Published in final edited form as:

Vascul Pharmacol. 2010 ; 52(5-6): 171–174. doi:10.1016/j.vph.2010.02.002.

New Insights Into Acute Lung Injury

Stephen M Black

Program in Pulmonary Disease, Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912, USA

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) were first identified over 40 years ago by Ashbaugh *et al* [1]. ALI and ARDS are forms of progressive respiratory failure characterized by an acute onset of dyspnea, decreased arterial oxygen pressure (hypoxemia), bilateral infiltrates on chest radiograms, and absence of clinical evidence of primary left heart failure. The American-European Consensus Conference on ARDS has led to a definition of ALI as a $\text{PaO}_2/\text{FiO}_2 < 300$ while ARDS is defined as < 200 for ARDS [2,3]. Thus, ARDS represents a subset of ALI patients with greater severity of symptoms. In ALI/ARDS, the integrity of the separation between the alveolus and the pulmonary circulation is compromised either by endothelial or epithelial injury or more commonly both. This damage leads to increased vascular permeability, alveolar flooding, and surfactant abnormalities (due to damage of type II pneumocytes). ALI/ARDS can occur in response to a number of insults that either directly or indirectly cause lung injury. The most common indirect insult leading to ALI is the release of lipopolysaccharide (LPS) from the outer cell wall of Gram-negative (G^-) bacteria producing sepsis [4]. Other common causes include severe trauma with shock, multiple transfusions, burn injury, pneumonia and aspiration of gastric contents. Based on a recent announcement from the National Institute of Health (NIH), ALI and its more severe form, acute respiratory distress syndromes (ARDS) affect approximately 150,000 people in the United States every year. Nearly 28,500 are likely to die. Some survivors recover completely. However, others may have lasting damage to their lungs and additional health problems. Sepsis represents the systemic inflammatory response to infection [5]. Severe sepsis is defined as sepsis complicated by organ dysfunction and hypotension (septic shock). Lungs are among the most frequently affected organs in severe sepsis leading to ALI and ARDS [6]. The incidence of sepsis has increased by 8.7% from 1979-2000 [6] and mortality ranges from 30-50% [7-9]. Clinical trials targeting inflammatory mediators have shown no survival benefit [10-15] and other strategies have failed to reduce morbidity associated with severe sepsis except for the survival benefit that has been identified with low tidal volume mechanical ventilation [16] and the use of recombinant activated protein C [17]. With an unacceptably high mortality rate up to 58% [18] it is evident that a clearer understanding of both the mechanisms involved in the pathogenesis of ALI/ARDS and the development of new therapies for the control of the disease are critical. Thus, this special issue has brought together five studies that are highlight the development of new reagents for the study of the mechanisms involved in ALI, elucidate new signaling pathways that may be involved in the pathophysiology of ALI, as well as evaluating potential new therapeutic agents to prevent the endothelial barrier disruption which is a hallmark of ALI. In this editorial I will briefly describe the major findings

Please address correspondence and proofs to: Stephen M. Black, Ph.D., Vascular Biology Center, 1459 Laney Walker Blvd, CB3201B, Medical College of Georgia, Augusta, GA 30912, sblack@mcg.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of each of the studies and attempt to highlight both the strengths and limitations of these individual studies and the field of ALI as a whole.

