Severe Influenza and Pneumococcal Pneumonia: Casting NETs to Assist Cell Suicide?

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**Abstract**  
We investigated the role of neutrophil extracellular traps (NETs) in primary influenza pneumonia and secondary pneumococcal pneumonia, and whether NETs induced during pulmonary influenza infection have functional significance against bacterial and fungal infections. NETs do not participate in killing of *Streptococcus pneumoniae* in vivo and in vitro. Dual-infected mice exhibit elevated bacterial load and enhanced lung pathogenesis compared to animals challenged with influenza virus or bacteria alone. The intensified NETs in dual-infected mice often appear as clusters frequently filled with partially degraded DNA. The severe pulmonary pathology and excessive NETs generation in dual infection correlate with exaggerated inflammation and damage to the alveolar-capillary barrier. NETs stimulation does not alter the gene expression of several antimicrobial proteins, and these NETs are not bactericidal. Fungicidal activity against *Candida albicans* is similar both in the presence or absence of NETs. These observations support the pathogenic effects of excessive neutrophils and NETs in acute lung injury (ALI) of primary influenza and secondary bacterial pneumonia by instigating alveolar-capillary damage. We developed a model of influenza virus infection of neutrophils by promoting differentiation of the MPRO promyelocytic cell line. Only a fraction of neutrophils are infectable by highly virulent influenza (HVI) H3N2 virus, but are not permissive for active viral replication. HVI infection of neutrophils augments early and late apoptosis. Global transcriptomic responses of neutrophils to HVI reveal that the interferon regulatory factor and interferon signaling pathways are the most significantly overrepresented pathways, with activation of related genes in HVI as early as 3 h. Mice infected with HVI virus reveal dysregulation of TREM1 signaling, cytokines and chemokines. Doxycycline treatment attenuates ALI of HVI virus-infected mice via the involvement of matrix metalloproteinases. In addition, combination therapy with hepatocyte growth factor and oseltamivir can confer greater protection against influenza pneumonia.

**Selected Publications**