DISPATCH

Immunity: Neutrophil Quorum at the Wound

Miguel Palomino-Segura and Andres Hidalgo*

Area of Cell and Developmental Biology, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid

*Correspondence: ahidalgo@cnic.es

How is neutrophil swarming initiated after an injury? A new study provides evidence of exquisite coordination between these immune cells, akin to quorum sensing in unicellular microorganisms, to protect tissues from invading pathogens.

Mechanisms of communication among 'equal' cells are common in nature and serve to orchestrate highly coordinated functions. Over 50 years ago, an intriguing type of cell– cell communication in bacteria was identified that depended on cell density and regulated the behavior of the entire population of cells. In this type of chemical communication, each cell synthesizes and secretes a membrane-permeable molecule, called the autoinducer, that can be detected by neighboring cells. Once the amount of the autoinducer reaches a critical threshold, it regulates gene expression and behavior of all proximal cells. This phenomenon — later termed quorum sensing — was shown to be critical to generate synchronized responses in bacteria, such as biofilm formation [1] (Figure 1A). This phenomenon, however, is not limited to unicellular organisms, as recent studies have described a similar type of communication in cells from multicellular organisms, such as immune cells [2]. Indeed, during immune reactions, some cell types can associate in clusters that may be subjected to a similar quorum-type regulation. For example, in response to infection and injury, neutrophils accumulate in the injured tissue and move in 'swarms' due to the coordinated release of a strong chemoattractant, leukotriene B4 (LTB4) [3,4]. Despite the enormous interest of the immune community in this behavior, the mechanism underlying this coordinated behavior, as well as its physiological purpose, has remained unknown. A new article from Poplimont *et al.* [5], published in this issue of *Current Biology*, now provides molecular, cellular, and functional evidence that links a quorum-sensing-type phenomenon with neutrophil swarming.

Neutrophil swarming is one of the most remarkable phenomena discovered by imaging living tissues in recent years. Unfortunately, because this behavior is difficult to replicate *in vitro*, the lack of tools to monitor biochemical signals *in vivo* has precluded the dissection of the molecular events leading to the coordinated synthesis of LTB4 during swarming. To overcome this caveat, Poplimont *et al*. [5] developed various genetic tools in the zebrafish model to track calcium fluxes and 5-lipoxygenase (5-LO), two potentially important components of the swarm. In particular, because 5-LO activation is a key event necessary for LTB4 synthesis [6], these authors generated a fluorescently tagged version of this enzyme to allow visualization of its active form when it translocates to the nuclear envelope. Imaging calcium fluxes, on the other hand, allowed the authors to score transfer of signals between proximal cells that accumulated in the nascent swarm.

Using this elegant imaging set-up, the authors show that LTB4 synthesis occurs in calcium-fluxing neutrophils near the wound, implying that this chemotactic signal emanates from clusters forming at the wound area. This study therefore represents a leap forward in the field as it demonstrates that initial neutrophil–neutrophil contacts are critical to initiate swarms.

When the findings of Poplimont *et al.* [5] are compared with models of cooperative response in bacteria, evidences supporting a quorum-sensing-type mechanism arise. In their model, neutrophils take advantage of local propagation of 'calcium alarm signals' to induce a synchronized response (i.e., LTB4 production) only when a sufficient number of cells are near the wound. It is interesting to speculate what specific signal plays the role of the autoinducer (a membrane-diffusive molecule) in this multicellular scenario. The authors find that ATP, a signal from dying cells, leaks out to the extracellular necrotic environment and enters neutrophils via connexin 43 hemichannels. Influxing ATP, in turn, activates a different type of channel that enables calcium entry in the incoming cells. By analogy with the classical concept of quorum sensing in bacteria, the various roles played by ATP suggest that this molecule functions as an autoinducer-like signal in this multicellular context: first, it is present at high levels in the area of injury; second, it diffuses from cell to cell and elicits calcium fluxes and subsequent signaling, i.e. it coordinates neutrophils by allowing the influx of additional signaling molecules (calcium); finally, when the number of cells thus influenced by ATP reaches a certain threshold, the coordinated production of LTB4 elicits a new type of behavior, in this case swarming towards the wound (Figure 1B).