The initial site affected during the development of ALI/ARDS is the endothelial cell (EC) layer lining the micro-vessels in the lung. The vascular endothelium is a single-cell layer that acts as a semi-selective barrier between the plasma and interstitial fluid. This function is critical for normal vessel wall homeostasis. Endothelial permeability is regulated by the balance between the contractile machinery within the cell and the elements that oppose contraction. The latter include tethering complexes that are responsible for cell-cell and cell-substrate contacts and systems granting cell rigidity that prevent cell collapse, such as actin filaments, microtubules and intermediate filaments [19,20]. One of the major limitations in the study of ALI/ARDS is the lack of a reproducible cell culture model that can be used to investigate how EC that line the micro-vessels in the pulmonary system are disrupted by ALI/ARDS. However, a significant number of published studies utilize pulmonary EC of either bovine or human origin but are isolated from the major vessels such as the pulmonary artery. These vessels are not normally affected by ALI/ARDS. Further, the commercially available EC isolated from human micro-vessels do not appear to maintain the archetypal properties of EC namely their cobblestone appearance. Further, the currently available EC lines require large quantities of agents such as LPS (~100 endotoxin units (EU)/ml) for measureable barrier disruption to occur. However, Catravas *et al* in this issue describe a new method for harvesting and culturing of human lung microvascular endothelial cells (HLMVEC). Further, they present convincing data demonstrating the identity of these cells and their response to appropriate stimuli. These HLMVEC appear to be of superior quality to other available cells exhibiting small size, characteristic cobblestone appearance, and a contact-inhibited monolayer. Further, these characteristics are maintained over multiple passages. The HLMVEC exhibited a tight monolayer with excellent transendothelial resistance (TER) (~1000M Ω) measured using an Applied Biosystems ECIS instrument. Most excitingly, freshly harvested HLMVEC demonstrated excellent sensitivity to a variety of barrier disrupting agents. For example as little as 1 EU/ml elicited a profound decrease in TER. These cells are likely to become the “gold-standard” for investigations into the mechanisms underlying the endothelial barrier disruption in ALI/ARDS.

Although extensive investigations have been carried out to delineate the mechanisms underlying the development of ALI/ARDS little of therapeutic value has resulted from these efforts. Thus, new signaling pathways may need to be elucidated and tested for their interventional potential. Thus, the remaining four studies in this series have begun to evaluate new mechanisms of endothelial protection in ALI/ARDS. In the first of these studies Sharma and colleagues have examined the role of protein nitration in the pathophysiology of ALI/ARDS. The nitration of tyrosine residues is mediated by reactive nitrogen species (RNS). Increased RNS production occurs when dysregulated nitric oxide (NO) production reacts with reactive oxygen species (ROS) such as superoxide. This reaction generates RNS, including peroxynitrite (ONOO⁻). ONOO⁻ leads to tyrosine nitration, a covalent modification that adds a nitro group (-NO₂) to one ortho carbon of the phenolic ring of tyrosine to form 3-nitrotyrosine (3-NT). This introduces a net negative charge to the tyrosine, altering structural properties and catalytic activity of the protein. There has been increasing interest in the effects of tyrosine nitration on changes in protein structure in diverse pathologic conditions. The nitration of tyrosine residues to form 3-NT residues is widely used as a marker of ONOO⁻ formation and Sharma *et al* have demonstrated that elevated levels of protein tyrosine nitration precedes the endothelial injury associated with the development of ALI in the LPS injected mouse. This increase in ONOO⁻ appears to be related to eNOS uncoupling mediated via an increase in the levels of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA). Further, the increases in ADMA occur secondary to a post-translational inhibition of the enzyme that degrades ADMA, dimethylarginine dimethylaminohydrolase (DDAH). Inhibition of DDAH

without alteration of its gene expression is becoming more widely appreciated as Lin and colleagues have shown that elevated glucose raises endothelial ADMA levels by inhibiting DDAH activity via a mechanism involving oxidative stress [21]. Similarly, LPS significantly increases the levels of ADMA and decreased DDAH activity in cultured medium from human endothelial cells [22]. Of great clinical interest is a recent study indicating that ADMA levels are elevated in patients with septic shock [23] suggesting that the ADMA-DAH pathway could be responsive to therapeutic intervention. Further, the studies of Church *et al* investigating the effect of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) signaling on eNOS activity are intriguing with respect to DDAH activity. PTEN is a lipid phosphatase that functions as a negative regulator of the phosphoinositide-3-kinase (PI3K) pathway. Although most studies on PTEN have focused on its role in cancer progression where it is found in the advanced stages of a number of cancers [24] it is now becoming apparent that PTEN can also modulate the blood vessel structure and function [25-27]. In addition, some recent studies have shown a role for PTEN in the progression of ALI as both the pharmacological inhibition of PTEN and its conditional ablation in the epithelia of the lung have been shown to reduce the severity of ALI [28,29]. The data indicating that enhancing PTEN signaling decreases NO generation from eNOS through the inhibition of Akt-mediated phosphorylation correlates well with the decrease in eNOS-derived NO that Sharma *et al* have shown to be an early event after I.P. LPS in the mouse lung. Although Church *et al* have focused on changes in serine phosphorylation to explain the decreases in NO induced by PTEN over-expression in COS-7 cells, the potential of PTEN to inhibit DDAH activity is an intriguing but unexplored possibility.

