

 TARGETED THERAPIES

Small wonder: nanoparticles feed hydroxychloroquine to activated neutrophils

Somanathapura K. NaveenKumar and Jason S. Knight 

Although neutrophils are vital components of the innate immune system, they can also contribute to the inflammation and autoantibody formation that characterize a number of rheumatic diseases. The ability to specifically target neutrophils, and in particular activated neutrophils, could enable the direct delivery of drugs for therapeutic modulation of neutrophil activity.

Refers to Cruz, M. A. et al. Nanomedicine platform for targeting activated neutrophils and neutrophil-platelet complexes using an α_1 -antitrypsin-derived peptide motif. *Nat. Nanotechnol.* <https://doi.org/10.1038/s41565-022-01161-w> (2022).

Neutrophils are an important part of the innate immune system, but they also contribute to the pathogenesis of a number of rheumatic diseases. The results of a newly published study¹ take us one step closer to the therapeutic targeting of neutrophils.

As the most abundant leukocytes in human blood, neutrophils are vital players in the host response to infection. Neutrophils have long been known to neutralize pathogens through a combination of phagocytosis and the production of reactive oxygen species such as hypochlorous acid. In 2004, neutrophils were also found to release microbicidal neutrophil extracellular traps (NETs), sticky spider-web-like structures composed of granule-derived effector proteins adorning a scaffold of massively decondensed chromatin². This decondensation occurs when reactive oxygen species trigger the migration of proteases to the nucleus, where they cleave histones³. In parallel, post-translational modifications alter the charge content of histones, most notably through citrullination mediated by peptidylarginine deiminases.

The loss of neutrophil homeostasis and/or unchecked neutrophil activation have been implicated in wide-ranging local and systemic disease states, including defective wound healing, immunothrombosis and COVID-19

(REF.⁴). For numerous rheumatic diseases, neutrophil-mediated inflammation is an important effector of tissue injury. Some obvious examples of this relationship include gout, Behçet disease and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Even when neutrophil activation is not obvious, residual activity — including NET release — contributes to the production of cytokines (such as type I interferons and IL-1 β) and formation of autoantibodies (including anti-DNA antibodies and anti-citrullinated histone antibodies) that are associated with diseases such as systemic lupus erythematosus and rheumatoid arthritis⁵. There is also strong evidence that neutrophils contribute to accelerated cardiovascular disease in many of these conditions, which can include acute venous and arterial thrombotic events, as are seen in antiphospholipid syndrome (APS)⁶.

Despite an emerging consensus that restraining neutrophil hyperactivity would sometimes be beneficial, the best approach to doing so, weighing the benefits and risks, has remained elusive. Some drugs that are already in use in the rheumatology clinic, such as colchicine and Janus kinase (JAK) inhibitors, clearly have direct neutrophil-inhibiting properties. Blockade of cytokines such as IL-23 and IL-17 by monoclonal antibodies would also

be expected to reduce neutrophil-mediated inflammation. Of course, development of these drugs has for the most part been optimized with other populations of leukocytes in mind. Therapeutic approaches such as inhibition of chemotaxis or adhesion have imperfect specificity for neutrophils and may carry a high risk of infection⁷. Although the repurposing of drugs such as dipyridamole (and even the use of supplements such as ginger) has also been considered, this concept is again essentially relying on effects that are off-target from the drug's canonical role⁸.

In an interesting study published in *Nature Nanotechnology*¹, Cruz and colleagues developed a nanomedicine-based platform that might eventually prove fruitful for the clinical treatment of neutrophil hyperactivity. Nanoparticles can be packaged with drugs, and the surfaces of the particles can be conjugated with ligands that specifically target them to disease-associated cells and tissues. Although this approach has been most extensively characterized in the context of cancer, some recent efforts have focused on developing neutrophil-targeting nanoparticles. However, these approaches have not always been unique to neutrophils (for example, targeting Fc or scavenger receptors that are also found on other cell types), and furthermore they have not had specificity for activated neutrophils.

