

Targeting Neutrophil Extracellular Traps in Acute Lung Injury

A Novel Therapeutic Approach in Acute Respiratory Distress Syndrome?

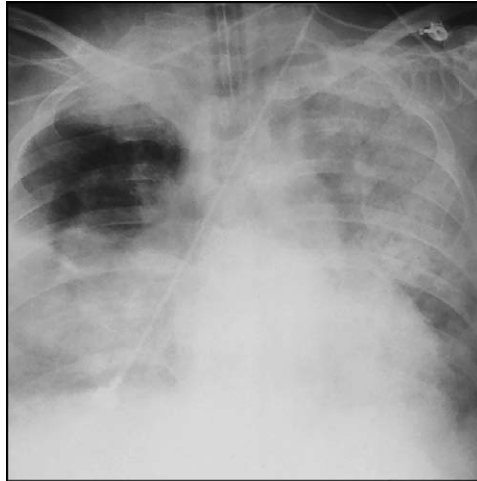
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THE pathogenesis of organ injury, including acute respiratory distress syndrome (ARDS), includes the liberation of endogenous molecular structures called “danger-associated molecular patterns” that are associated with inflammation and tissue injury. Targeting danger-associated molecular patterns thus may be a potential approach for novel ARDS therapies. Extracellular deoxyribonucleic acid (DNA) forms neutrophil extracellular traps (NET), which act like a danger-associated molecular pattern in that they are associated with inflammation and tissue injury.

However, we have learned from the blockade of specific cytokines or the use of activated protein C in sepsis and ARDS that there are very narrow therapeutic opportunities, and a single therapy may not be useful for these syndromes caused by partly very distinct pathomechanisms.

Given this background, the current study in animals by Yildiz *et al.*¹ investigated the role of NETs in the pathogenesis of acute lung injury in mice. They used a double-hit model of intratracheal lipopolysaccharide challenge followed by ventilator-induced lung injury (VILI). In this widely used model, they found correlates for VILI-induced NET formation *in vivo*; however, NET degradation by DNase treatment had no impact on the development of lung injury. Hence, the authors propose that NETs do not contribute functionally to the pathogenesis of lung injury in this animal model.

Neutrophil extracellular traps are decondensed DNA expelled mainly by neutrophils during a process termed NETosis. NETosis is a tightly controlled cell death pathway requiring among others neutrophil elastase, myeloperoxidase,



“Targeting danger-associated molecular patterns ... may be a potential approach for novel ARDS therapies.”

and reactive oxygen species. Various intracellular components such as histones, proteases, and antimicrobial peptides are bound to the expelled DNA. NETs can bind various pathogens, immobilize or “trap” them, and ultimately promote their killing.² They trap bacteria during bacteremia, thereby counteracting the dissemination of infections.^{3,4} Patients with chronic granulomatous disease (lacking reactive oxygen species production due to NADPH oxidase NOX2 deficiency) or complete myeloperoxidase deficiency cannot form NETs. Their enhanced susceptibility to fungal infections may be explained by the lack of NETs, as hyphae of *Candida* species escape phagocytosis due to their size and require NETs for clearance.⁵ However, NETs also exhibit harmful effects.

NETs and especially NET-bound components such as histones are cytotoxic causing endothelial damage and vascular permeability.⁶ NETs are also potent activators of coagulation and have been identified as drivers of organ failure in sepsis due to disruption of the microcirculation.³ The role of NETs in sterile organ damage such as VILI is not fully understood.

In the current study, Yildiz *et al.* addressed this important question in a well-established VILI model. They found that lipopolysaccharide induced infiltration of neutrophils regardless of additional VILI. However, the combination of lipopolysaccharide and high tidal volume ventilation induced a significant increase in DNA content in the bronchoalveolar lavage. Together with enhanced amounts of citrullinated histone 3, they concluded that NETosis is induced by VILI in lipopolysaccharide-treated animals. When they next treated

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animals with DNase in order to degrade NETs, they observed no significant impact on lung injury in their model.

The detection of NETs, especially *in vivo*, remains challenging. One approach, which was also used by Yildiz *et al.*, has been to quantify extracellular DNA. However, DNA may also be released during necrosis of various other cell types, especially during traumatic tissue injury. Yildiz *et al.* supported their findings by additionally staining citrullinated histone 3, a widely used marker for NETs.⁷ However, histone citrullination alone may not be specific for NETosis, and costaining of myeloperoxidase or neutrophil elastase, histone, and DNA together with a neutrophil marker may be more suitable to ensure specificity of NET staining. Lipopolysaccharide alone may also induce NETosis, which cannot be accurately discriminated from additional VILI-induced NET formation in the current study. Barring these technical challenges, the study by Yildiz *et al.* clearly suggests that NETosis is a feature of VILI, yet NETs may not play a major role for the development of lung injury in this particular animal model.