The Sharma study also highlights the limitations of current investigations: the lack of easily available means to identify post translational modifications of proteins. This is a major roadblock to our understanding of the potential mechanistic contributions of these modifications to disease processes. New methodologies are needed that go beyond the limitations of current analytical approaches that focus on measuring either global changes in protein nitration (as in the Sharma study) or merely identifying individual proteins that are susceptible to nitration. Rather priority should be placed on moving towards identifying both the individual tyrosine residues targeted by nitration and the effect these nitration events have on the structure-function relationship of the nitrated protein.

Recently, attention has been given to the therapeutic potential of purinergic agonists in the treatment of cardiovascular and pulmonary diseases [30-32] and the study in this issue from Umapathy *et al* has evaluated the barrier protective properties of adenosine *in vitro* using cultured human PAEC. Extracellular purines can function as intercellular signaling molecules when released from different sources in the body [33] and accumulating experimental data suggest that purines could be barrier-protective agents against the effects of ALI, as they are present in the EC microenvironment *in vivo* and they decrease permeability *in vitro*. The dominant pathway modulating the levels of extracellular adenosine is the extracellular catabolism of ATP to adenosine through the progressive action of a number of ectonucleotidases [34,35]. In this study Umapathy *et al* clearly demonstrate that the addition of adenosine in physiologically relevant concentrations (1-5 μ M) significantly increases the TER of cultured human PAEC. Further, using an elegant siRNA strategy they confirm that the mechanism of action is mediated via A_{2A} receptors and a cAMP-dependent signaling pathway that produces changes in F-actin via the activation of myosin light chain phosphatase (MLCP). This report also emphasizes an important limitation in our current knowledge of the role of phosphatases in the pathophysiology of ALI. This is primarily due to the inherent technical difficulties in studying the action of protein phosphatases. MLCP is a type 1 Ser/Thr PPase and the holoenzyme is composed of 3 subunits: a catalytic subunit (CS1) of 38 kD that was identified as CS1 δ isoform (currently CS1 β) and two non-catalytic subunits of 20-21 and 110-130 kD [36-39]. The 110-130 kD non-catalytic subunit, called myosin PPase targeting subunit

1 (MYPT1), binds to CS1 and this targets CS1 to MLC and provides substrate specificity [40,41]. Human MYPT1 and its splice variants are encoded by a single gene on human chromosome 12q15-q21.2 [42]. It is well established that MLC is the major substrate for MLCP. However, recent data have revealed that MYPT1 can also bind directly to the ezrin/radixin/moesin (ERM) family of actin-binding proteins [43,44]. ERM proteins act as linkers between the actin cytoskeleton and plasma membrane proteins, and as signal transducers in responses involving cytoskeletal remodeling [45] as Umapathy *et al* have shown with adenosine. Established a role of the ERMs in the barrier protective effect of adenosine could yield novel potential therapeutic targets for ALI. However, it should be noted that a major limitation of the Umapathy study is the fact that there was no *in vivo* confirmation of the barrier enhancement or protective effects *in vivo*. Hopefully these important confirmatory studies will be soon forthcoming.

One of the major limitations in our understanding of ALI/ARDS is that much of the work is carried out using LPS as a model of G^- sepsis and studies on G^+ infections are less common in the literature. Thus, the final study from Xiong *et al* is potentially very powerful and timely in that it has identified a new signaling mechanism and potential therapy for the treatment of G^+ associated bacterial infections using the G^+ pore forming toxin, Listeriolysin-O (LLO) isolated from *Listeria monocytogenes*, a bacterium that causes a severe food-borne disease in neonates characterized by meningitis and meningo-encephalitis. The data presented indicate that the oxidative stress associated with LLO is mediated via the activation of PKC α and that its pharmacologic inhibition using GÖ6976, attenuates the LLO-mediated decreases in TER in the HLMVEC isolated by Catravas *et al*. Further, and of significant therapeutic interest, the authors have shown that the lectin like domain of TNF-the TIP peptide- prevents the LLO-mediated increase in oxidative stress, at least in part, by preventing the up-regulation of Nox-4. However, the mechanism by which the TIP peptide exerts its barrier protective effect has been only partially elucidated as the TIP peptide prevented the LLO-mediated activation of PKC α but did not prevent the influx of intracellular calcium known to play a key role its activation. Similar to the report by Umapathy *et al.*, this study was also *in vitro* and did not evaluate the barrier protective effects of the TIP peptide *in vivo* in LLO challenged mice. Again it is hoped that these studies will be forthcoming soon.

