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Update on the spider and the fly: An extended commentary on “Oxidized LDL induced extracellular trap formation in human neutrophils via TLR-PKC-IRAK-MAPK and NADPH-Oxidase activation”

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Neutrophils belong to the first line innate immune defense against microbes and have the capacity to eliminate them via several redundant mechanisms. The process of extracellular traps (ETs) in animals seems confined to cells derived from hematopoietic myeloid cells, most notably but not exclusively from neutrophils (NETs) [1]. In just over a decade ETs have gained traction, have been described in primordial host defense systems conserved through evolution, and have become a focus in the field of neutrophil biology and innate immunity as well as in allergy and autoimmunity (Fig. 1) [2–6]. Their formation by neutrophils, labeled NETosis, is characterized by the release of weblike structures which contribute to the containment and killing of pathogens. They are composed of decondensed nuclear chromatin, histones, usually nuclear double stranded DNA (mitochondrial DNA in basophils), granular proteins including myeloperoxidase, elastase, and protease-3, and numerous antimicrobial proteins and peptides [4,7,8]. NETs are released to the extracellular space in response to an impressive number of microbial and non-microbial stimuli, including bacteria, fungi, protozoa, viruses, reactive oxygen species (ROS), antibodies, antigen–antibody complexes, lipopolysaccharides (LPS), and activated platelets [1,4,8].

Besides ROS producing enzymes, specific signal transduction events and several enzymatic activities are needed to initiate NETosis [4,8,9]. Phorbol-12-myristate (PMA), the most classically studied initiator, activates PKC, NADPH oxidase, Rac 2 and Raf/MEK/ERK signaling pathways [10]. As future data on ETosis biology emerges, it is clear that parallel initiator signaling pathways exist [9,11–13]. Features of ETosis include the nuclear transport of azurophilic granular elastase and MPO [14,15] and activations of petidylarginine

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deaminase 4 [16–18] which act together with NADPH oxidase [4], or in some instance mitochondrial generated ROS [12], to decondense and citrullinate core histones, relieving their electrostatic coiling and weakening their binding to DNA. Much is left to understand about these nuclear processes. Involvements of cytoskeletal tubulin and actin dynamics are thought to play a role in the nuclear transportation of elastase and MPO and in the later less characterized executioner molecular pathways of nuclear envelope disintegration, plasma membrane rupture, and functional propulsion of the ET into the extracellular matrix [4,8].

NETosis is generally recognized as a novel form of cell death related to autophagy but distinct from apoptosis and necrosis, because of its dependence on chromatin decondensation, increase in membrane permeability and its relative independence from necrosis—inducing or apoptosis—inducing stimuli [4,7,19]. However, neutrophils releasing NETs while maintaining their cellular membrane integrity (called “vital NETosis”) and maintaining chemotaxis and bacterial phagocytosis have been reported [20]. Like several other potent neutrophil constituents and metabolic pathways, NETs represent a two-edged sword [21]. NETs are important in host anti-microbial processes, yet when overly activated or when activated by non-microbial initiators, they have considerable potential to cause harm to host tissues. A pathophysiological role of NETs has been implicated to play an important role in inflammation which is seen in allergic, infectious, respiratory tract (RT), vascular, and autoimmune diseases [3,4,8,18,21–23].

This latter potential has led to aggressive efforts to further delineate the myriad of factors initiating neutrophil “NETosis,” including characterizations of receptor and intracellular pathways transducing their formation [3,4,8]. The inferential goal is that if causative receptor and transducing mechanisms of NETosis can be identified, this could add to the development of directly targeted therapies addressing both infectious and non-infectious inflammatory processes in which NETs play either a helpful or harmful role [24–27]. Although much has been learned about the molecular mechanisms of NETosis at the cellular level, it is recognized that animal studies and clinical trials will probably have to be the arbitrator of the expected Janus effects of therapeutically modulating NETosis.