The work of Poplimont *et al*. [5] describes a cooperative model that needs to be integrated with previous studies of neutrophil swarming. Swarm formation is divided into several sequential phases: initial scouting of individual neutrophils, followed by an amplification phase, final cluster formation, and resolution [4,7]. While the coordination/quorum model presented in the Poplimont *et al*. study aligns easily with the initial phases of swarming, it raises new questions about its relevance in later phases. For example, how are these signals balanced during cluster stabilization? It is possible that the ATP-to-neutrophil ratio, which should be high in nascent clusters, rapidly declines as new neutrophils arrive, eventually reaching values in which the released ATP may not be sufficient to promote more LTB4 synthesis, thereby preventing excessive cluster growth. In contrast to this autoregulatory mechanism, reinforced calcium fluxes by newly recruited neutrophils may promote swarm growth. In addition, because calcium fluxes at the leading edge of migrating neutrophils contribute to define their direction of movement [8], it is tempting to speculate that calcium fluxes present in the growing cluster favor migration towards areas with high neutrophil density, further favoring swarming patterns.

Likewise, while this quorum-type process is useful to initiate swarms, how is it turned off during the resolution phase? Are regulatory mechanisms, such as quorum 'quenching' [9], triggered by the neutrophils themselves or other neighboring cells? The recent finding that neutrophils from chronic granulomatous disease patients, which produce very low amounts of reactive oxygen species (ROS), form larger swarms [10] has suggested a role for ROS in limiting swarms, perhaps by modulating ion channel activity [11]. Similar links may exist with neutrophil extracellular traps, the formation of which is amplified by ATP, depends on ROS, and varies depending on population density [12,13]. Also relevant to the resolution phase, it is unclear whether monocytes or macrophages recruited to neutrophil swarms at late stages contribute to resolve this positive feedback cascade [7]. Interestingly, another quorum-type mechanism has been

proposed for 3 monocyte-derived cells [14]; in this case, accumulation of cells that produce nitric oxygen (NO) may allow for the attainment of NO levels that prevent phagocyte accumulation by blocking cellular respiration and by decreasing ATP:ADP ratios. It is intriguing to consider that these two quorum-type mechanisms, including the one reported by Poplimont *et al*. [5], could encounter and antagonize each other during the resolution phase of inflammation.

A fundamental question addressed by this study is the extent to which swarming is beneficial for the host. This is relevant in the light of studies showing that resident macrophages actively 'cloak' small, recurrent lesions to curb amplification of tissue damage by swarming neutrophils [15]. Does this imply that swarming is always a maladaptive response of the immune system? By using a model of infection with *Pseudomonas aeruginosa* around preformed wounds, Poplimont *et al.* [5] show that neutrophil-specific depletion of connexin 43 compromises cell–cell communication, swarm formation, and exacerbates bacterial spread and mortality **.** These findings suggest that neutrophil coordination leading to swarming at wounds is protective, as it allows for the formation of 'plugs' that contain bacterial entry through the wound. This is in line with other reports suggesting that swarming protects the host by physically isolating sterile tissue from potential invasion by microorganisms [7]. In the context of the study showing active cloaking of lesions [15], the work from Poplimont *et al.* [5] satisfactorily explains why a critical task of tissue macrophages is to prevent leakage of danger signals like ATP from small necrotic areas. Along these lines, this work also raises the possibility of preventing neutrophil swarms in the context of pathological inflammation by targeting mechanisms of cross-communication as a new form of immunotherapy.

Many more new questions arise from the discovery that neutrophils use quorum-type responses to form antimicrobial or tissue-damaging swarms. Nonetheless, this study by Poplimont *et al.* [5] is an exciting first step towards understanding group behavior and synchronized responses in the mammalian immune system and should encourage interest in exploring similar coordination in other immune cells and in different pathophysiological scenarios.