In the new study¹, liposome-based nanoparticles were labelled with a peptide derived from the reactive-centre loop of alpha-1 antitrypsin, an abundant inhibitor of neutrophil elastase and other serine proteases in solution. Given that neutrophil elastase is only found on the neutrophil surface upon activation and degranulation, the authors posited that this approach would direct the nanoparticles to activated (but not resting) neutrophils. The peptide showed good specificity for elastase as compared with other neutrophil-derived and plasma proteases such as proteinase 3 and plasmin. Peptide-coated nanoparticles associated with the surface of mouse and human neutrophils activated with *N*-formylmethionine-leucyl-phenylalanine in vitro, and a minority of the nanoparticles were internalized and trafficked to lysosomes. When injected into mice, peptide-labelled (but not unlabelled) nanoparticles could be found in close association with lipopolysaccharide-activated neutrophils.

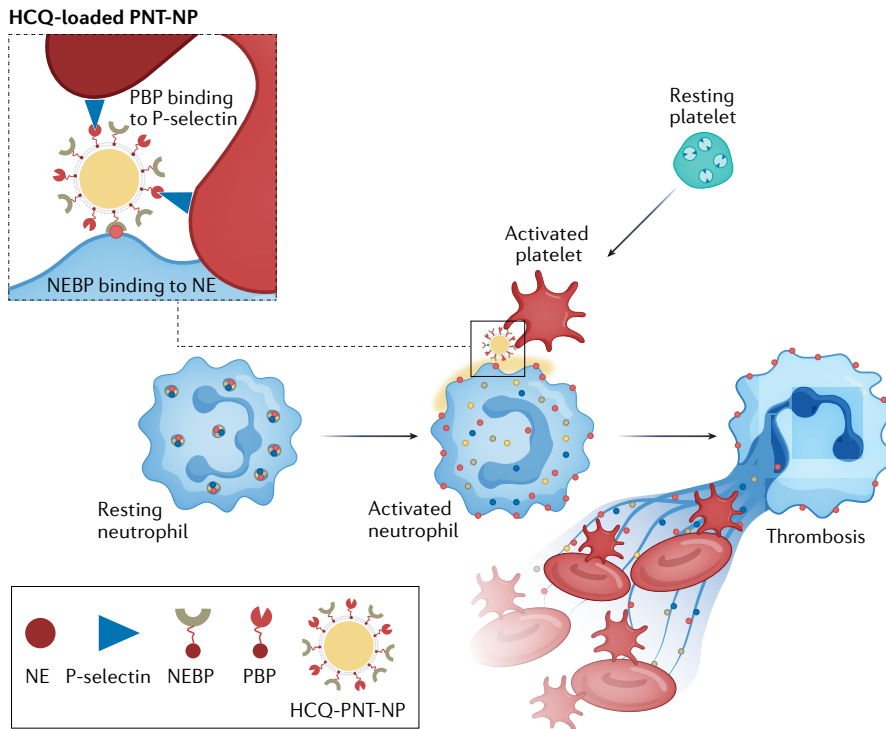


Fig. 1 | Illustration of therapeutic nanoparticle targeting. Hydroxychloroquine (HCO)-containing platelet-neutrophil-targeted nanoparticles (PNT-NP) are conjugated with a neutrophil elastase binding peptide (NEBP) derived from alpha-1 antitrypsin, which engages neutrophil elastase (NE) on activated neutrophils, and with P-selectin-binding peptide (PBP), which interacts with P-selectin on activated platelets. HCO-PNT-NP have the potential to reduce thrombus formation.

Communication between neutrophils and platelets, including the formation of neutrophil-platelet aggregates, is known to occur in both physiological (for example, in the resolution of infection) and pathological (such as thrombosis) disease states⁹. Through the formation of such aggregates, platelets support various neutrophil effector functions such as chemotaxis and NET release. In the study conducted by Cruz et al.¹, the researchers asked what would happen if nanoparticles were coated with ligands that recognized both neutrophils and platelets (FIG. 1). To add platelet specificity, a peptide was selected on the basis of its known affinity for P-selectin, which comes to the platelet surface upon activation and degranulation. Interestingly, the resultant heteromultivalent nanoparticles, which were capable of targeting both neutrophils and platelets, demonstrated synergistic binding efficacy in activated neutrophil-platelet co-cultures, compared with nanoparticles that targeted either neutrophils or platelets alone.