Experimental studies have linked NETs to acute lung injury.^{8,9} A crucial mechanism seems to be the interaction of neutrophils and platelets, which rapidly induces NETosis leading to trapping of neutrophils on the endothelium, activation of coagulation, and vascular permeability.⁹ A recent study by Rossaint *et al.*¹⁰ showed different results regarding the role for NETs in VILI. In contrast to the current study by Yildiz *et al.*, they found that inhibition of NETosis as well as disintegration of NETs by DNase treatment was highly protective against VILI. Several reasons may account for the different results. One important reason is that in the study by Yildiz *et al.*, the lung was saturated with neutrophils at the initiation of VILI due to the prior lipopolysaccharide challenge, whereas in the study by Rossaint *et al.*, neutrophils were recruited during VILI. Reduction of lung injury due to various interventions was at any time accompanied by a significant reduction of pulmonary neutrophil numbers, indicating that NETs may substantially contribute to pulmonary leukocyte recruitment during VILI. Recognizing the major role of neutrophils in the pathogenesis of VILI, these results could imply that the disruption of pulmonary neutrophil recruitment rather than direct NET toxicity may account for the beneficial effects of NET inhibition.¹⁰ Accordingly, no beneficial effect could be detected in the study by Yildiz *et al.* because lung neutrophilia was already induced before VILI due to lipopolysaccharide.

The studies by Yildiz *et al.* and others have established that NETosis takes place during lung injury without any doubt. However, it has to be pointed out that only a small fraction of alveolar neutrophils undergo NETosis. Thus, it is unclear whether the alveolus is the right compartment to target when designing therapeutic intervention strategies based on NET disruption.

However, in the microcirculation, a single neutrophil undergoing NETosis—expelling NETs within the capillary—may

have a tremendous effect as it may rapidly occlude the vessel by local activation of coagulation as suggested in the study by Clark *et al.*³ Along this line, the vascular system may be a promising compartment for targeting NETosis.

By using sterile inflammatory models such as VILI, the role of NETs as crucial components of the innate immune system is omitted. Notably, NET degradation by treatment with DNase in mice with polymicrobial sepsis resulted in higher bacterial counts and worsening of lung injury.¹¹ Furthermore, NETs are essential to control fungal infections, and NETosis seems to be tightly controlled rather than being an unspecific event in host defense.¹²

Thus, targeting NETs as adjuvant therapy in lung injury should take into account the effects on host defense against infections.

Overall, NETs remain an interesting target for adjuvant therapy in ARDS and sepsis. However, ARDS outcome may be hard to improve by future adjuvant therapies that are applied in a “one-size-fits-all” manner. We have to recognize that the syndrome that we call ARDS is caused by multiple different disease processes with very unique pathomechanisms. Thus, targeting NET-induced neutrophil sequestration may be very efficient in transfusion-related acute lung injury or in shock with significant microcirculatory failure, whereas being less efficient or even of risk in patients with pneumonia or invasive fungal infections.

Although we have learned a lot about NET biology and its impact on certain models of acute lung injury, there still remains a lot of preclinical work ahead of us. We have to further deepen our mechanistic understanding of NETs in ARDS, taking its different underlying conditions into account when testing potential therapeutic concepts in respective clinically relevant animal models. The study by Yildiz *et al.* is an important contribution in this process, which will hopefully lead us to success by further combining the visions of basic science with our clinical knowledge and experience.

Competing Interests

The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article.

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References

1. Yildiz C, Palaniyar N, Otulakowski G, Khan MA, Post M, Kuebler WM, Tanswell K, Belcastro R, Masood A, Engelberts D, Kavanagh BP: Mechanical ventilation induces neutrophil extracellular trap formation. *ANESTHESIOLOGY* 2015; 122:864–75
2. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A: Neutrophil extracellular traps kill bacteria. *Science* 2004; 303:1532–5
3. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes

- P: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007; 13:463–9
4. Massberg S, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, Brinkmann V, Lorenz M, Bidzhekov K, Khandagale AB, Konrad I, Kennerknecht E, Reges K, Holdenrieder S, Braun S, Reinhardt C, Spannagl M, Preissner KT, Engelmann B: Reciprocal coupling of coagulation and innate immunity *via* neutrophil serine proteases. *Nat Med* 2010; 16:887–96
 5. Branzk N, Papayannopoulos V: Molecular mechanisms regulating NETosis in infection and disease. *Semin Immunopathol* 2013; 35:513–30
 6. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, Lohmeyer J, Preissner KT: Neutrophil extracellular traps directly induce epithelial and endothelial cell death: A predominant role of histones. *PLoS One* 2012; 7:e32366
 7. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, Hayama R, Leonelli L, Han H, Grigoryev SA, Allis CD, Coonrod SA: Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol* 2009; 184:205–13
 8. Sayah DM, Mallavia B, Liu F, Ortiz-Muñoz G, Caudrillier A, DerHovanessian A, Ross DJ, Lynch JP III, Saggart R, Ardehali A, Ware LB, Christie JD, Belperio JA, Looney MR; LTOG Investigators: Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med* 2014 [Epub ahead of print]
 9. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, Toy P, Werb Z, Looney MR: Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* 2012; 122:2661–71
 10. Rossaint J, Herter JM, Van Aken H, Napirei M, Döring Y, Weber C, Soehnlein O, Zarbock A: Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. *Blood* 2014; 123:2573–84
 11. Meng W, Paunel-Görgülü A, Flohé S, Hoffmann A, Witte I, MacKenzie C, Baldus SE, Windolf J, Lögters TT: Depletion of neutrophil extracellular traps *in vivo* results in hypersusceptibility to polymicrobial sepsis in mice. *Crit Care* 2012; 16:R137
 12. Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, Papayannopoulos V: Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat Immunol* 2014; 15:1017–25