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CELL MIGRATION

Arraying neutrophils in swarms

Microscale arrays of protein–polysaccharide clusters enable the functional characterization of human-neutrophil migration.

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Neutrophils (phagocytic granulocytes) are the most abundant circulatory white blood cells in the body. Within a few hours of tissue damage or infection, they can exit the blood vasculature and infiltrate the inflamed tissue to engulf and destroy bacteria and fungi[1]. Because neutrophil development and migration are tightly controlled in healthy individuals, abnormal values in absolute blood neutrophil count can indicate acquired or congenital neutrophil disorders. In addition, the functionality of human blood neutrophils can be tested through the characterization of cell-surface markers by flow cytometry and via *in vitro* measurements of phagocytosis, respiratory burst, microbial killing and cell adhesion[2]. Yet these assays do not provide any insight into how neutrophils coordinate their dynamics and effector processes, as occur in inflamed or infected tissues. Because of technical limitations in the identification of neutrophils at single-cell resolution and in the direct observation of their live dynamics in humans, information about how human-neutrophil populations coordinate the antimicrobial tissue response remains scarce. Writing in *Nature Biomedical Engineering*, Daniel Irimia and colleagues now describe an elegant *in vitro* solution for monitoring the swarming response of human neutrophils, enabling the detailed analysis of neutrophil-derived factors released during swarming[3].

Animal models have helped to uncover the extravascular tissue dynamics of neutrophils in inflamed mammalian tissues. Over the past decade, intravital imaging of mouse strains with cell-specific fluorescent reporters has allowed the direct live observation of neutrophils in tissues of living anesthetized mice[4] and the discovery of a special form of neutrophil-population dynamics, termed neutrophil swarming, where groups of individual neutrophils undergo phases of highly directed and coordinated movement before clustering (Fig. 1a). To date, neutrophil swarms have been seen in several mouse models of tissue wounding, of sterile inflammation and of infection with various pathogens

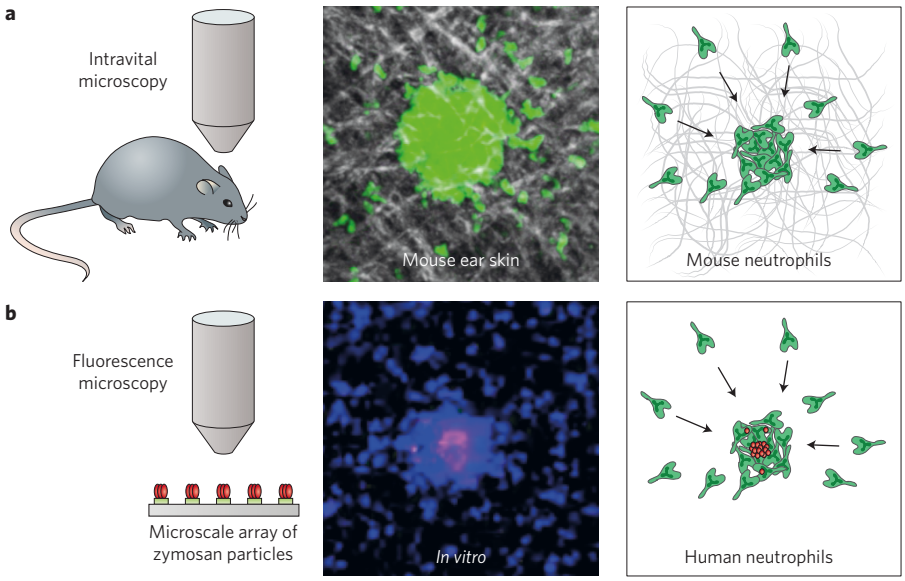

Figure 1 | Comparison of mouse-neutrophil swarming *in situ* and human-neutrophil swarming *in vitro*. **a**, Two-photon intravital microscopy of inflamed and infected mouse tissues has uncovered neutrophil swarming dynamics, such as the formation of a neutrophil cluster in over 1 hour in response to local tissue damage in the dermis of the mouse ear. **b**, Microscale arrays of clusters of zymosan particles (red) trigger the swarming behaviour of neutrophils isolated from human blood[3]. Micrographs (middle) represent an area of approximately 0.1 mm^2. Micrograph in **b** courtesy of E. Reátegui and D. Irimia.

(including bacteria, fungi and parasites[4,5]). The formation of these dense neutrophil clusters is host-protective, contributing to the isolation of healthy tissue from sites of wounding and infection. In contrast, excessive accumulation of neutrophils can interfere with the integrity and functionality of an organ. Therefore, neutrophil swarms are considered an essential process of the body's innate immune response, and mediate a fine balance between tissue protection and destruction.

Detailed analyses of the molecular mechanisms behind neutrophil swarming are especially challenging in the complexity of mouse tissues, and impossible in humans. The systemic or local administration of blocking antagonists or chemical inhibitors globally interfere with protein function in all cells in the treated tissues and are often inconclusive regarding cell-specific effects. Moreover, the exact local concentration of blocking reagents at a certain tissue region can only poorly be controlled, which makes conclusions difficult when blocking effects are not observed. Furthermore, the exact tissue geometry and cellular composition is specific for a particular microscopic field of view, which limits comparisons of neutrophil behaviour between different tissue sites[6]. Hence, most intravital-imaging studies in mice have remained descriptive, categorizing swarms according to their morphology, dynamics and persistence. Systematic screening of candidate molecules for mediating neutrophil swarming has recently been carried out in mice and provided an initial map of signals controlling swarm formation[7]. However, no single signal could be identified that uniquely mediates swarming, suggesting that there exists a complex, multilayered network of pro- and

anti-inflammatory signals that fine-tune the neutrophil swarming response.

By using soft-lithography techniques, Irimia and co-authors developed large microscale arrays of fluorescent particle clusters of zymosan (protein–carbohydrate complexes, prepared from the cell wall of the non-pathogenic yeast *Saccharomyces cerevisae*, that represent a classic experimental fungal stimulus for immune cells) amenable to study neutrophil swarms of any species, including human neutrophils. The clusters of zymosan particles were manufactured by first micropatterning a cationic copolymer polyelectrolyte of acrylamide before precisely trapping controlled numbers of the negatively charged zymosan particles. The zymosan particles can bind to specific cell-surface receptors on neutrophils to activate their oxidative burst, the secretion of pro-inflammatory mediators and phagocytosis (uptake of the particle by the phagocyte). The authors found that both the size and the spacing of zymosan-particle clusters were critical for triggering neutrophil swarm formation. Smaller clusters of one or two zymosan particles were taken up by individual neutrophils and did not induce swarming. However, clusters of more than three zymosan particles and spacings larger than 20 μm between clusters were required for inducing neutrophil swarming.

Irimia and co-authors' microarrays allow for the synchronized induction of thousands of neutrophil swarms. Thus, these microarrays in combination with live-cell imaging represent a high-throughput, standardized and precisely controlled experimental system to study neutrophil swarming (Fig. 1b). The authors confirmed existing data from mouse studies and provide new molecular insight into the swarming process of human neutrophils in response to zymosan particles. In particular, they show that human neutrophils undergo three distinct dynamic phases of swarming: scouting, swarm growth, and swarm stabilization (as had been observed for mouse neutrophils in models of small ear-skin injuries[8]). By micropatterning glass slides with zymosan particles of different size next to each other, the authors mimicked *in vivo* situations of transient neutrophil swarming, where cells move out of smaller swarm centres and are attracted to larger nearby clusters (the formation of multiple transient swarms is a common phenomenon of mouse tissues diffusely infected with bacteria or fungi[2]). The authors also show that multiple chemoattractants synergize to optimize the formation of human-neutrophil swarms. Moreover, they quantified molecules released by swarming neutrophils into the medium supernatant by lipid and protein profiling and identified unexpected candidate molecules for the modulation of swarming. A major advantage of the *in vitro* microarray approach is that the low volume of supernatant enables a sensitive readout for measuring low amounts of signalling molecules released by neutrophils at different stages during swarming. Notably, the authors used the approach for the analysis of clinical samples, identifying impaired swarming behaviour of neutrophils in patients suffering trauma, autoimmune disease and sepsis. They also show that neutrophils isolated from patients recovering from trauma regained their ability for optimal neutrophil swarming. Hence, the technology also holds promise for therapeutic screening and monitoring in the clinic.

Irimia and colleagues' microarray technology builds on recent developments in the fields of microfabrication and microfluidics, and aims at mimicking aspects of immune-cell migration in physiological tissues[9–11]. Similar micropatterning technology could be used for the investigation of neutrophil swarming in response to other microbial stimuli and even living pathogens, to provide insight into how pathogens shape the secretome and swarming behaviour of neutrophils, and could also serve as a basis for new treatments to enhance the ability of neutrophils to contain areas of tissue infection. Moreover, the addition of other immune cell types (such as monocytes and macrophages) is likely to bring Irimia and co-authors' technology even closer to the physiological situation, where tissue bystander cells may contribute to neutrophil swarming dynamics. Hence, the authors' functional assay for the characterization of human neutrophils may hold predictive value for neutrophil migration in infected human tissues. ❐

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References

1. Nauseef, W. M. & Borregard, N. *Nat. Immunol.* **15,** 602–611 (2014).
2. Dinauer, M. C. *Methods Mol. Biol.* **1124,** 501–515 (2014).
3. Reátegui, E. *et al. Nat. Biomed. Eng.* **1,** 0094 (2017).
4. Kienle, K. & Lämmermann, T. *Immunol. Rev.* **273,** 76–93 (2016).
5. Lämmermann, T. *J. Leukoc. Biol.* **100,** 55–63 (2016).
6. Lämmermann, T. & Germain, R. N. *Semin. Immunopathol.* **36,** 227–251 (2014).
7. Lämmermann, T. *et al. Nature* **498,** 371–375 (2013).
8. Ng, L. G. *et al. J. Invest. Dermatol.* **131,** 2058–2068 (2011).
9. Irimia, D. & Ellett, F. *J. Leukoc. Biol.* **100,** 291–304 (2016).
10. Sackmann, E. K., Fulton, A. L. & Beebe, D. J. *Nature* **507,** 181–189 (2014).
11. Vargas, P. *et al. J. Immunol. Methods.* **432,** 30–34 (2016).