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Akt2 deficiency as therapeutic strategy protects against acute lung injury

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Abstract

Acute respiratory distress syndrome (ARDS) currently has limited effective treatments, however, recent evidence suggest that modulation of alveolar macrophage responses may be an effective method for protection or repair of lung injury. Vergadi *et al.* are the first to demonstrate that depletion of Akt2 kinase and microRNA-146a induction in mice resulted in polarization of alveolar macrophages towards an M2 activation phenotype and resulted in less severe injury following acid-induced lung injury. However, this M2 polarization also resulted in increased lung bacterial load following infection with *Pseudomonas aeruginosa*.

Keywords

acute respiratory distress syndrome; Akt2; microRNA-146a; acute lung injury; alveolar macrophages; M1 and M2 macrophages

Introduction

Acute respiratory distress syndrome (ARDS) is a major cause of respiratory failure among critically ill patients with few treatment options. Alveolar macrophages participate in the pathogenesis of ARDS through initiating the inflammatory response, promoting neutrophil infiltration and tissue damage [1–7]. Two macrophage phenotypes are associated with acute inflammation. The pro-inflammatory M1 macrophages express inducible nitric-oxide synthetase (iNOS) and secrete IL-12 β . In contrast, less inflammatory M2 macrophages highly express arginase-1 (Arg-1), found in-inflammatory zone-1 (Fizz1), and chitinase-3-like-3 (Ymn1) proteins [3, 8, 9]. M2 macrophages are associated with the resolution phase of inflammatory response. However, the exact mechanisms contributing to these phenotypes are currently unresolved. Several signaling molecules related to toll-like receptor activation (TLR) and cytokine signaling are associated with M1 activation, while C/EBP β up-regulation is associated with M2 activation [10, 11].

Of interest to this paper, Akt2 belongs to a family of serine/threonine protein kinases involved in the regulation of macrophage activation [11, 12]. Recent studies found depletion of Akt2 in peritoneal macrophages abrogated M1 activation and promoted M2 macrophage activation by inducing C/EBP β , a transcriptional regulator of Arg-1, through reduction in microRNA(miR)- 155 expression [11, 12]. MiR-155 directly targets C/EBP β and promotes M1 activation [13]. In addition, TLR4 signaling has contributed to alveolar macrophage

activation in animal models of acute lung injury (ALI) [6, 14]. TLR4 downstream signaling molecules such as IRAK1, TNFR-associated factor (TRAF)-6, and IRF5 are regulated by miR-146a [15, 16]. However, the role of miR-146a during macrophage activation and inflammation has not been investigated. Vergadi *et al.* study utilized acid-induced lung injury in WT and Akt2^{-/-} mice to evaluate biological effects of Akt2, miR-155, and miR-146a on macrophage activation and to establish therapeutic potential for Akt2 depletion in aseptic lung injury.

Summary of Methods & Results

Vergadi *et al.* compared the development of acid aspiration-induced lung injury in wild-type (WT) and Akt2^{-/-} mice to evaluate the roles of Akt2 deficiency in inducing protective M2 macrophages. To induce aseptic ALI, mice were administered hydrochloric acid solution or control sodium chloride saline via tracheal intubation and aspiration. Mice were then sacrificed to measure pressure-volume curves of the respiratory systems and bronchoalveolar lavage fluid (BALF) and lung tissues were collected and harvested for analyses. Aseptic ALI in WT mice was characterized by severe lung inflammation, as indicated by decreased inspiratory capacity, increased BALF protein concentrations, macrophage and neutrophil infiltration, and inflammatory cytokine accumulation in BALF. ALI severity peaked at 12 hours post-aspiration and returned to baseline by 72 hours. Evaluation of mouse respiratory systems indicated decreased inspiratory capacity and increased BALF protein concentrations in WT mice compared to Akt2^{-/-} mice. Neutrophil and macrophage infiltration and representative inflammatory cytokines (IL-6, TNF α , CXCL-1, and IL-1 β) were reduced in Akt2^{-/-} BALF compared to WT. Histological examination of Akt2^{-/-} lungs 12 hours post-acid aspiration indicated only mild histological alterations.