In conclusion, this special issue focuses on the role of the endothelium in ALI/ARDS and highlights the complexity of the disease process and the problems with developing single therapies targeted at alleviating the significant morbidity and mortality associated with ALI/ARDS. Several new signaling pathways that could have therapeutic potential (DDAH, PTEN, PKC α) are investigated in human microvascular endothelial cells that are isolated from human pulmonary microvessels and are significantly more relevant to this etiology of this disease. Finally, new agents are tested for their ability to prevent endothelial barrier disruption *in vitro* (adenosine, TIP peptide) and *in vivo* (peroxynitrite scavengers) against agents derived from both G^- and G^+ bacteria. It is hoped that as these studies progress they will begin to elucidate mechanistic similarities and differences between the effects of G^- and G^+ infections and that these mechanisms will provide a pathway to new and efficacious therapeutic strategies that integrates multiple approaches.

Acknowledgments

This work was supported in part by grants HL60190 (to SMB), HL67841, HL72123, HL70061, HL084739, and R21HD057406 all from the National Institutes of Health, by a Transatlantic Network Development Grant from the *Fondation Leducq*, and by a Programmatic Development award from the from the Cardiovascular Discovery Institute of the Medical College of Georgia.

Literature Cited

1. Ashbaugh DG, B D, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet* 1967;2(7511): 319–323. [PubMed: 4143721]
2. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;149(3 Pt 1):818–824. [PubMed: 7509706]
3. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, LeGall JR, Morris A, Spragg R. Report of the American-European Consensus conference on acute respiratory distress syndrome: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Consensus Committee. *J Crit Care* 1994;9(1):72–81. [PubMed: 8199655]
4. Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;151(2 Pt 1):293–301. [PubMed: 7842182]
5. Jacobi J. Pathophysiology of sepsis. *Am J Health Syst Pharm* 2002;59:S3–8. [PubMed: 11885412]
6. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003;348(16):1546–1554. [PubMed: 12700374]
7. Angus DC, Birmingham MC, Balk RA, Scannon PJ, Collins D, Kruse JA, Graham DR, Dedhia HV, Homann S, MacIntyre N. E5 murine monoclonal antiendotoxin antibody in gram-negative sepsis: a randomized controlled trial. E5 Study Investigators *Jama* 2000;283(13):1723–1730.
8. Annane D, Sebille V, Troche G, Raphael JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *Jama* 2000;283(8):1038–1045. [PubMed: 10697064]
9. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study *Jama* 1995;273(2):117–123.
10. Abraham E, Anzueto A, Gutierrez G, Tessler S, San Pedro G, Wunderink R, Dal Nogare A, Nasraway S, Berman S, Cooney R, Levy H, Baughman R, Rumbak M, Light RB, Poole L, Allred R, Constant J, Pennington J, Porter S. Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. *Lancet* 1998;351(9107):929–933. [PubMed: 9734938]
11. Abraham E, Reinhart K, Svoboda P, Seibert A, Olthoff D, Dal Nogare A, Postier R, Hempelmann G, Butler T, Martin E, Zwingelstein C, Percell S, Shu V, Leighton A, Creasey AA. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit Care Med* 2001;29(11): 2081–2089. [PubMed: 11700399]
12. Dhainaut JF, Tenaillon A, Hemmer M, Damas P, Le Tulzo Y, Radermacher P, Schaller MD, Sollet JP, Wolff M, Holzappel L, Zeni F, Vedrinne JM, de Vathaire F, Gourlay ML, Guinot P, Mira JP. Confirmatory platelet-activating factor receptor antagonist trial in patients with severe gram-negative bacterial sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. BN 52021 Sepsis Investigator Group. *Crit Care Med* 1998;26(12):1963–1971. [PubMed: 9875905]
13. Fink MP. Therapeutic options directed against platelet activating factor, eicosanoids and bradykinin in sepsis. *J Antimicrob Chemother* 1998;41:81–94. [PubMed: 9511090]
14. Fisher CJ Jr, Opal SM, Lowry SF, Sadoff JC, LaBrecque JF, Donovan HC, Lookabaugh JL, Lemke J, Pribble JP, Stromatt SC, et al. Role of interleukin-1 and the therapeutic potential of interleukin-1 receptor antagonist in sepsis. *Circ Shock* 1994;44(1):1–8. [PubMed: 7704933]
15. McCloskey RV, Straube RC, Sanders C, Smith SM, Smith CR. Treatment of septic shock with human monoclonal antibody HA-1A. A randomized, double-blind, placebo-controlled trial. CHESST Trial Study Group. *Ann Intern Med* 1994;121(1):1–5. [PubMed: 8198341]
16. Jain R, DalNogare A. Pharmacological therapy for acute respiratory distress syndrome. *Mayo Clin Proc* 2006;81(2):205–212. [PubMed: 16471076]
17. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ Jr. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344(10):699–709. [PubMed: 11236773]

18. MacCallum NS, Evans TW. Epidemiology of acute lung injury. *Curr Opin Crit Care* 2005;11(1):43–49. [PubMed: 15659944]
19. Dudek SM, Birukov KG, Zhan X, Garcia JG. Novel interaction of cortactin with endothelial cell myosin light chain kinase. *Biochem Biophys Res Commun* 2002;298(4):511–519. [PubMed: 12408982]
20. Birukov KG, Birukova AA, Dudek SM, Verin AD, Crow MT, Zhan X, DePaola N, Garcia JG. Shear stress-mediated cytoskeletal remodeling and cortactin translocation in pulmonary endothelial cells. *Am J Respir Cell Mol Biol* 2002;26(4):453–464. [PubMed: 11919082]
21. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 2002;106(8):987–992. [PubMed: 12186805]
22. Xin HY, Jiang DJ, Jia SJ, Song K, Wang GP, Li YJ, Chen FP. Regulation by DDAH/ADMA pathway of lipopolysaccharide-induced tissue factor expression in endothelial cells. *Thromb Haemost* 2007;97(5):830–838. [PubMed: 17479195]
23. O'Dwyer MJ, Dempsey F, Crowley V, Kelleher DP, McManus R, Ryan T. Septic shock is correlated with asymmetrical dimethyl arginine levels, which may be influenced by a polymorphism in the dimethylarginine dimethylaminohydrolase II gene: a prospective observational study. *Crit Care* 2006;10(5):R139. [PubMed: 17002794]
24. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;275(5308):1943–1947. [PubMed: 9072974]
25. Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, Bodurtha J, Crowe C, Curtis MA, Dasouki M, Dunn T, Feit H, Geraghty MT, Graham JM Jr, Hodgson SV, Hunter A, Korf BR, Manchester D, Miesfeldt S, Murday VA, Nathanson KL, Parisi M, Pober B, Romano C, Eng C, et al. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum Mol Genet* 1999;8(8):1461–1472. [PubMed: 10400993]
26. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL. Modulation of hypoxia-inducible factor 1 α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 2000;60(6):1541–1545. [PubMed: 10749120]
27. Tsigkos S, Zhou Z, Kotanidou A, Fulton D, Zakynthinos S, Roussos C, Papapetropoulos A. Regulation of Ang2 release by PTEN/PI3-kinase/Akt in lung microvascular endothelial cells. *J Cell Physiol* 2006;207(2):506–511. [PubMed: 16447257]
28. Lai JP, Bao S, Davis IC, Knoell DL. Inhibition of the phosphatase PTEN protects mice against oleic acid-induced acute lung injury. *Br J Pharmacol* 2009;156(1):189–200. [PubMed: 19134000]
29. Tiozzo C, De Langhe S, Yu M, Londhe VA, Carraro G, Li M, Li C, Xing Y, Anderson S, Borok Z, Bellusci S, Minoo P. Deletion of Pten Expands Lung Epithelial Progenitor Pools and Confers Resistance to Airway Injury. *Am J Respir Crit Care Med*. 2009
30. Burnstock G. Purinergic receptors as future targets for treatment of functional GI disorders. *Gut* 2008;57(9):1193–1194. [PubMed: 18719131]
31. Raju NC, Eikelboom JW, Hirsh J. Platelet ADP-receptor antagonists for cardiovascular disease: past, present and future. *Nat Clin Pract Cardiovasc Med* 2008;5(12):766–780. [PubMed: 18957959]
32. Smyth SS, Woulfe DS, Weitz JI, Gachet C, Conley PB, Goodman SG, Roe MT, Kuliopulos A, Moliterno DJ, French PA, Steinhubl SR, Becker RC. G-protein-coupled receptors as signaling targets for antiplatelet therapy. *Arterioscler Thromb Vasc Biol* 2009;29(4):449–457. [PubMed: 19023091]
33. Burnstock G, Williams M. P2 purinergic receptors: modulation of cell function and therapeutic potential. *J Pharmacol Exp Ther* 2000;295(3):862–869. [PubMed: 11082418]
34. Eltzschig HK, Thompson LF, Karhausen J, Cotta RJ, Ibla JC, Robson SC, Colgan SP. Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood* 2004;104(13):3986–3992. [PubMed: 15319286]

35. Thomson S, Bao D, Deng A, Vallon V. Adenosine formed by 5'-nucleotidase mediates tubuloglomerular feedback. *J Clin Invest* 2000;106(2):289–298. [PubMed: 10903345]
36. Alessi D, MacDougall LK, Sola MM, Ikebe M, Cohen P. The control of protein phosphatase-1 by targeting subunits. The major myosin phosphatase in avian smooth muscle is a novel form of protein phosphatase-1. *European journal of biochemistry / FEBS* 1992;210(3):1023–1035. [PubMed: 1336455]
37. Shimizu H, Ito M, Miyahara M, Ichikawa K, Okubo S, Konishi T, Naka M, Tanaka T, Hirano K, Hartshorne DJ, et al. Characterization of the myosin-binding subunit of smooth muscle myosin phosphatase. *The Journal of biological chemistry* 1994;269(48):30407–30411. [PubMed: 7982954]
38. Shirazi A, Iizuka K, Fadden P, Mosse C, Somlyo AP, Somlyo AV, Haystead TA. Purification and characterization of the mammalian myosin light chain phosphatase holoenzyme. The differential effects of the holoenzyme and its subunits on smooth muscle. *The Journal of biological chemistry* 1994;269(50):31598–31606. [PubMed: 7989330]
39. Hartshorne DJ, Ito M, Erdodi F. Myosin light chain phosphatase: subunit composition, interactions and regulation. *Journal of muscle research and cell motility* 1998;19(4):325–341. [PubMed: 9635276]
40. Hubbard MJ, Cohen P. On target with a new mechanism for the regulation of protein phosphorylation. *Trends in biochemical sciences* 1993;18(5):172–177. [PubMed: 8392229]
41. Johnson D, Cohen P, Chen MX, Chen YH, Cohen PT. Identification of the regions on the M110 subunit of protein phosphatase 1M that interact with the M21 subunit and with myosin. *European journal of biochemistry / FEBS* 1997;244(3):931–939. [PubMed: 9108268]
42. Takahashi N, Ito M, Tanaka J, Nakano T, Kaibuchi K, Odai H, Takemura K. Localization of the gene coding for myosin phosphatase, target subunit 1 (MYPT1) to human chromosome 12q15-q21. *Genomics* 1997;44(1):150–152. [PubMed: 9286714]
43. Fukata Y, Kimura K, Oshiro N, Saya H, Matsuura Y, Kaibuchi K. Association of the myosin-binding subunit of myosin phosphatase and moesin: dual regulation of moesin phosphorylation by Rho-associated kinase and myosin phosphatase. *The Journal of cell biology* 1998;141(2):409–418. [PubMed: 9548719]
44. Kimura K, Fukata Y, Matsuoka Y, Bennett V, Matsuura Y, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of the association of adducin with actin filaments by Rho-associated kinase (Rho-kinase) and myosin phosphatase. *The Journal of biological chemistry* 1998;273(10):5542–5548. [PubMed: 9488679]
45. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. *Nature reviews* 2002;3(8):586–599.