To assess the potential therapeutic relevance of this approach, nanoparticles with

neutrophil, platelet or multivalent specificity were loaded with the antimalarial autophagy inhibitor hydroxychloroquine, which is known to interfere with NET release¹⁰. The nanoparticles were then tested in a model of inferior vena cava flow-restriction-mediated thrombosis, similar to the model used elsewhere to study APS⁸. Compared with nanoparticles that were either not targeted to cells or not loaded with drug, nanoparticles targeted to any of neutrophils, platelets or neutrophil-platelet aggregates were able to reduce thrombus size in the inferior vena cava model.

Although some progress has been made in defining different neutrophil subsets functionally (such as N1 and N2 neutrophils in the context of cancer), these cells are yet to be fully defined by unique surface markers that would enable them to be specifically targeted⁷. The results of this new study therefore represent an interesting step forward for neutrophil-specific therapeutics, directing nanoparticles only to activated neutrophils with cell-surface expression of neutrophil elastase. One can envision how this approach

might be useful in the setting of acute sterile (or overly exuberant infectious) neutrophil activation, including in patients with emergent rheumatic complications such as diffuse alveolar haemorrhage, catastrophic APS, adult-onset Still's disease and likely others, where drugs could be delivered directly to neutrophils in a way that might help mitigate off-target effects. This concept could be most appealing when one considers molecules such as dipyridamole and phosphodiesterase inhibitors, which have an ability to restore neutrophil homeostasis by boosting intracellular cyclic AMP concentrations, but which have numerous other effects when delivered systemically. Going forward, beyond further refining the pharmacokinetics and targeting associated with this approach, an additional consideration is the extent to which nanoparticle internalization and trafficking will need to be optimized in order to maximize the therapeutic benefits.

Somanathapura K. NaveenKumar
and Jason S. Knight

Division of Rheumatology,
University of Michigan, Ann Arbor, MI, USA.

e-mail: jsknight@med.umich.edu

<https://doi.org/10.1038/s41584-022-00854-9>

1. Cruz, M. A. et al. Nanomedicine platform for targeting activated neutrophils and neutrophil-platelet complexes using an α_1 -antitrypsin-derived peptide motif. *Nat. Nanotechnol.* <https://doi.org/10.1038/s41565-022-01161-w> (2022).
2. Brinkmann, V. et al. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
3. Papayannopoulos, V., Metzler, K. D., Hakim, A. & Zychlinsky, A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J. Cell Biol.* **191**, 677–691 (2010).
4. Zuo, Y. et al. Neutrophil extracellular traps in COVID-19. *JCI Insight* **5**, e138999 (2020).
5. Liu, Y. & Kaplan, M. J. Neutrophils in the pathogenesis of rheumatic diseases: fueling the fire. *Clin. Rev. Allergy Immunol.* **60**, 1–16 (2021).
6. Tambralli, A., Gockman, K. & Knight, J. S. NETs in APS: current knowledge and future perspectives. *Curr. Rheumatol. Rep.* **22**, 67 (2020).
7. Nemeth, T., Sperandio, M. & Mocsai, A. Neutrophils as emerging therapeutic targets. *Nat. Rev. Drug Discov.* **19**, 253–275 (2020).
8. Ali, R. A. et al. Adenosine receptor agonism protects against NETosis and thrombosis in antiphospholipid syndrome. *Nat. Commun.* **10**, 1916 (2019).
9. Denorme, F., Rustad, J. L. & Campbell, R. A. Brothers in arms: platelets and neutrophils in ischemic stroke. *Curr. Opin. Hematol.* **28**, 301–307 (2021).
10. Smith, C. K. et al. Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: an additional proatherogenic mechanism in systemic lupus erythematosus. *Arthritis Rheumatol.* **66**, 2532–2544 (2014).

Competing interests

The authors declare no competing interests