Pathological role of neutrophilextracellulartraps-associated DNA in sterileinflammation

Hudecová L., Bečka M., Borka G., Celec P., Pastorek M.

Institute of MolecularBiomedicine, Faculty of Medicine, ComeniusUniversity, Bratislava

Background: Neutrophil extracellular traps (NETs) formed from DNA coupled with neutrophil proteins are formed in an immune response against bacteria. NETs are however also formed in response to tissue damage and exacerbate sterile inflammation through the toll-like receptor 9 (TLR9) signaling. TLR9 recognizes hypomethylated DNA and its activation is augmented by antimicrobial proteins like cathelicidin that protects NETs against nucleases. We hypothesized that NETs rich in cathelicidin would strongly activate TLR9, with their composition determined by the initiating stimuli.

Methods: NETs mediated TLR9 activation was analyzed using an *in vitro* reporter. The immunogenic and cytotoxic potential of NETs was analyzed by live-cell microscopy and flow cytometry. NETs induced by mitochondria and E. coli were analyzed by flow cytometry and dot blot for the presence of cathelicidin and qPCR for DNA composition. SYTOX Green™ was used to analyze the resistance of NETs to DNase I degradation.

Results: We have observed that NETs induced TLR9-mediated NETs formation with cathelicidin enhancing the response up to the concentration of ~0.2 μg/ml, beyond which they induced necrosis. Both mtDNA and ncDNA isolated from NETs were not able to activate TLR9 on their own and only mtDNA in combination with cathelicidin was able to induce very low NETs formation. Regarding their composition, NETs induced by *E. coli* contained more cathelicidin than those induced by mitochondria. However, cathelicidin-deficient NETs were more potent at inducing further NETs formation but less cytotoxic than cathelicidin-positive NETs.

Conclusion: We conclude that the immunogeneicity of NETs depends on the stimuli that induced their formation and cathelicidin concentration. In addition, we also propose that a vicious cycle of NETs formation can be at least attenuated through negative feedback loop where a higher concentration of NETs is cytotoxic rather than immunogenic.

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