In the current issue of CMI, Chirivi et al. describe the isolation and characterization of a monoclonal antibody that may find applications in numerous inflammatory conditions. This new antibody, called tACPA, inhibits the completion of a specific type of neutrophil cell death. Neutrophils, which are the most abundant white blood cells, die in several different ways. In response to inflammatory stimuli, which may range from bacterial, viral and fungal pathogens to endogenous danger signals, neutrophils die via the release of nuclear chromatin through a breach in the nuclear and plasma membranes. On the outside of the cell, the unwinding of the genomic DNA generates a meshwork of chromatin that carries many of the toxic components of neutrophil granules. Brinkmann et al. named this externalized meshwork a neutrophil extracellular trap (NET) when they first recognized that NETs immobilize and inactivate foreign pathogens. The TACPA antibody developed by Chirivi et al. is innovative in that its proposed mechanism of action relies on the specific recognition of NET chromatin.

The release of NETs follows a complex choreography of enzymatic activities and structural alterations that include modifications of histones in nucleosomes. The structure of a nucleosome (Fig. 1), the basic unit of organization for the genome, consists of eight core histones, two each of H2A, H2B, H3 and H4, which, like spokes on a wheel, form a flat disc of proteins that twist the DNA into a tight coil. Nucleosomes pack against each other in the nuclear so that the full length of the chromosomal DNA is accommodated inside the close confines of the interphase nucleus. The tight packing of chromatin is only possible if the negatively charged phosphate groups along both strands of the DNA are matched by positive charges on lysine and arginine residues contained in histones.

One unique enzymatic modification of histones that precedes the release of NETs involves the conversion of arginine residues in the amino termini of core histones into citrulline residues. The conversion, carried out by peptidyl arginine deiminase 4 (PAD4), is referred to as citrullination. Citrullination reduces the positive charge of histones and thereby frees the termini of histones from interactions with the DNA (Fig. 1). Citrullination of histones by PAD4 is induced in neutrophils that respond to inflammatory stimuli and leads to the release of NETs. Conversely, autoimmuneigtivity against citrullinated histones is observed in several autoimmune diseases, which also present antibodies to other components of NETs. Importantly, NETs are also implicated in biological processes such as blood clotting disorders, wound repair and complications arising from infectious diseases, such as sepsis. The antibodies described by Chirivi et al. could therefore find applications in different clinical situations. Indeed, Chirivi et al. describe the benefits of using tACPA, their antibody to citrullinated histones, in experimental models of autoimmune disease, including rheumatoid arthritis, pulmonary fibrosis, inflammatory bowel disease and even sepsis.

Therefore, it is useful to consider more closely how TACPA may bind to its citrullinated antigens. The structure of the nucleosome compared with an IgG and the relative sizes of the two are shown in Fig. 1 (the bracket equals 10 nm). Because the H2A and H4 histones have homologous amino termini, citrullination generates four identical peptide sequences that project from the octamer of core histones. The TACPA antibody of Chirivi et al. therefore has four equivalent target sequences that may fit well within its combining sites. If so, the binding of TACPA could act as a “clamp” to prevent the nucleosome from unravelling. This may be a probable mechanism to account for the observations of Chirivi et al.

Despite the remarkable scope of research presented by Chirivi et al., the authors did not pursue the detailed molecular mechanism whereby TACPA was able to modify the disease process. They suggested two possible ways that TACPA could reduce the pathogenic effects of NETs. One possibility is that the antibody prevents the total dispersal of NETs. The second is that the specific binding of TACPA leads to the deposition of a highly specific mark on the NET chromatin, such that phagocytes effectively clear the dissipating, amorphous NET chromatin.

If, as indicated by the results of Chirivi et al., TACPA can prevent the release of NETs, the relative stoichiometry at which TACPA is able to accomplish this task remains an unsolved problem. The sheer complexity of nuclear chromatin and the numbers of nucleosomes that each cell releases far exceed the available number of antibody molecules. How could only a small number of antibodies prevent the release of nuclear chromatin? One possible way this could happen is perhaps akin to a small number of determined fighters, who can prevent a large army from passing through a narrow river valley. If the encounter between the small number of antibodies and the large excess of nuclear chromatin occurs at a very restrained opening in the plasma membrane, a small number of TACPA antibodies could perhaps block the full extent of NET release.

There is a possible molecular basis for such a scenario. Recent studies have revealed that one mechanism of NET release requires the formation of membrane pores by a multimer of proteins called gasdermin D. Proteolytic cleavage of gasdermin D activates the protein to multimerize and insert into the plasma membrane, thus forming an 18 nm pore structure. The diameter of the gasdermin D pore could be just large enough to allow small numbers of nucleosomes to escape to the outside of the cells. This could also...
be the ideal place where tACPA could block the further escape of NETs from the cell.

Future studies will need to confirm the broad efficacy of tACPA in various human disease processes and more precisely describe the specific molecular mechanism of its action. Moreover, at least some forms of NETosis proceed with the concomitant proteolytic cleavage of H2A and H4 termini.11 Whether tACPA can win the race against neutrophil proteases that have the advantage of acting on the inside of NETotic neutrophils remains an interesting question.

ADDITIONAL INFORMATION

Competing interests: The author declares no competing interests.

REFERENCES

1. Chirivi, R. G. S. et al. Cell Mol. Immunol. https://doi.org/10.1038/s41423-020-0381-3 (2020).
2. Brinkmann, V. et al. Science 303, 1532–1535 (2004).
3. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Nature 389, 251–260 (1997).
4. Hagiwara, T., Hidaka, Y. & Yamada, M. Biochemistry 44, 5827–5834 (2005).
5. Neeli, I., Khan, S. N. & Radic, M. J. Immunol. 180, 1895–1902 (2008).
6. Gupta, S. & Kaplan, M. J. Nat. Rev. Nephrol. 12, 402–413 (2016).
7. Sørensen, O. E. & Borregaard, N. J. Clin. Investig. 126, 1612–1620 (2016).
8. Wong, S. L. et al. Nat. Med. 21, 815–819 (2015).
9. Xu, J. et al. Nat. Med. 15, 1318–1321 (2009).
10. Chen, K. W. et al. Sci. Immunol. 3, eaar6676 (2018).
11. Pieterse, E. et al. Ann. Rheum. Dis. 77, 1790–1798 (2018).