To elucidate the mechanisms by which Akt2 deficiency protects from acid-induced ALI, alveolar M1 and M2 macrophage markers were evaluated in WT and Akt2^{-/-} mice following acid aspiration. During the acute inflammatory phase of the acid-induced ALI, WT alveolar macrophages expressed mRNA and protein for iNOS and IL-12 β , which returned to basal levels by 48 hours after injury. However, these M1 markers following acid aspiration in Akt2^{-/-} macrophages were significantly lower when compared to WT. In addition, WT alveolar macrophages showed elevated gene expression of *Arg1*, *Fizz1*, and *Ym1* following acid-induced ALI with maximum gene expression at 12 hours post-exposure. Interestingly, gene expression of M2 markers, *Arg1* and *Fizz1*, were elevated in Akt2^{-/-} alveolar macrophages following both normal saline and acid aspiration when compared to WT. To prove whether Akt2 was involved in polarization of M1 towards M2 macrophage phenotype *in vitro*, short inhibitory (si)RNA targeting Akt2 were transfected into isolated WT alveolar macrophages and resulted in up-regulation of M2-associated proteins C/EBP β , *Fizz1*, and *Arg1*.

Since macrophage activation and inflammation responses depend on TLR signaling, first the impact of Akt2 deficiency on miR-155 and miR-146a expression and then the mechanism by which Akt2 regulates macrophage activation phenotypes through expression of miR-155 or miR-146a following acid-induced ALI were evaluated. Akt2^{-/-} alveolar macrophages

showed reduced miR-155 expression and up-regulation of its target gene C/EBP β following acid or saline exposure, as well as following exposure to BALF from WT mice exposed to acid or saline. Therefore, Vergadi *et al.* concluded miR-155 was not involved in the initial M1 activation of alveolar macrophages in their acid aspiration model. Conversely, increased miR-146a expression and decreased target gene expression were observed in Akt2 $^{-/-}$ mice with acid-induced ALI in comparison with WT. To test how Akt2 regulates macrophage activation, WT alveolar macrophages were transfected with siAkt2, which also exhibited elevated miR-146a and reduced target gene expression. Furthermore, direct modulation of miR-146a expression *in vitro*, transfecting either miR-146a mimics or inhibitor into WT or Akt2 $^{-/-}$ alveolar macrophages also showed miR-146a suppression modestly increased target gene expression of TRAF6 and IRF5 in Akt2 $^{-/-}$ macrophages but had no effect in WT. Furthermore, miR-146a transfection inhibited TLR4 activation-induced iNOS, up-regulated M2 marker gene expression, and reduced pro-inflammatory cytokine expression in WT macrophages. Conversely, inhibition of miR-146a in Akt2 $^{-/-}$ alveolar macrophages resulted in elevated iNOS and inflammatory cytokines, but showed neither reduction in expression of C/EBP β or elevation in *Arg-1* and *Fizz1* after TLR4 activation.

To test whether inhibition of Akt2 and induction of miR-146a contributed to inhibition of M1 activation in alveolar macrophages and protection against ALI *in vivo*, WT mice received either siAkt2 or miR-146a mimic intratrachally prior to acid-induced injury. In addition, Akt2 $^{-/-}$ mice received miR-146a inhibitor intratracheally prior to acid-induced ALI to determine whether miR-146a was sufficient for protection. Following ALI, siAkt2 or miR-146a mimic treated alveolar macrophages expressed reduced iNOS MFI and percentage of positive alveolar macrophages when compared to those treated with scrambled RNA. In addition, the administration of miR-146a inhibitor to Akt2 $^{-/-}$ mice resulted in a partial reversal of iNOS suppression in Akt2 $^{-/-}$ alveolar macrophages following ALI. Finally, Vergadi *et al.* investigated whether Akt2 depletion would also prevent ALI following administration of live bacteria. Interestingly, bacterial load of *P. aeruginosa* was significantly higher in Akt2 $^{-/-}$ mice compared with WT mice. Furthermore, inflammatory cytokine concentrations in BALF were not significantly different between WT and Akt2 $^{-/-}$ mice. However, when inflammatory cytokines were normalized to *P. aeruginosa* colony forming units, they showed reduced concentrations in Akt2 $^{-/-}$ compared to WT BALF. Infiltrating neutrophils and macrophages were fewer and expression of reduced iNOS and elevated Arg1 MFI were observed in Akt2 $^{-/-}$ compared with WT. Structural damage was slightly less severe in Akt2 $^{-/-}$ mice compared with WT mice although they both showed severe distortion of lung architecture.

Conclusion

ARDS is an important contributor to acute respiratory failure resulting in severe morbidity and mortality. Several clinical disorders can precipitate ARDS, including pneumonia, sepsis, aspiration of gastric contents, and major trauma [17]. Because ARDS has such broad clinical phenotypes, it has been challenging to translate results of cell and animal studies to therapies in the clinical setting [17, 18]. Nonetheless, experimental and clinical studies have made progress towards understanding the mechanisms critical for the resolution of lung injury.

One promising avenue for investigation is the role of alveolar macrophages in the initiation, maintenance, and resolution of inflammation [9].

Alveolar macrophages are well established to have a role in ARDS. Resolution of lung inflammation requires induction of regulatory mechanisms to terminate inflammatory response. Along these lines, it is known that suppression of M1 activation and iNOS, as well as induction of M2 macrophages can confer protection in ARDS [7, 19, 20]. The paper by Vergadi *et al.* indicates roles for Akt2 and miR-146a during initiation and resolution of ALI. Vergadi *et al.* associated Akt2 deficiency and increased expression of miR-146a with promotion of M2 macrophage activation *in vitro* and *in vivo* mouse model of aseptic ALI. To our knowledge, the current study is the first to evaluate the role of Akt2^{-/-} for the amelioration of acute lung injury in an aseptic model of disease. It would be interesting to investigate further potential contributing factors to M2 macrophage phenotype associated with Akt and miR-146a and the function of Akt2 and miR-146a during chronic inflammation. Moreover, since mice and humans differ in their immunological responses to septic and aseptic challenges, applications of these current findings would require rigorous experimentation prior to clinical trials.

Future perspective

In the paper under evaluation, Vergadi *et al.* demonstrated that Akt2 ablation resulted in reduced lung inflammation and injury following aseptic ALI, likely as a consequence of increased number of M2 alveolar macrophages prior to injury. However, Akt2 suppression clearly compromises the innate immune response of alveolar macrophages to live bacteria. These data suggest that Akt2 ablation or miR-146a overexpression in alveolar macrophages is not recommended for pneumonia- or sepsis-related ARDS, but may instead be used for protection against aseptic ALI, such as following gastric acid aspiration. The use of modulating macrophage activation phenotypes has potential applications for prevention of aseptic ALI; however, numerous challenges still remain in developing effective and safe immunotherapies for ARDS patients.

Bibliography

1. Frank JA, Wray CM, Mcauley DF, Schwendener R, Matthay MA. Alveolar macrophages contribute to alveolar barrier dysfunction in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2006; 291(6):L1191–1198. [PubMed: 16877636]
2. Eyal FG, Hamm CR, Parker JC. Reduction in alveolar macrophages attenuates acute ventilator induced lung injury in rats. *Intensive Care Med.* 2007; 33(7):1212–1218. [PubMed: 17468847]
3. Johnston LK, Rims CR, Gill SE, Mcguire JK, Manicone AM. Pulmonary macrophage subpopulations in the induction and resolution of acute lung injury. *Am J Respir Cell Mol Biol.* 2012; 47(4):417–426. [PubMed: 22721830]
4. Janssen WJ, Barthel L, Muldrow A, et al. Fas determines differential fates of resident and recruited macrophages during resolution of acute lung injury. *Am J Respir Crit Care Med.* 2011; 184(5):547–560. [PubMed: 21471090]
5. Dhaliwal K, Scholefield E, Ferenbach D, et al. Monocytes control second-phase neutrophil emigration in established lipopolysaccharide-induced murine lung injury. *Am J Respir Crit Care Med.* 2012; 186(6):514–524. [PubMed: 22822022]
6. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell.* 2008; 133(2):235–249. [PubMed: 18423196]

