Beyond Life and Death: The Ultimate Cargo of Aged Neutrophils

Summary: Neutrophils secrete a variety of mediators throughout their lifespan but are mostly associated with pro-inflammatory functions. In this issue of *Cell*, Hsu et al. describe a new class of extracellular vesicles produced solely by aged neutrophils, eliciting anti-inflammatory effects that extend beyond neutrophil lifespan.

Neutrophils are short-lived innate effector cells typically known for their pro-inflammatory functions, releasing powerful mediators like reactive oxygen species (ROS), defensins, cytokines and extracellular traps (NETs). Developed and matured within the bone marrow, neutrophils are produced in large numbers to maintain their presence, with new cells replacing aged ones. Neutrophils acquire enhanced antimicrobial capabilities as they age, following a circadian pattern, and show increased migratory and phagocytic functions during acute inflammation. However, prolonged retention of aged neutrophils can exacerbate vascular injuries, highlighting the need for an intrinsic disarming mechanism to mitigate their toxicity and NET-forming capacity (Adrover et al., 2020). Thus, the accumulation of aged neutrophils has been considered detrimental in disease states. However, in this issue of *Cell*, Hsu et al. provide compelling evidence that aged neutrophils can exert anti-inflammatory functions through secreting a newly identified class of extracellular vesicles, which the authors termed Large Aged Neutrophil-Derived Vesicles (LAND-Vs) (This paper).

Specifically, Hsu et al. show that aged neutrophils innately secrete large CD55+ vesicles that dampen complement activation, reducing inflammatory responses while preserving effective bacterial clearance. This novel anti-inflammatory mechanism demonstrates how neutrophils can extend their functional impact during immune responses despite their short lifespans. The group was able to isolate these LAND-Vs from both murine and human neutrophils, using them to modulate disease severities in their disease models. Inhibition of Rho-GTPases or CD55 expression ameliorates LAND-V formation and its protection, highlighting their significance. Of note, more LAND-Vs were found in patients with milder forms of COVID-19, signifying their strong association with the host's ability to resolve inflammation. The authors also validated their importance by demonstrating their role in a sepsis model, providing additional evidence that LAND-Vs represent a previously undetected bona fide vesicle structure.

The field of extracellular vesicles (EVs) is expanding, and discriminatory markers to identify each class of EV are still lacking. To prove that LAND-Vs are unlike any known EV, Hsu et al. performed several experiments demonstrating their uniqueness in terms of marker expression, vesicle composition, and biogenesis pathways. This was critical evidence, especially to distinguish LAND-Vs from similarly large EVs like migrasomes (Zhang et al., 2023) and exophers. Like LAND-Vs, the appearance of elongated neutrophil derived

structures (ENDS) during septic conditions suggests an overlap or synergy with LAND-Vs (Marki et al., 2020), an area for further investigation. Other EV types similarly possess anti-inflammatory mechanisms, such as microvesicles (Headland et al., 2015) and apoptotic bodies containing alpha-defensins (Miles et al., 2009). Thus, any overlapping characteristics among EVs can suggest shared functions, or are possible altered versions of the same EV type. LAND-Vs are argued to be specifically produced in aged neutrophils, an attribute distinguishing from other classes of EVs. Pro-inflammatory mediators were able to induce their release, attributing to the stage-specificity of LAND-V function.

Notably, a unique characteristic of LAND-Vs is that their abundance peaks during the resolution phase of inflammation, coinciding with the decline in neutrophil numbers. This observation suggests a role for LAND-Vs in restoring homeostasis. In parallel, LAND-Vs, the phagocytosis of apoptotic neutrophils by efferocytic macrophages promotes a reparative polarization state (Fadok et al., 1998), further suppressing inflammation. These mechanisms highlight a dynamic balance between the neutrophil effector phase and the resolution phase. The discovery of LAND-Vs by Hsu and colleagues further refines this intricate interplay, where the prolonged lifespan of neutrophils enhances their effector function, while LAND-Vs help regulate the degree of inflammation.

Besides the reported role of LAND-Vs in complement deactivation via CD55, their potential functions in various diseases remain unclear. It is likely that other membrane proteins, similarly sequestered on lipid rafts, are present in LAND-Vs and may exert biological effects that have yet to be defined. Additionally, the authors have identified CD47 as an additional marker on LAND-Vs, functioning as a "don't eat me" signal, which may further enhance their immunosuppressive activity by preventing their clearance. Considering the ability to generate LAND-Vs by aged neutrophils in culture, as demonstrated by Hsu et al., this finding further supports the potential for scalable production of LAND-Vs as novel anti-inflammatory therapeutic agents. However, further research is needed to fully elucidate their roles in various disease settings. A key area of investigation is their function in cancer, where tumor neutrophils exhibit prolonged lifespans and aged phenotypes (Ng et al., 2024).It remains unclear if these neutrophils secrete LAND-Vs and how these vesicles influence the immunosuppressive microenvironment.

Neutrophil function has been typically studied in their mature circulating forms. However, emerging evidence suggest that neutrophils can exert distinct functions at various stages of their life cycle. For example, the late precursor pre-neutrophils (preNeus) can secrete macrophage colony stimulating factor (M-CSF), promoting monopoiesis through hypocretin signaling (McAlpine et al., 2019). Immature neutrophils, while usually retained in the bone marrow, are mobilized during stress conditions to reinforce the pool of neutrophils available. These immature neutrophils are resistant to apoptosis and secrete higher levels of pro-inflammatory cytokines (Drifte et al., 2013). Interestingly, LAND-Vs are produced exclusively by aged neutrophils, independent of external stimuli. The authors demonstrated that pro-inflammatory signals do not alter LAND-V production, further supporting that this process is driven specifically by neutrophil aging. Taken together, neutrophils are increasing

recognized as multi-faceted cells, regulated by maturation and time, allowing their massive numbers to be fully utilized throughout life cycle (Figure 1). Beyond the array of mediators they release, neutrophils also shed parts of themselves to modulate immune responses. Even after their demise, they continue to exert influence through multiple mechanisms. The discovery of LAND-Vs highlights yet another aspect of neutrophil function, paving the way for further research into their expanding roles in immunity.

References

Adrover, J. M., Aroca-Crevillén, A., Crainiciuc, G., Ostos, F., Rojas-Vega, Y., Rubio-Ponce, A., Cilloniz, C., Bonzón-Kulichenko, E., Calvo, E., Rico, D., Moro, M. A., Weber, C., Lizasoaín, I., Torres, A., Ruiz-Cabello, J., Vázquez, J., & Hidalgo, A. (2020). Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation. *Nature Immunology*, *21*(2), 135–144. https://doi.org/10.1038/s41590-019-0571-2

Drifte, G., Dunn-Siegrist, I., Tissières, P., & Pugin, J. (2013). Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. *Critical Care Medicine*, *41*(3), 820–832. https://doi.org/10.1097/CCM.0b013e318274647d

Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y., & Henson, P. M. (1998). Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *Journal of Clinical Investigation*, *101*(4), 890–898. https://doi.org/10.1172/JCI1112

Headland, S. E., Jones, H. R., Norling, L. V., Kim, A., Souza, P. R., Corsiero, E., Gil, C. D., Nerviani, A., Dell'Accio, F., Pitzalis, C., Oliani, S. M., Jan, L. Y., & Perretti, M. (2015). Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. *Science Translational Medicine*, *7*(315), 315ra190-315ra190. https://doi.org/10.1126/scitranslmed.aac5608

Marki, A., Buscher, K., Lorenzini, C., Meyer, M., Saigusa, R., Fan, Z., Yeh, Y.-T., Hartmann, N., Dan, J. M., Kiosses, W. B., Golden, G. J., Ganesan, R., Winkels, H., Orecchioni, M., McArdle, S., Mikulski, Z., Altman, Y., Bui, J., Kronenberg, M., … Ley, K. (2020). Elongated neutrophil-derived structures are blood-borne microparticles formed by rolling neutrophils during sepsis. *Journal of Experimental Medicine*, *218*(3), e20200551. https://doi.org/10.1084/jem.20200551

McAlpine, C. S., Kiss, M. G., Rattik, S., He, S., Vassalli, A., Valet, C., Anzai, A., Chan, C. T., Mindur, J. E., Kahles, F., Poller, W. C., Frodermann, V., Fenn, A. M., Gregory, A. F., Halle, L., Iwamoto, Y., Hoyer, F. F., Binder, C. J., Libby, P., … Swirski, F. K. (2019). Sleep modulates haematopoiesis and protects against atherosclerosis. *Nature*, *566*(7744), 383–387. https://doi.org/10.1038/s41586-019-0948-2

Miles, K., Clarke, D. J., Lu, W., Sibinska, Z., Beaumont, P. E., Davidson, D. J., Barr, T. A., Campopiano, D. J., & Gray, M. (2009). Dying and Necrotic Neutrophils Are Anti-Inflammatory Secondary to the Release of α-Defensins1. *The Journal of Immunology*, *183*(3), 2122–2132. https://doi.org/10.4049/jimmunol.0804187

Ng, M. S. F., Kwok, I., Tan, L., Shi, C., Cerezo-Wallis, D., Tan, Y., Leong, K., Calvo, G. F., Yang, K., Zhang, Y., Jin, J., Liong, K. H., Wu, D., He, R., Liu, D., Teh, Y. C., Bleriot, C., Caronni, N., Liu, Z., … Ng, L. G. (2024). Deterministic reprogramming of neutrophils within tumors. *Science*, *383*(6679), eadf6493. https://doi.org/10.1126/science.adf6493

Zhang, X., Yao, L., Meng, Y., Li, B., Yang, Y., & Gao, F. (2023). Migrasome: A new functional extracellular vesicle. *Cell Death Discovery*, *9*(1), 1–7. https://doi.org/10.1038/s41420-023-01673-x