair process. These experimental models will also enable us to test novel treatment modalities which seem promising in patients with ALI/ARDS.

Correspondence
PD Dr. Thomas Geiser
Div. of Pulmonary Medicine
Inselspital
CH-3010 Bern
E-Mail: Thomas.Geiser@insel.ch

References

1 Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334–49.
2 Berthiaume Y, Lesur O, Dagenais A. Treatment of adult respiratory distress syndrome: plea for rescue therapy of the alveolar epithelium. Thorax 1999;54:150–60.
3 Geiser T, Jarreau PH, Atabai K, Matthay MA. Interleukin-1β increases in vitro alveolar epithelial repair. Am J Physiol Lung Cell Mol Physiol 2000;279:L1184–90.
4 Geiser T, Atabai K, Jarreau PH, Ware LB, Pugin J, Matthay MA. Pulmonary oedema fluid from patients with acute lung injury augments alveolar epithelial repair by an interleukin-1β dependent mechanism. Am J Respir Crit Care Med 2001; 163:1384–8.
5 Atabai K, Ishigaki M, Geiser T, Ueki I, Matthay M, Ware LB. Keratinocyte growth factor can enhance alveolar epithelial repair by nonmitogenic mechanisms. Am J Physiol Lung Cell Mol Physiol 2002;283:L163–9.
6 Uhal, B. Cell cycle kinetics in the alveolar epithelium. Am J Physiol Lung Cell Mol Physiol 1997;272:L1031–L1045.
7 Matthay MA. Function of the alveolar epithelial barrier under pathologic conditions. Chest 1994;105:67S–74S.
8 Bachofen M, Weibel ER. Alterations of the gas exchange apparatus in adult respiratory insufficiency associated with septicemia. Am Rev Respir Dis 1977;116:589–615.
9 Adamson IY, Young L, Bowden DH. Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. Am J Pathol 1988;130:377–83.
10 Pittet JF, Mackersie RC, Martin TR, Matthay MA. Biological markers of acute lung injury: prognostic and pathogenetic significance. Am J Respir Crit Care Med 1997;155:1187–205.
11 Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar oedema in humans. Am Rev Resp Dis 1990;142:1250–7.
12 Martin T. Overview of cytokine networks in lung injury. Am J Respir Crit Care Med 1996;(Monograph):19–28.
13 Pugin J, Ricou B, Steinberg KP, Suter PM, Martin TR. Proinflammatory activity in bronchoalveolar lavage fluids from patients with ARDS, a prominent role for interleukin-1. Am J Respir Crit Care Med 1996;153:1850–6.
14 Kheradmand F, Folkesson HG, Shum L, Derynk R, Pytela R, Matthay MA. Transforming growth factor-α enhances alveolar epithelial cell repair in a new in vitro model. Am J Physiol Lung Cell Mol Physiol 1994;267:L728–38.
15 Panos RJ, Rubin JS, Aaronson SA, Mason RJ. Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin-binding growth factors for alveolar type II cells in fibroblast conditioned medium. J Clin Invest 1993;92:969–77.
16 Ulich TR, Yi ES, Longmuir K, Yin S, Biltz R, Morris CF, et al. Keratinocyte growth factor is a growth factor for type II pneumocytes in vivo. J Clin Invest 1994;93:1298–306.
17 Barazzone C, Donati YR, Rochat, AF, Vesin C, Kan CD, Pache JC, et al. Keratinocyte growth factor protects the alveolar epithelium and endothelium from oxygen-induced injury in mice. Am J Pathol 1999;154:1479–87.
18 Deterding RR, Havill AM, Toshiyuki Y, Middleton SC, Jacoby CR, Shannon JM, et al. Prevention of bleomycin-induced lung injury in rats by keratinocyte growth factor. Proc Assoc Am Phys 1997;109:254–68.
19 Adamson IY, Bakowska J. Relationship of keratinocyte growth factor and hepatocyte growth factor levels in rat lung lavage fluid to epithelial cell regeneration after bleomycin. Am J Pathol 1999;155:949–54.
20 Ohmichi H, Matsumoto K, Nakamura T. In vivo mitogenic action of HGF on lung epithelial cells: pulmotrophic role in lung regeneration. Am J Physiol Lung Cell Mol Physiol 1996;270: L1031–9.
21 Verghese GM, McCormick-Shannon K, Mason RJ, Matthay MA. Hepatocyte growth factor and keratinocyte growth factor in the pulmonary oedema fluid of patients with acute lung injury. Biologic and clinical significance. Am J Respir Crit Care Medicine 1998;158:386–94.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

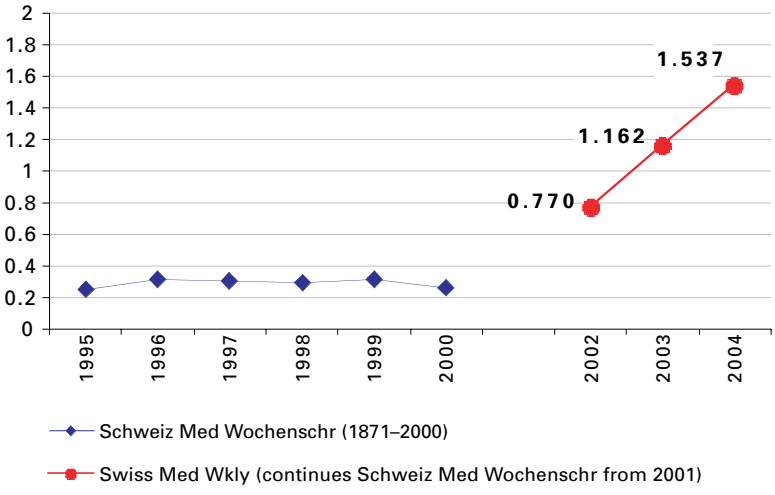
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch