Antiviral Research 99 (2013) 113–118

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Commentary

The lung microvascular endothelium as a therapeutic target in severe influenza

Susan M. Armstrong [a,d], Samira Mubareka [b], Warren L. Lee [a,c,d,*]

[a] Institute of Medical Science, University of Toronto, Canada
[b] Sunnybrook Research Institute, Toronto, Canada
[c] Division of Respirology and Interdepartmental Division of Critical Care Medicine, University of Toronto, Canada
[d] Keenan Research Centre of The Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Canada

ARTICLE INFO

Article history:
Received 27 March 2013
Revised 3 May 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
Influenza
Vascular stability
Acute lung injury
Endothelial permeability
Vascular leak

ABSTRACT

Severe infections with influenza virus are characterized by acute respiratory distress syndrome (ARDS), a life-threatening disorder in which the alveolocapillary membrane in the lung becomes leaky. This leads to alveolar flooding, hypoxemia and respiratory failure. Recent data suggest that influenza virus can exert both direct and indirect effects on the lung endothelium, activating it and inducing microvascular leak. These findings raise the possibility that enhancing lung endothelial barrier integrity or modulating lung endothelial activation may prove therapeutically useful for severe influenza. In this paper, we review evidence that lung endothelial activation and vascular leak are a "final common pathway" in severe influenza, as has been reported in bacterial sepsis, and that enhancing endothelial barrier function may improve the outcome of illness. We describe a number of experimental therapies that have shown promise in preventing or reversing increased vascular leak in animal models of sepsis or influenza.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Most cases of influenza are mild and self-limited, but a small percentage require hospitalization; infants, the elderly and persons with underlying chronic illness are especially vulnerable to fatal illness (Thompson et al., 2003, 2004; Lee et al., 2010). The development of severe influenza reflects a combination of pathologic processes, including the spread of viral infection from the upper to the lower respiratory tract, bacterial superinfection of injured mucosal surfaces and the effect of host inflammatory responses on pulmonary function. Attempts to treat severe influenza by blocking viral replication are hindered by the few available antivirals, the increasing prevalence of drug-resistant variants and the limited efficacy of antiviral therapy once extensive pulmonary injury has occurred. New treatments are therefore needed to complement antiviral therapy by targeting deleterious host responses.

The inflammatory response to infection is defined primarily by altered vascular function, in which the endothelial barrier opens to permit the passage of immune cells, antibody and complement molecules and other substances from the bloodstream into the tissues. In the lungs, this standard response to injury results in the shift of fluid into alveolar spaces, which in the case of severe infection may lead to progressive respiratory compromise (acute respiratory distress syndrome, ARDS). Therapies that counteract the adverse effects of pulmonary inflammation on endothelial function might therefore be of significant clinical benefit. Although the concept of targeting host responses has long been accepted in the field of sepsis (Brun-Buisson et al., 1995; Beutler, 1993), it has only recently found favor in influenza research (Steinberg et al., 2012). In this paper, we review evidence that lung endothelial activation and vascular leak is a "final common pathway" in severe influenza, as it is in bacterial sepsis (Goldenberg et al., 2011; Lee and Slutsky, 2010), and that enhancing endothelial barrier function may improve the outcome of illness. We describe a number of experimental therapies that have shown promise in preventing or reversing increased vascular leak in animal models of sepsis or influenza (see Table 1). We also note recent experimental data suggesting that influenza virus can spread from the respiratory epithelium to infect adjacent endothelial cells, providing a further rationale for novel therapies targeting the pulmonary vasculature.

2. Mechanisms of increased endothelial permeability

How influenza virus infection causes ARDS is poorly understood. The virus infects the bronchial epithelium, leading to epithelial injury, apoptosis and frank desquamation (Kuiken and Taubenberger, 2008). In uncomplicated infections, these changes to the airway epithelium are transient and the process of repair

* Corresponding author. Address: 30 Bond Street, LKS Room 613, Toronto, ON, Canada M5B 1W8. Tel.: +1 416 864 6060 77655.
E-mail address: warren.lee@utoronto.ca (W.L. Lee).

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.05.003

is evident within days. However, in primary viral pneumonia, influenza virus spreads from the upper to the lower respiratory tract (Mauad et al., 2010), infecting the distal lung, particularly type I pneumocytes and ciliated bronchiolar epithelium. This leads to damage to the alveoli including frank alveolar denudement (Kuiken and Taubenberger, 2008). Type II pneumocytes and alveolar macrophages can also be infected. In the 1957 influenza pandemic, primary viral pneumonia accounted for about 20% of deaths (Kuiken and Taubenberger, 2008), with the remainder attributed to bacterial superinfection and pneumonia. More recent data indicate that the predominant cause of death in the influenza A virus (H1N1pdm09) pandemic was ARDS (Mauad et al., 2010), either alone or in association with bacterial superinfection (Gill et al., 2010). In seasonal influenza outbreaks, 10–15% of deaths are directly attributable to virus-induced pneumonia and respiratory failure (Schanzer et al., 2007; Thompson et al., 2003). However, the mechanism of ARDS in virus-induced pneumonia is not clear, since lung epithelial apoptosis alone is not sufficient to induce leak of the lung alveolocapillary membrane (Mura et al., 2010).

Influenza virus is known to interfere with alveolar fluid clearance, by interfering with the function of the epithelial sodium channel (ENaC), which regulates fluid absorption from the alveolar space (Chen et al., 2004; Kunzelmann et al., 2000; Lazrak et al., 2009; Wolk et al., 2008). Indeed, a decrease in alveolar fluid clearance correlates with poor outcomes in patients with ARDS (Ware and Matthay, 2001). These effects would diminish or delay the resolution of pulmonary edema, and may also contribute to its formation (Lee et al., 2007). However, attempts to increase alveolar fluid clearance pharmacologically as a treatment for ARDS have proved disappointing (Gao Smith et al., 2012). Furthermore, the effect of the virus on the *other* half of the alveolocapillary membrane, the lung endothelium, remains largely unexplored. Indeed, a loss of lung endothelial barrier function is a major determinant of the formation of pulmonary edema in ARDS (Maniatis and Orfanos, 2008).

The average thickness of the alveolocapillary membrane is just over 1 μm, including the alveolar epithelium, the thin interstitium and the lung microvascular endothelium (Weibel and Knight, 1964). In some regions, the barrier is as thin as 100–200 nm. Hence the epithelium and endothelium are in very close proximity, making an interaction between the virus and the endothelium plausible. Infection of the alveolar epithelium leads to cell death and the release of new virions (Kuiken and Taubenberger, 2008; Mori et al., 1995), exposing the endothelium to viral particles and to epithelial and leukocyte paracrine factors (Fig. 1).

There are multiple overlapping mechanisms by which influenza virus could induce increased lung endothelial permeability. One of the main contributors is pro-inflammatory cytokines produced by leukocytes, lung epithelium and the lung endothelium. For instance, infections with both H5N1 avian influenza virus (Schmolke et al., 2009) and influenza A (H1N1pdm09) virus (Bermejo-Martin et al., 2009) have been associated with markedly elevated circulating cytokines, including TNFα and IL-6, which are well known to cause increased endothelial permeability (Ferro et al., 2000; Maruo et al., 1992). While leukocytes have traditionally been considered the major source of pro-inflammatory cytokines, Teijaro et al. have recently implicated lung endothelial cells as being key regulators of the process, if not the actual source (Teijaro et al., 2011). Their data suggest that a small-molecule agonist of the S1P (sphingosine-1-phosphate) receptor (subtype 1) is sufficient to protect against lethal influenza, largely by reducing pro-inflammatory cytokine production. The agent also inhibited the recruitment of neutrophils and macrophages/monocytes to the lung. Remarkably, impairment of leukocyte recruitment did not account for the blunted cytokine storm, suggesting that endothelial cells may have been the source of the cytokines. Although S1P itself is known to reduce endothelial permeability (Garcia et al., 2001), the authors did not assess vascular leakage in the lung making it unclear what role (if any) barrier enhancement played in the benefit from the drug. Nonetheless, the results make a compelling case that both the lung endothelium and cytokines are critical to the pathogenesis of severe influenza.

The recruitment of leukocytes to the lung has also been postulated to be involved in ARDS after influenza. Neutrophils in particular have been implicated in causing lung endothelial damage, through the generation of neutrophil extracellular traps (Narasaraju et al., 2011), the secretion of elastase (Lee and Downey,

Table 1
Selected pharmacologic agents that have been evaluated for their ability to block experimental vascular leak in laboratory animals.

Agent	Therapeutic target	Animal models of vascular leak tested	Reference
Slit2N	Activates Robo4; causes retention of VE-cadherin at cell–cell junctions; may affect leukocytes	LPS-induced acute lung injury in C57BL/6 mice; CLP in C57BL/6 mice; H5N1 infection in BALB/c mice	London et al. (2010)
Doxycycline	Induction of VE-cadherin expression and decrease in VE-cadherin phosphorylation	IL-2 associated pulmonary edema in C57Bl/6J mice; tumour vascular permeability in C57Bl/6J mice; DTH reactions in C57Bl/6J mice; H3N2 infection of BALB/c mice	Fainaru et al. (2008)Ng et al. (2012)
Vasculotide	Tie2 receptor agonist; reduces VE-cadherin loss and cytoskeletal rearrangement; may affect leukocyte recruitment and cytokine levels	CLP sepsis in C57Bl/6 mice; LPS-induced lung injury in C57Bl/6 mice	David et al. (2011), Kumpers et al. (2011)
Activated protein C	Effect on permeability mediated through the EPCR and PAR1 receptors; may decrease endothelial cell apoptosis	Endotoxemia (LPS given parenterally) in C57Bl/6, $EPCR^{6/6}$, and $PAR1^{-/-}$ mice; sepsis models (IP infection by *S. aureus* or *E. coli*, and colon ascendens stent implantation)	Kerschen et al. (2007)
Atrial naturetic peptide (ANP)	Acts on atrial naturetic peptide receptor (NPR)-A; strengthens cortical actin through activation of Rac and inhibition of Rho; inhibits changes in VE-cadherin	Lung injury induced by *Staphylococcus aureus*-derived peptidoglycan and/or lipoteichoic acid in C57Bl/6 and $NPPA^{-/-}$ (ANP knockout) mice; LPS-induced lung injury in C57Bl/6 and $NPPA^{-/-}$ mice; histamine-induced vascular permeability in the cremaster muscle of C57Bl/6 mice	Birukova et al. (2010), Xing et al. (2011)
Sphingosine-1-phosphate (S1P)	Binds to endothelial differentiation gene (Edg) family of receptors; thickens cortical actin through activation of Rac; reduces pro-inflammatory cytokine production	Lung injury in the context of acute necrotizing pancreatitis in Wistar rats; ischemia–reperfusion injury in the kidneys of C57Bl/6 mice; LPS-induced acute lung injury in C57Bl/6 mice; lung injury caused by high tidal volume mechanical ventilation in C57Bl/6 mice and beagle dogs	Awad et al. (2006), Liu et al. (2008), McVerry et al. (2004), Peng et al. (2004)

Abbreviations: LPS, lipopolysaccharide; CLP, cecal ligation and perforation.

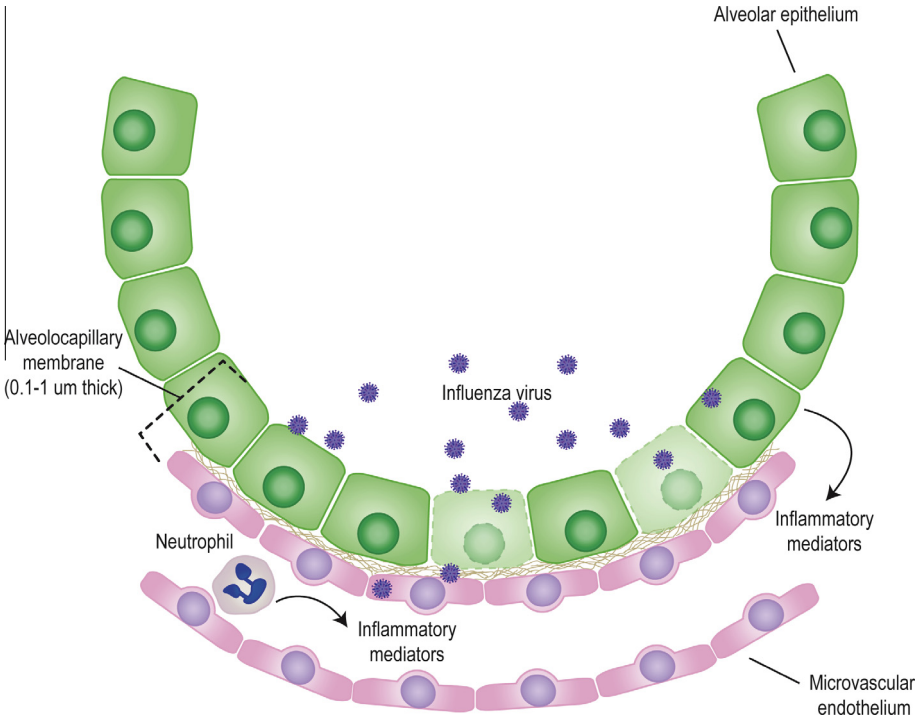

Fig. 1. The alveolar epithelium and microvascular endothelium are in very close proxiity. Together with the interstitium, they form the alveolocapillary membrane, which ranges in diameter from 100 nm to just over 1 μm. When influenza virus infects the alveolar epithelium, it replicates and induces apoptosis, releasing new virions that could infect the endothelium. Infected epithelial cells also release inflammatory mediators, which could induce endothelial leak. Influenza virus infection is associated with leukocyte infiltration into the lung; neutrophils in particular release cytokines, reactive oxygen species, elastase and nucleic acids (e.g., neutrophil extracellular traps), which may all contribute to endothelial barrier disruption.

2001a) and the generation of reactive oxygen species (Lee and Downey, 2001b). In contrast, macrophages appear to be protective against influenza (Cao et al., 2012), since macrophage-depleted mice displayed worsened lung injury and increased neutrophil accumulation in the lung (Narasaraju et al., 2011).

After infection with H5N1 avian influenza virus, endothelial cell activation and barrier dysfunction may be related to the transcription factor NF-κB (Schmolke et al., 2009). Schmolke et al. found that the H5N1 avian influenza virus induces many genes in the endothelium, most of which are downstream of NF-κB. This transcription factor is of particular interest because of elegant work by others showing that *endothelial cell-specific* blockade of NF-κB activation reduces lung edema, neutrophil infiltration, and mortality after *E. coli* bacteremia or after cecal ligation and perforation (Xu et al., 2010; Ye et al., 2008). This benefit was independent of bacterial clearance. While the mechanism of increased vascular permeability in response to endothelial NF-κB activation has not been elucidated, many of the downstream genes, such as cytokines, are pro-inflammatory and can induce leak. Elegant work suggests that excessive endothelial activation leads to endothelial apoptosis in association with decreased survival from sepsis (Minami et al., 2009). An effect of the influenza virus infection on lung endothelial NF-κB could therefore lead to loss of the endothelial barrier. Recent research also suggests that cytokines can induce endothelial leak independently of NF-κB (Zhu et al., 2012) and it will be interesting to determine if this pathway is involved in influenza virus infection.

Last, but not least, endothelial barrier dysfunction could result from direct cytopathic effects of the virus. Human endothelial cells are known to express α2,6-linked sialic acid residues, the receptor for human influenza virus (Abe et al., 1999; Yao et al., 2008); expression increases when endothelial cells are stimulated with cytokines, as might occur in serious infections (Hanasaki et al., 1994). In fact, influenza virus infection of the lung endothelium has been observed *in vitro* and causes endothelial cell death (Armstrong et al., 2012), cytokine production (Visseren et al., 1999; Wang et al., 2010) as well as a decrease in the expression of endothelial cell junctional proteins (Armstrong et al., 2012; Wang et al., 2010) that lead to increased permeability. While the respiratory epithelium is the primary target of influenza virus, the proximity of the lung microvascular endothelium to the alveolar epithelium, combined with the damage that the virus causes to the epithelium, provides a plausible route through which it could come into contact with the endothelium. However, the relative contribution of direct endothelial infection to the pathogenesis of severe influenza is unknown.

In summary, loss of the endothelial barrier through both indirect (paracrine) and direct (cytopathic) mechanisms may contribute to pathology from influenza.

3. Is blocking endothelial leak a viable therapeutic strategy in severe influenza?

Although almost all patients with severe influenza are treated with an antiviral drug, treatment is only partially effective at reducing mortality (McGeer et al., 2007). This supports the notion that the severity of illness and patient outcomes are dependent on factors related to the host rather than just the infectious agent (Marcelin et al., 2011). Whether lung endothelial activation and barrier dysfunction contribute to the morbidity and mortality of severe influenza should be directly tested, as it would have marked implications for therapy.

While numerous putative endothelial barrier-enhancing agents exist (Goldenberg et al., 2011) (see Table 1), much of the work is based on cell culture experiments and must be repeated in animals. The most widely used animal model of severe influenza is

C57BL/6 mice, which are inoculated with murine-adapted influenza viruses by the droplet or aerosol route. The phenotype in affected animals is characterized by weight loss and lethargy. Lung pathology is notable for infiltration by neutrophils and macrophages and the development of peribronchial inflammation, alveolar damage, hemorrhage and edema (Narasaraju et al., 2011), and is more pronounced for H5N1 and the 1918 H1N1 influenza virus (Perrone et al., 2008). Although this model is widely accepted, a recent provocative study has called into question the accuracy of mouse models of human inflammatory diseases (Seok et al., 2013). In addition to mice, ferrets have long been utilized to study influenza pathogenesis, vaccination, and antiviral agents. Their utility has been limited by the lack of reagents, but recent advances including the characterization of the host response to the virus (Leon et al., 2013) and sequencing of the ferret genome (Broad-Institute, 2013) will likely broaden the use of this model by the influenza community.

Very few endothelial barrier-enhancing compounds have been tested in animal models of influenza, in part because the importance of the vascular barrier has only recently been appreciated, but also because endothelial-specific reagents are few in number. Slit2N was shown *in vitro* to enhance the retention of VE-cadherin at endothelial cell–cell junctions and, when given intravenously to mice at the time of H5N1 virus infection, improved survival and reduced lung inflammation without altering lung cytokine levels or viral titers (London et al., 2010) (see Fig. 2, reprinted with permission). While promising, it remains unclear if Slit's benefit is mediated mostly through endothelial barrier-enhancement or through effects on leukocytes (Ye et al., 2010). Another putative barrier-enhancing compound is the antibiotic doxycycline. Doxycycline has been reported to decrease vascular leak by inducing expression of VE-cadherin, the major constituent of intercellular adherens junctions (Fainaru et al., 2008). Interestingly, it was also recently found to decrease lung injury after H3N2 influenza virus (Ng et al., 2012). In that study, doxycycline was administered orally and had no effect on flu-induced weight loss, but lung permeability and leukocyte recruitment were significantly lower in the treated group. The authors did not examine VE-cadherin levels and attributed the benefit to inhibition of matrix metalloproteases, which may contribute to alveolocapillary barrier dysfunction. It is also unfortunate that no effect of doxycycline on survival was reported in that study and that viral titers were paradoxically highest in the animals receiving doxycycline.

There are other barrier-enhancing agents which have not yet been tested in the context of influenza virus infection. Vasculotide is a synthetic Tie2 agonist that has been shown to reduce the loss of VE-cadherin and the cytoskeleton rearrangement that occurs *in vitro* to endothelial cells exposed to endotoxin. Tie2 is the cell-

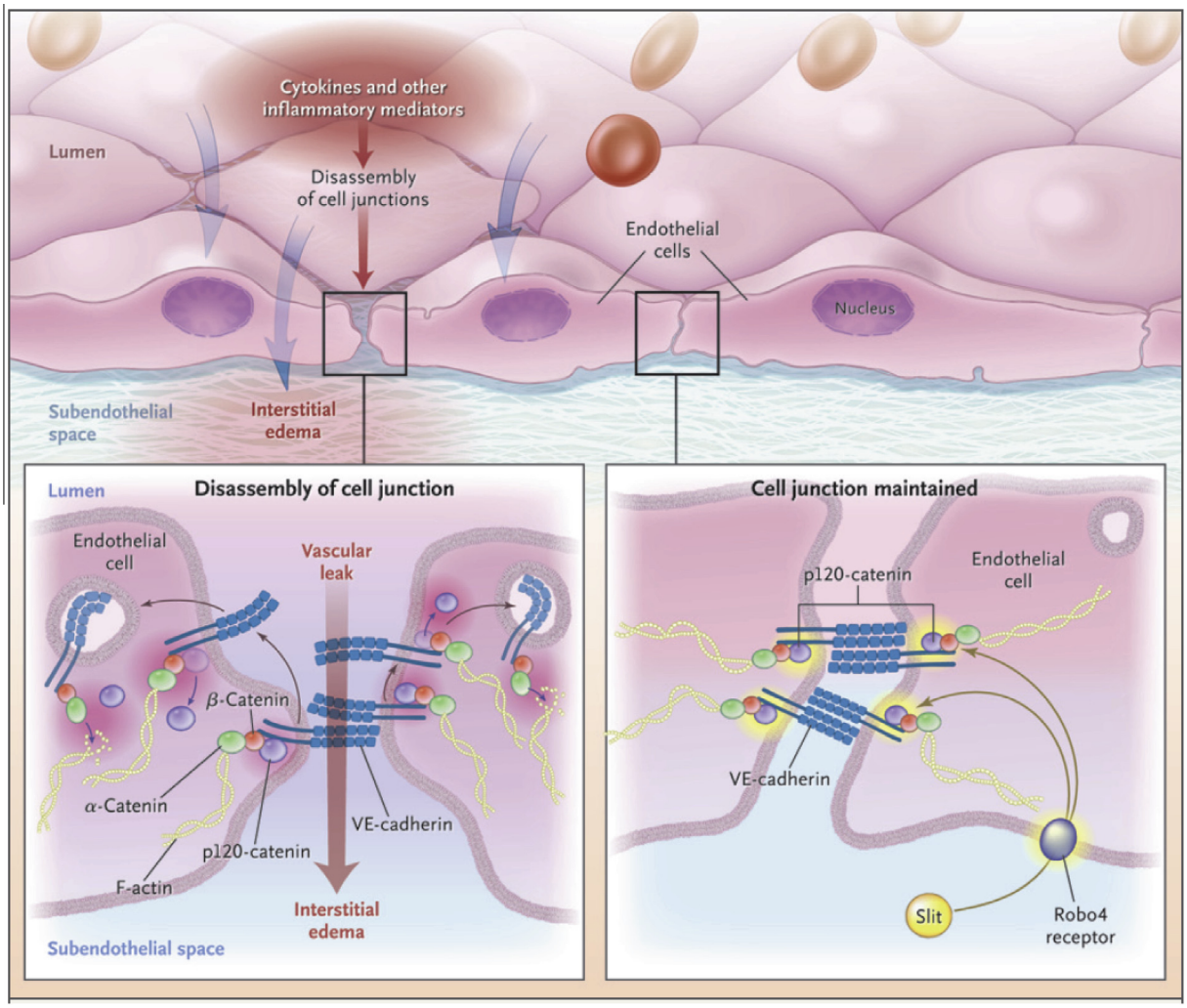

Fig. 2. Cytokines and other inflammatory mediators cause disassembly of endothelial cell–cell junctions. This results in leakage of vascular fluid into the interstitial space, producing edema. Mechanisms of junctional disassembly include internalization of VE-cadherin and rearrangement of the actin cytoskeleton. One barrier-enhancing agent, Slit2N, acts via the Robo4 receptor to foster retention of VE-cadherin at the cell membrane, thereby sparing the cell–cell junction from disruption. From Lee and Slutsky (2010), Copyright© (2010) Massachusetts Medical Society. Reprinted with permission.

surface receptor for angiopoietins, hormones that regulate endothelial permeability and activation (Wong et al., 1997). Intriguingly, Vasculotide improved survival and reduced lung microvascular leak, both in mice injected with endotoxin and in mice subjected to cecal ligation and perforation (David et al., 2011; Kumpers et al., 2011). Although these findings are from models of sepsis, it is worth noting that endotoxemia is essentially a model of systemic hypercytokinemia (Wang et al., 1999), similar to that which has been postulated to occur in severe H1N1 and H5N1 influenza (London et al., 2010). In collaboration with Dr. Dan Dumont, one of our labs (WLL) is currently studying whether Vasculotide is effective in a murine model of severe influenza.

Lastly, activated protein C, which had been approved for use in sepsis, was recently withdrawn from the market for lack of efficacy. One of its major adverse effects was bleeding. Intriguingly, however, modified protein C derivatives have been generated that possess little effect on coagulation, but are still capable of reducing vascular leak, lung inflammation and mortality after endotoxemia and cecal ligation and perforation (Kerschen et al., 2007). Whether these non-anticoagulant versions of activated protein C would be beneficial in severe influenza is unknown. Other barrier-enhancing agents, such as atrial naturetic peptide (ANP) and S1P, have been tested in animals in various models of vascular leak (Awad et al., 2006; Birukova et al., 2010; Furst et al., 2008; Liu et al., 2008; McVerry et al., 2004; Peng et al., 2004; Xing et al., 2011). However, neither has yet been tested in the context of influenza.

A final point is that, to reflect clinical practice, barrier-enhancing agents should ideally be administered to animals in combination with antiviral drugs. This approach is appealing, because antiviral drugs alone only partially reduce human mortality, and enhancing the lung endothelial barrier is likely to be more effective when viral replication has been halted. A potential experiment could involve administering a suboptimal dose of an antiviral drug, which when given alone permits the development of clinical illness (Nguyen et al., 2012); the effect of adding a barrier-enhancing agent such as Vasculotide on outcomes such as weight loss, pulmonary edema and acute lung injury could then be assessed. Objective scoring criteria for acute lung injury have been described (Matute-Bello et al., 2001) and could be used in a blinded study to quantify changes in lung histopathology. In all such experiments, it will be important to exclude any direct effects of barrier-enhancing agents on viral replication and leukocyte function (e.g. chemotaxis, cytokine production), which might confound the results.

4. Conclusions

A number of lines of evidence suggest that the lung endothelium plays a critical role in the pathogenesis of severe influenza. Loss of endothelial barrier integrity, combined with impaired alveolar fluid clearance, leads to alveolar flooding and hypoxemia, while the state of endothelial activation regulates diverse processes including cytokine production, leukocyte recruitment and cellular apoptosis. Investigators should consider testing whether enhancement of the endothelial barrier or modulation of endothelial activation can improve the outcome of severe influenza. Barrier-enhancing agents could be tested alone or in combination with antiviral drugs. Importantly, agents that reduce lung vascular leak should theoretically be less susceptible to the development of viral drug resistance, as they target the host, not the pathogen.

Acknowledgements

Research in Dr. Lee's lab is supported by the Institute of Circulatory and Respiratory Health of the Canadian Institutes of Health Research, the Physicians' Services Incorporated Foundation and an Early Researcher Award from the Government of Ontario. Susan M. Armstrong receives funding from the Canadian Institutes of Health Research and the McLaughlin Centre for Molecular Medicine.

References

Abe, Y., Smith, C.W., Katkin, J.P., Thurmon, L.M., Xu, X., Mendoza, L.H., Ballantyne, C.M., 1999. Endothelial alpha 2,6-linked sialic acid inhibits VCAM-1-dependent adhesion under flow conditions. J. Immunol. 163, 2867–2876.

Armstrong, S.M., Wang, C., Tigdi, J., Si, X., Dumpit, C., Charles, S., Gamage, A., Moraes, T.J., Lee, W.L., 2012. Influenza infects lung microvascular endothelium leading to microvascular leak: role of apoptosis and claudin-5. PLoS One 7, e47323.

Awad, A.S., Ye, H., Huang, L., Li, L., Foss Jr, F.W., Macdonald, T.L., Lynch, K.R., Okusa, M.D., 2006. Selective sphingosine 1-phosphate 1 receptor activation reduces ischemia-reperfusion injury in mouse kidney. Am. J. Physiol. Renal. Physiol. 290, F1516–1524.

Bermejo-Martin, J.F., Ortiz de Lejarazu, R., Pumarola, T., Rello, J., Almansa, R., Ramirez, P., Martin-Loeches, I., Varillas, D., Gallegos, M.C., Seron, C., Micheloud, D., Gomez, J.M., Tenorio-Abreu, A., Ramos, M.J., Molina, M.L., Huidobro, S., Sanchez, E., Gordon, M., Fernandez, V., Del Castillo, A., Marcos, M.A., Villanueva, B., Lopez, C.J., Rodriguez-Dominguez, M., Galan, J.C., Canton, R., Lietor, A., Rojo, S., Eiros, J.M., Hinojosa, C., Gonzalez, I., Torner, N., Banner, D., Leon, A., Cuesta, P., Rowe, T., Kelvin, D.J., 2009. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. Crit. Care 13, R201.

Beutler, B., 1993. Endotoxin, tumor necrosis factor, and related mediators: new approaches to shock. New Horiz. 1, 3–12.

Birukova, A.A., Xing, J., Fu, P., Yakubov, B., Dubrovskyi, O., Fortune, J.A., Klibanov, A.M., Birukov, K.G., 2010. Atrial natriuretic peptide attenuates LPS-induced lung vascular leak: role of PAK1. Am. J. Physiol. Lung Cell. Mol. Physiol. 299, L652–L663 (Broad-Institute, 2013).

Brun-Buisson, C., Doyon, F., Carlet, J., Dellamonica, P., Gouin, F., Lepoutre, A., Mercier, J.C., Offenstadt, G., Regnier, B., 1995. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU group for severe Sepsis. Jama 274, 968–974.

Cao, W., Taylor, A.K., Biber, R.E., Davis, W.G., Kim, J.H., Reber, A.J., Chirkova, T., De La Cruz, J.A., Pandey, A., Ranjan, P., Katz, J.M., Gangappa, S., Sambhara, S., 2012. Rapid differentiation of monocytes into type I IFN-producing myeloid dendritic cells as an antiviral strategy against influenza virus infection. J. Immunol. 189, 2257–2265.

Chen, X.J., Seth, S., Yue, G., Kamat, P., Compans, R.W., Guidot, D., Brown, L.A., Eaton, D.C., Jain, L., 2004. Influenza virus inhibits ENaC and lung fluid clearance. Am. J. Physiol. Lung Cell. Mol. Physiol. 287, L366–L373.

David, S., Ghosh, C.C., Kumpers, P., Shushakova, N., Van Slyke, P., Khankin, E.V., Karumanchi, S.A., Dumont, D., Parikh, S.M., 2011. Effects of a synthetic PEGylated Tie-2 agonist peptide on endotoxemic lung injury and mortality. Am. J. Physiol. Lung Cell. Mol. Physiol. 300, L851–L862.

Fainaru, O., Adini, I., Benny, O., Bazinet, L., Pravda, E., D'Amato, R., Folkman, J., 2008. Doxycycline induces membrane expression of VE-cadherin on endothelial cells and prevents vascular hyperpermeability. FASEB J. 22, 3728–3735.

Ferro, T., Neumann, P., Gertzberg, N., Clements, R., Johnson, A., 2000. Protein kinase C-alpha mediates endothelial barrier dysfunction induced by TNF-alpha. Am. J. Physiol. Lung Cell. Mol. Physiol. 278, L1107–L1117.

Furst, R., Bubik, M.F., Bihari, P., Mayer, B.A., Khandoga, A.G., Hoffmann, F., Rehberg, M., Krombach, F., Zahler, S., Vollmar, A.M., 2008. Atrial natriuretic peptide protects against histamine-induced endothelial barrier dysfunction in vivo. Mol. Pharmacol. 74, 1–8.

Gao Smith, F., Perkins, G.D., Gates, S., Young, D., McAuley, D.F., Tunnicliffe, W., Khan, Z., Lamb, S.E., 2012. Effect of intravenous beta-2 agonist treatment on clinical outcomes in acute respiratory distress syndrome (BALTI-2): a multicentre, randomised controlled trial. Lancet 379, 229–235.

Garcia, J.G., Liu, F., Verin, A.D., Birukova, A., Dechert, M.A., Gerthoffer, W.T., Bamberg, J.R., English, D., 2001. Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. J. Clin. Invest. 108, 689–701.

Gill, J.R., Sheng, Z.M., Ely, S.F., Guinee, D.G., Beasley, M.B., Suh, J., Deshpande, C., Mollura, D.J., Morens, D.M., Bray, M., Travis, W.D., Taubenberger, J.K., 2010. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch. Pathol. Lab. Med. 134, 235–243.

Goldenberg, N.M., Steinberg, B.E., Slutsky, A.S., Lee, W.L., 2011. Broken barriers: a new take on sepsis pathogenesis. Sci. Transl. Med. 3, 88ps25.

Hanasaki, K., Varki, A., Stamenkovic, I., Bevilacqua, M.P., 1994. Cytokine-induced beta-galactoside alpha-2,6-sialyltransferase in human endothelial cells mediates alpha 2,6-sialylation of adhesion molecules and CD22 ligands. J. Biol. Chem. 269, 10637–10643.

Kerschen, E.J., Fernandez, J.A., Cooley, B.C., Yang, X.V., Sood, R., Mosnier, L.O., Castellino, F.J., Mackman, N., Griffin, J.H., Weiler, H., 2007. Endotoxemia and sepsis mortality reduction by non-anticoagulant activated protein C. J. Exp. Med. 204, 2439–2448.

Kuiken, T., Taubenberger, J.K., 2008. Pathology of human influenza revisited. Vaccine 26 (Suppl. 4), D59–D66.

Kumpers, P., Gueler, F., David, S., Slyke, P.V., Dumont, D.J., Park, J.K., Bockmeyer, C.L., Parikh, S.M., Pavenstadt, H., Haller, H., Shushakova, N., 2011. The synthetic tie2

agonist peptide vasculotide protects against vascular leakage and reduces mortality in murine abdominal sepsis. Crit. Care 15, R261.
Kunzelmann, K., Beesley, A.H., King, N.J., Karupiah, G., Young, J.A., Cook, D.I., 2000. Influenza virus inhibits amiloride-sensitive Na^+ channels in respiratory epithelia. Proc. Natl. Acad. Sci. USA 97, 10282–10287.
Lazrak, A., Iles, K.E., Liu, G., Noah, D.L., Noah, J.W., Matalon, S., 2009. Influenza virus M2 protein inhibits epithelial sodium channels by increasing reactive oxygen species. FASEB J. 23, 3829–3842.
Lee, W.L., Downey, G.P., 2001a. Leukocyte elastase: physiological functions and role in acute lung injury. Am. J. Respir. Crit. Care Med. 164, 896–904.
Lee, W.L., Downey, G.P., 2001b. Neutrophil activation and acute lung injury. Curr. Opin. Crit. Care 7, 1–7.
Lee, W.L., Slutsky, A.S., 2010. Sepsis and endothelial permeability. N. Engl. J. Med. 363, 689–691.
Lee, J.W., Fang, X., Dolganov, G., Fremont, R.D., Bastarache, J.A., Ware, L.B., Matthay, M.A., 2007. Acute lung injury edema fluid decreases net fluid transport across human alveolar epithelial type II cells. J. Biol. Chem. 282, 24109–24119.
Lee, N., Choi, K.W., Chan, P.K., Hui, D.S., Lui, G.C., Wong, B.C., Wong, R.Y., Sin, W.Y., Hui, W.M., Ngai, K.L., Cockram, C.S., Lai, R.W., Sung, J.J., 2010. Outcomes of adults hospitalised with severe influenza. Thorax 65, 510–515.
Leon, A.J., Banner, D., Xu, L., Ran, L., Peng, Z., Yi, K., Chen, C., Xu, F., Huang, J., Zhao, Z., Lin, Z., Huang, S.H., Fang, Y., Kelvin, A.A., Ross, T.M., Farooqui, A., Kelvin, D.J., 2013. Sequencing, annotation, and characterization of the influenza ferret infectome. J. Virol. 87, 1957–1966.
Liu, H.B., Cui, N.Q., Wang, Q., Li, D.H., Xue, X.P., 2008. Sphingosine-1-phosphate and its analogue FTY720 diminish acute pulmonary injury in rats with acute necrotizing pancreatitis. Pancreas 36, e10–15.
London, N.R., Zhu, W., Bozza, F.A., Smith, M.C., Greif, D.M., Sorensen, L.K., Chen, L., Kaminoh, Y., Chan, A.C., Passi, S.F., Day, C.W., Barnard, D.L., Zimmerman, G.A., Krasnow, M.A., Li, D.Y., 2010. Targeting Robo4-dependent Slit signaling to survive the cytokine storm in sepsis and influenza. Sci. Transl. Med. 2, 23ra19.
Maniatis, N.A., Orfanos, S.E., 2008. The endothelium in acute lung injury/acute respiratory distress syndrome. Curr. Opin. Crit. Care 14, 22–30.
Marcelin, G., Aldridge, J.R., Duan, S., Ghoneim, H.E., Rehg, J., Marjuki, H., Boon, A.C., McCullers, J.A., Webby, R.J., 2011. Fatal outcome of pandemic H1N1 2009 influenza virus infection is associated with immunopathology and impaired lung repair, not enhanced viral burden, in pregnant mice. J. Virol. 85, 11208–11219.
Maruo, N., Morita, I., Shirao, M., Murota, S., 1992. IL-6 increases endothelial permeability in vitro. Endocrinology 131, 710–714.
Matute-Bello, G., Frevert, C.W., Kajikawa, O., Skerrett, S.J., Goodman, R.B., Park, D.R., Martin, T.R., 2001. Septic shock and acute lung injury in rabbits with peritonitis: failure of the neutrophil response to localized infection. Am. J. Respir. Crit. Care Med. 163, 234–243.
Mauad, T., Hajjar, L.A., Callegari, G.D., da Silva, L.F., Schout, D., Galas, F.R., Alves, V.A., Malheiros, D.M., Auler Jr, J.O., Ferreira, A.F., Borsato, M.R., Bezerra, S.M., Gutierrez, P.S., Caldini, E.T., Pasqualucci, C.A., Dolhnikoff, M., Saldiva, P.H., 2010. Lung pathology in fatal novel human influenza A (H1N1) infection. Am. J. Respir. Crit. Care Med. 181, 72–79.
McGeer, A., Green, K.A., Plevneshi, A., Shigayeva, A., Siddiqi, N., Raboud, J., Low, D.E., 2007. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. Clin. Infect. Dis. 45, 1568–1575.
McVerry, B.J., Peng, X., Hassoun, P.M., Sammani, S., Simon, B.A., Garcia, J.G., 2004. Sphingosine 1-phosphate reduces vascular leak in murine and canine models of acute lung injury. Am. J. Respir. Crit. Care Med. 170, 987–993.
Minami, T., Yano, K., Miura, M., Kobayashi, M., Suehiro, J., Reid, P.C., Hamakubo, T., Ryeom, S., Aird, W.C., Kodama, T., 2009. The down syndrome critical region gene 1 short variant promoters direct vascular bed-specific gene expression during inflammation in mice. J. Clin. Invest. 119, 2257–2270.
Mori, I., Komatsu, T., Takeuchi, K., Nakakuki, K., Sudo, M., Kimura, Y., 1995. In vivo induction of apoptosis by influenza virus. J. Gen. Virol. 76 (Pt 11), 2869–2873.
Mura, M., Binnie, M., Han, B., Li, C., Andrade, C.F., Shiozaki, A., Zhang, Y., Ferrara, N., Hwang, D., Waddell, T.K., Keshavjee, S., Liu, M., 2010. Functions of type II pneumocyte-derived vascular endothelial growth factor in alveolar structure, acute inflammation, and vascular permeability. Am. J. Pathol. 176, 1725–1734.
Narasaraju, T., Yang, E., Samy, R.P., Ng, H.H., Poh, W.P., Liew, A.A., Phoon, M.C., van Rooijen, N., Chow, V.T., 2011. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am. J. Pathol. 179, 199–210.
Ng, H.H., Narasaraju, T., Phoon, M.C., Sim, M.K., Seet, J.E., Chow, V.T., 2012. Doxycycline treatment attenuates acute lung injury in mice infected with virulent influenza H3N2 virus: involvement of matrix metalloproteinases. Exp. Mol. Pathol. 92, 287–295.
