RESEARCH HIGHLIGHT

Knowing when to stop: MICL self-regulates neutrophil NETosis

Hanjoo Brian Shim[1,2], Justin François Deniset[1,2,3,4,6] and Paul Kubes[1,2,5,6]✉

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In a recent study published in *Nature*, Malamud et al. identified how neutrophil MICL recognizes neutrophil extracellular traps (NETs). This recognition suppresses further neutrophil activation and NET production, thereby preventing a vicious cycle of inflammation.

Neutrophils are circulating immune cells that rapidly migrate into infected or injured tissues. Upon recruitment, these cells amplify inflammation by releasing cytokines, proteases, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs) — a sticky web of DNA containing histones and other effector molecules. Of these programs, NETs may be the most potent because they can non-specifically kill cells by disrupting their cell membranes.[1,2] During a bacterial or fungal infection, NETs are helpful as they efficiently immobilize and clear the pathogens. However, NETs cause excessive tissue damage and are likely harmful in cases of sterile inflammation, autoimmunity or viral infection.[1,2] Despite the significant polarizing influence NETs can have in various inflammatory contexts, our understanding of how NET release (NETosis) is regulated remains far from complete. In a recent article published in *Nature*, Malamud et al.[3] report evidence of a new MICL (myeloid inhibitory C-type lectin-like)-NET axis that prevents unregulated NETosis in mouse and human neutrophils.

Malamud and colleagues investigated the function of MICL in a mouse model of rheumatoid arthritis using *Micl*$^{-/-}$ mice.[3] Their observations revealed enhanced disease severity and neutrophil infiltration in the joints of *Micl*$^{-/-}$ mice, indicating that MICL plays a role in suppressing the neutrophil response.[3] Moreover, not only were neutrophil numbers increased, but also the neutrophils in the *Micl*$^{-/-}$ mice displayed heightened activation and NET deposition.[3] Importantly, when NETs were removed with DNase or NETosis was inhibited using a small-molecule inhibitor, the enhanced disease severity in *Micl*$^{-/-}$ mice was attenuated.[3] These results demonstrated that MICL is crucial in preventing excessive neutrophil-mediated inflammation and tissue damage.

While these findings are consistent with previous studies,[4] Malamud et al. took their investigation further by examining the signaling pathways of MICL, NETs, and NETosis, uncovering novel insights into the process. MICL is known to bind uric acid crystals (monosodium urate (MSU)) released by dying cells and this interaction suppresses neutrophil ROS production.[4] Using MSU as the stimulus, Malamud and colleagues discovered that ROS generated by NADPH oxidase induced NETosis in MICL-deficient neutrophils.[3] Building on this finding, the researchers next investigated whether MICL could directly bind to NETs, employing several in vitro ELISAs and cell binding assays.[3] This line of inquiry may have stemmed from the fact that MICL is a promiscuous receptor known to bind seemingly unrelated ligands.[5] Nevertheless, these experiments revealed NETs as a new ligand of MICL, as MICL directly bound the DNA in NETs.[3]

According to their findings, the authors propose a model in which activated neutrophils release ROS which then induces NETosis. MICL then binds and recognizes the NETs to halt further ROS and NET production. Without proper MICL function, the neutrophil response enters an inflammatory positive feedback loop with unregulated ROS and NET generation.[3]

Human patients with rheumatoid arthritis have anti-MICL autoantibodies in circulation and a previous mouse study suggested a pathogenic role for these anti-MICL inhibiting antibodies.[6] However, there has been little direct evidence in humans. To this end, the authors exposed isolated human neutrophils to patient serum containing the autoantibodies in vitro.[3] This experiment revealed that the autoantibodies elevated ROS and NET production in response to MSU.[3] The authors concluded that the enhanced neutrophil activation was indicative of autoantibodies causing MICL dysfunction.[3] Based on these findings, the authors further interrogated the pathogenicity of anti-MICL antibodies in a cohort of 200 rheumatoid arthritis patients and a positive correlation between circulating anti-MICL autoantibodies and disease severity was found.[3] Taken together, these findings support the idea that anti-MICL autoantibodies may at the very least aggravate if not induce rheumatoid arthritis by disrupting the newly discovered MICL-NET axis. The human data are a very nice example of the mouse model validating human disease, even though laboratory mice are kept in specific pathogen-free condition while humans are not. It would be interesting to see whether in wild mice exposed to many more fungal pathogens and in humans with larger amounts of commensal fungal agents, MICL levels would change and arthritis severity and prevalence would also differ.

The researchers also found that anti-MICL autoantibodies may be involved in other NET-related conditions as they observed positive correlations in cohorts of COVID-19 and systemic lupus erythematosus (SLE) patients, suggesting that MICL and NETs could have broader relevance beyond rheumatoid arthritis.[3] Future studies could examine how the anti-MICL autoantibody levels are distributed within these autoimmune patients. For example, would levels of MICL be changed in subsets of SLE patients with DNase deficiencies. Given that NETs help protect

[1]Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada. [2]Calvin, Phoebe, and Joan Snyder Institute for Chronic Diseases, University of Calgary, Calgary, AB, Canada. [3]Department of Cardiac Sciences, University of Calgary, Calgary, AB, Canada. [4]Libin Cardiovascular Institute, University of Calgary, Calgary, AB, Canada. [5]Department of Biomedical and Molecular Science, Queen's University, Kingston, ON, Canada. [6]These authors contributed equally: Justin François Deniset, Paul Kubes.
✉email: p.kubes@queensu.ca

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against fungal and bacterial infections, the authors next investigated whether MICL dysfunction and excessive NETosis could enhance neutrophil responses to the fungi *Aspergillus fumigatus*. In experiments exposing mouse neutrophils to *A. fumigatus* in vitro, *Micl*$^{-/-}$ neutrophils produced more NETs compared to controls.[3] Moreover, *Micl*$^{-/-}$ mice showed improved survival, better fungal clearance and reduced fungal dissemination following a blood stream *A. fumigatus* infection.[3] These findings not only suggest that MICL dysfunction could boost antimicrobial immunity, but also raise the possibility that blocking MICL could be useful in fungal disease. This highlights the broader relevance and functional duality of MICL in neutrophil responses.

The work by Malamud et al. is exciting for many reasons. First, this study aligns with a growing body of research investigating how neutrophils can regulate their own activity. For instance, a recent study by Kienle et al. showing that neutrophils self-limit their swarming behavior through GPCR desensitization, highlights another mechanism of neutrophil self-regulation.[7] Second, MICL and NETs have not been studied extensively, and thus their findings open the door for a better understanding of neutrophils, MICL, and NETosis in the context of stroke, myocardial infarction, and cancer.[1,2] However, some important questions remain unanswered. Neutrophils are known to change their expression of surface receptors upon recruitment and activation; therefore, it would have been informative to analyze MICL expression in their flow cytometry data. This is particularly relevant because the same research group previously reported that neutrophils in a mouse model of rheumatoid arthritis downregulate MICL within 48 h of recruitment.[8] Does this suggest that MICL's role is limited to newly recruited neutrophils? Additionally, MICL is also expressed on monocytes and macrophages present at inflammation sites.[3] Although their numbers were similar between *Micl*$^{-/-}$ and wild-type mice, their functions including phagocytosis and cytokine secretion might still be altered. Addressing these questions with lineage-specific MICL knockout mice could provide a more comprehensive understanding of the MICL-NET axis and its role in inflammation, paving the way for future therapeutic advances.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Paul Kubes.