A recent FRBM issue, Awasthi and colleagues have rigorously demonstrated that aliquots of isolated human LDL, heavily oxidized by 24 h exposures to CuSO₄ in PBS, induced NET formation in neutrophils isolated from human blood and seeded and adhered on precoated coverslips [28]. Their studies used PMA or native LDL as positive and negative controls. They convincingly show that the highly oxidized LDL triggered neutrophil NET formation via activations of TLR2 and TLR6, but not TLR4 receptors which are also capable of stimulating NETs [29]. Awasthi et al. also characterized proximal downstream effector molecular pathways including a sequence of NADPH oxidase-dependent activated PKC-IRAK-MAPK signaling mediators. They then interrogated a spectrum of oxylipids (including oxysterols) contained in their highly oxidized LDL preparations and demonstrated their relative potential for triggering NETosis. They demonstrated that oxidized phospholipids, known to be pro-inflammatory [30–32], were the most potent oxidized lipid LDL component to elicit NETosis. Oxysterols, suspected of participating in the pathobiology of chronic inflammatory diseases [33,34] were considerably less potent contributors to NETosis. The enlightening inclusion of 92 relevant references put the present

findings into perspective in this rapidly expanding field of biology relevant to human disease (Fig. 1).

Oxidized lipids thus join the intriguing lists of reported triggers of ET formation [1,4,8]. As stated by Awasthi et al. most inflammatory foci will contain a myriad of bioactive molecules impacting numerous receptor and signaling pathways relating to neutrophil function and fate. Of relevance to their studies, recent literature has emphasized the pleiotropic biological effects of molecular species derived from oxidized unsaturated fatty acids [30–32,35]. Confounding interpretations of the specific roles that oxidized lipids many play in an inflammatory mileau is the co-existence of varying concentrations of anti-inflammatory resolving lipids derived from unsaturated fatty acids and capable of modulating numerous pro-inflammatory pathways [36–38].

As previously mentioned, overly active NET formation (or ineffective clearance) is believed to be linked to deleterious effects on host tissues in numerous inflammatory diseases [4,8]. Cystic fibrosis (CF) and asthma represent two ET-related RT diseases where ETosis is believed to contribute to disease mechanisms. In CF, the absence of CFTR function severely compromises RT innate inflammatory-immune processes which play critical roles in disease pathobiology. The natural history of the disease is characterized by progressively intense RT bacterial colonization and unresolved inflammation [39,40]. RT secretions in CF contain a plethora of neutrophils exhibiting numerous activation states (including dysfunctional phenotypes), an abundance of DNA attributed to NETs and their cytotoxic histones, as well as necrotic and apoptotic neutrophils along with an impressive accumulation of numerous cytokines, chemokines, acute phase proteins, antimicrobial peptides, oxidized lipids including oxysterols and ROS [40–42]. Many of these constituents are capable of inducing the massive NETosis shown to be present in the RT of CF subjects [43,44]. Importantly, CF patients also appear to have a deficiency of anti-inflammatory pro-resolution factors such as surfactant protein-D and the forementioned specialized pro-resolving mediators derived from omega-3 and omega-6 fatty acids [42,45]. It is readily apparent that effective therapy targeting NETosis could possibly benefit CF, a disease in which new anti-inflammatory therapies are aggressively being sought in order to complement the new CFTR correctors and potentiators which are revolutionizing the treatment of this disease [46,47]. In a similar vein, both eosinophilic and neutrophilic ETs are reported in asthmatic airway secretions [3]. Just as in CF, their specific contributions to the pathophysiology of asthma are still not completely or well understood.

In conclusion, it can be expected that oxidized LDL species and especially selective oxidized phospholipids are capable of affecting neutrophil NETosis in inflammatory mileau containing both ROS and unsaturated lipids. As discussed by Awasthi et al. the degree to which they contribute will depend on the overall repertoire of inflammatory cells and ET-influencing compounds in the inflammatory space, confounded by the presence and cross-talk of many other bioactive inflammatory compounds, and the context of the inflamed host tissue. Further work will be needed to substantiate the role of the oxidized lipids studied by Awasthi and colleagues in modulating NETosis within complex inflammatory mileau. Unraveling the myriad of interactive signals and pathways influencing NETosis and designing therapeutic approaches to modulate its formation and clearance will continue to

present formidable challenges. This may require robust agendas of biologic and metabolipidomic profiling approaches to identify targets on which to base new therapeutic approaches designed to dampen, or in some cases strengthen, NET formations. Such approaches should lead to a better understanding of inflammatory human disease and novel future therapeutic approaches.

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Fig. 1.

The number of publications on NETs and ET in the literature as reported in PubMed from 2004 through 2015. The 2016 data are pro-rated based on 64 publications as of February 2016. Our selection criteria included all original scientific papers, reviews, and editorials, as well as human and animal studies.