Nguyen, J.T., Smee, D.F., Barnard, D.L., Julander, J.G., Gross, M., de Jong, M.D., Went, G.T., 2012. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. PLoS One 7, e31006.
Peng, X., Hassoun, P.M., Sammani, S., McVerry, B.J., Burne, M.J., Rabb, H., Pearse, D., Tuder, R.M., Garcia, J.G., 2004. Protective effects of sphingosine 1-phosphate in murine endotoxin-induced inflammatory lung injury. Am. J. Respir. Crit. Care Med. 169, 1245–1251.
Perrone, L.A., Plowden, J.K., Garcia-Sastre, A., Katz, J.M., Tumpey, T.M., 2008. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. PLoS Pathog. 4, e1000115.
Schanzer, D.L., Tam, T.W., Langley, J.M., Winchester, B.T., 2007. Influenza-attributable deaths, Canada 1990–1999. Epidemiol. Infect. 135, 1109–1116.
Schmolke, M., Viemann, D., Roth, J., Ludwig, S., 2009. Essential impact of NF-kappaB signaling on the H5N1 influenza A virus-induced transcriptome. J. Immunol. 183, 5180–5189.
Seok, J., Warren, H.S., Cuenca, A.G., Mindrinos, M.N., Baker, H.V., Xu, W., Richards, D.R., McDonald-Smith, G.P., Gao, H., Hennessy, L., Finnerty, C.C., Lopez, C.M., Honari, S., Moore, E.E., Minei, J.P., Cuschieri, J., Bankey, P.E., Johnson, J.L., Sperry, J., Nathens, A.B., Billiar, T.R., West, M.A., Jeschke, M.G., Klein, M.B., Gamelli, R.L., Gibran, N.S., Brownstein, B.H., Miller-Graziano, C., Calvano, S.E., Mason, P.H., Cobb, J.P., Rahme, L.G., Lowry, S.F., Maier, R.V., Moldawer, L.L., Herndon, D.N., Davis, R.W., Xiao, W., Tompkins, R.G., 2013. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc. Natl. Acad. Sci. USA.
Steinberg, B.E., Goldenberg, N.M., Lee, W.L., 2012. Do viral infections mimic bacterial sepsis? The role of microvascular permeability: a review of mechanisms and methods. Antiviral Res. 93, 2–15.
Teijaro, J.R., Walsh, K.B., Cahalan, S., Fremgen, D.M., Roberts, E., Scott, F., Martinborough, E., Peach, R., Oldstone, M.B., Rosen, H., 2011. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell 146, 980–991.
Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Cox, N., Anderson, L.J., Fukuda, K., 2003. Mortality associated with influenza and respiratory syncytial virus in the United States. Jama 289, 179–186.
Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Bridges, C.B., Cox, N.J., Fukuda, K., 2004. Influenza-associated hospitalizations in the United States. Jama 292, 1333–1340.
Visseren, F.L., Verkerk, M.S., Bouter, K.P., Diepersloot, R.J., Erkelens, D.W., 1999. Interleukin-6 production by endothelial cells after infection with influenza virus and cytomegalovirus. J. Lab. Clin. Med. 134, 623–630.
Wang, H., Bloom, O., Zhang, M., Vishnubhakat, J.M., Ombrellino, M., Che, J., Frazier, A., Yang, H., Ivanova, S., Borovikova, L., Manogue, K.R., Faist, E., Abraham, E., Andersson, J., Andersson, U., Molina, P.E., Abumrad, N.N., Sama, A., Tracey, K.J., 1999. HMG-1 as a late mediator of endotoxin lethality in mice. Science 285, 248–251.
Wang, S., Le, T.Q., Kurihara, N., Chida, J., Cisse, Y., Yano, M., Kido, H., 2010. Influenza virus-cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza. J. Infect. Dis. 202, 991–1001.
Ware, L.B., Matthay, M.A., 2001. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 163, 1376–1383.
Weibel, E.R., Knight, B.W., 1964. A morphometric study on the thickness of the pulmonary air-blood barrier. J. Cell Biol. 21, 367–396.
Wolk, K.E., Lazarowski, E.R., Traylor, Z.P., Yu, E.N., Jewell, N.A., Durbin, R.K., Durbin, J.E., Davis, I.C., 2008. Influenza A virus inhibits alveolar fluid clearance in BALB/c mice. Am. J. Respir. Crit. Care Med. 178, 969–976.
Wong, A.L., Haroon, Z.A., Werner, S., Dewhirst, M.W., Greenberg, C.S., Peters, K.G., 1997. Tie2 expression and phosphorylation in angiogenic and quiescent adult tissues. Circ. Res. 81, 567–574.
Xing, J., Moldobaeva, N., Birukova, A.A., 2011. Atrial natriuretic peptide protects against Staphylococcus aureus-induced lung injury and endothelial barrier dysfunction. J. Appl. Physiol. 110, 213–224.
Xu, H., Ye, X., Steinberg, H., Liu, S.F., 2010. Selective blockade of endothelial NF-kappaB pathway differentially affects systemic inflammation and multiple organ dysfunction and injury in septic mice. J. Pathol. 220, 490–498.
Yao, L., Korteweg, C., Hsueh, W., Gu, J., 2008. Avian influenza receptor expression in H5N1-infected and noninfected human tissues. FASEB J. 22, 733–740.
Ye, X., Ding, J., Zhou, X., Chen, G., Liu, S.F., 2008. Divergent roles of endothelial NF-kappaB in multiple organ injury and bacterial clearance in mouse models of sepsis. J. Exp. Med. 205, 1303–1315.
Ye, B.Q., Geng, Z.H., Ma, L., Geng, J.G., 2010. Slit2 regulates attractive eosinophil and repulsive neutrophil chemotaxis through differential srGAP1 expression during lung inflammation. J. Immunol. 185, 6294–6305.
Zhu, W., London, N.R., Gibson, C.C., Davis, C.T., Tong, Z., Sorensen, L.K., Shi, D.S., Guo, J., Smith, M.C., Grossmann, A.H., Thomas, K.R., Li, D.Y., 2012. Interleukin receptor activates a MYD88-ARNO-ARF6 cascade to disrupt vascular stability. Nature 492, 252–255.