7. Kudoh I, Miyazaki H, Ohara M, Fukushima J, Tazawa T, Yamada H. Activation of alveolar macrophages in acid-injured lung in rats: different effects of pentoxifylline on tumor necrosis factor-alpha and nitric oxide production. *Crit Care Med.* 2001; 29(8):1621–1625. [PubMed: 11505142]
8. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol.* 2008; 8(12):958–969. [PubMed: 19029990]
9. Herold S, Mayer K, Lohmeyer J. Acute lung injury: how macrophages orchestrate resolution of inflammation and tissue repair. *Front Immunol.* 2011; 2:65. [PubMed: 22566854]
10. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity.* 2010; 32(5):593–604. [PubMed: 20510870]
11. Androulidaki A, Iliopoulos D, Arranz A, et al. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. *Immunity.* 2009; 31(2):220–231. [PubMed: 19699171]
12. Arranz A, Doxaki C, Vergadi E, et al. Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. *Proc Natl Acad Sci U S A.* 2012; 109(24):9517–9522. [PubMed: 22647600]
13. He M, Xu Z, Ding T, Kuang DM, Zheng L. MicroRNA-155 regulates inflammatory cytokine production in tumor-associated macrophages via targeting C/EBPbeta. *Cell Mol Immunol.* 2009; 6(5):343–352. [PubMed: 19887047]
14. Reino DC, Pisarenko V, Palange D, et al. Trauma hemorrhagic shock-induced lung injury involves a gut-lymph-induced TLR4 pathway in mice. *PLoS One.* 2011; 6(8):e14829. [PubMed: 21829592]
15. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A.* 2006; 103(33):12481–12486. [PubMed: 16885212]
16. Williams AE, Perry MM, Moschos SA, Larner-Svensson HM, Lindsay MA. Role of miRNA-146a in the regulation of the innate immune response and cancer. *Biochem Soc Trans.* 2008; 36(Pt 6): 1211–1215. [PubMed: 19021527]
17. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994; 149(3 Pt 1):818–824. [PubMed: 7509706]
18. Walkey AJ, Summer R, Ho V, Alkana P. Acute respiratory distress syndrome: epidemiology and management approaches. *Clin Epidemiol.* 2012; 4:159–169. [PubMed: 22866017]
19. Jian MY, Koizumi T, Kubo K. Effects of nitric oxide synthase inhibitor on acid aspiration-induced lung injury in rats. *Pulm Pharmacol Ther.* 2005; 18(1):33–39. [PubMed: 15607125]
20. Farley KS, Wang LF, Razavi HM, et al. Effects of macrophage inducible nitric oxide synthase in murine septic lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2006; 290(6):L1164–1172. [PubMed: 16414981]

Executive Summary

- Akt2-deficient alveolar macrophages showed reduced miR-155 expression that was not associated with the activation of alveolar macrophages following acid-induced lung injury.
- Depletion of Akt2 and miR-146a induction in mice resulted in polarization of alveolar macrophages towards an M2 phenotype *in vitro* and *in vivo*.
- Akt2 kinase deficiency in mice resulted in less severe ALI following acid-induced lung injury, however, this Akt2 deficiency resulted in increased lung bacterial load and did not significantly reduce ALI following infection with *Pseudomonas aeruginosa*.