References

1. Nealson, K.H., and Hastings, J.W. (1979). Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. *43*, 496–518.

2. Antonioli, L., Blandizzi, C., Pacher, P., Guilliams, M., and Haskó, G. (2019). Rethinking communication in the immune system: the quorum sensing concept. Trends Immunol. *40*, 88–97.

3. Lämmermann, T., Afonso, P. V, Angermann, B.R., Wang, J.M., Kastenmüller, W., Parent, C. a, and Germain, R.N. (2013). Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. Nature *498*, 371–375.

4. Ng, L.G., Qin, J.S., Roediger, B., Wang, Y., Jain, R., Cavanagh, L.L., Smith, A.L., Jones, C.A., de Veer, M., Grimbaldeston, M.A., *et al.* (2011). Visualizing the neutrophil response to sterile tissue injury in mouse dermis reveals a threephase cascade of events. J. Invest. Dermatol. *131*, 2058–2068.

5. Poplimont, H., Georgantzoglou, A., Boulch, M. Walker, H.A., Coombs, C., Foteini 4 Papaleonidopoulou, F., and Sarris, M. (2020). Neutrophil swarming in damaged tissue is orchestrated by connexins and cooperative calcium alarm signals. Curr. Biol. 30, XXX-XXX.

6. Goulet, J.L., Snouwaert, J.N., Latour, A.M., Coffman, T.M., and Koller, B.H. (1994). Altered inflammatory responses in leukotriene-deficient mice. Proc. Natl. Acad. Sci. USA *91*, 12852–12856.

7. Kienle, K., and Lämmermann, T. (2016). Neutrophil swarming: an essential process of the neutrophil tissue response. Immunol. Rev. *273*, 76–93.

8. Beerman, R.W., Matty, M.A., Au, G.G., Looger, L.L., Choudhury, K.R., Keller, P.J., and Tobin, D.M. (2015). Direct In vivo manipulation and imaging of calcium transients in neutrophils identify a critical role for leading-edge calcium flux. Cell Rep. *13*, 2107–2117.

9. Pietschke, C., Treitz, C., Forêt, S., Schultze, A., Künzel, S., Tholey, A., Bosch, T.C.G., and Fraune, S. (2017). Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts. Proc. Natl. Acad. Sci. USA *114*, E8488–E8497.

10. Alex, H., Scherer, A., Kreuzburg, S., Abers, M.S., Zerbe, C.S., Dinauer, M.C., Mansour, M.K., and Irimia, D. (2020). Neutrophil swarming delays the growth of clusters of pathogenic fungi. Nat. Commun.

11, 2031. 11. Kiselyov, K., and Muallem, S. (2016). ROS and intracellular ion channels. Cell Calcium *60*, 108–114.

12. Sofoluwe, A., Bacchetta, M., Badaoui, M., Kwak, B.R., and Chanson, M. (2019). ATP amplifies NADPH-dependent and -independent neutrophil extracellular trap formation. Sci. Rep. *9*, 16556.

13. Schauer, C., Janko, C., Munoz, L.E., Zhao, Y., Kienhöfer, D., Frey, B., Lell, M., Manger, B., Rech, J., Naschberger, E., *et al.* (2014). Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat. Med. *20*, 511–517.

14. Postat, J., Olekhnovitch, R., Lemaître, F., and Bousso, P. (2018). A metabolismbased quorum sensing mechanism contributes to termination of inflammatory responses. Immunity *49*, 654–665.e5. 15. Uderhardt, S., Martins, A.J., Tsang, J.S., Lämmermann, T., and Germain, R.N. (2019). Resident macrophages cloak tissue microlesions to prevent neutrophildriven inflammatory damage. Cell *177*, 541–555.e17.

Figure 1. Coordinated responses in bacteria and neutrophils. (A) Quorum sensing in bacteria. Each bacterium produces a membrane-diffusible molecule called the autoinducer. When bacteria grow over time, cell density increases together with the concentration of the autoinducer in the extracellular milieu. At a certain concentration threshold, the autoinducer triggers a coordinated biological response (e.g. biofilm formation) in the autoinducer-rich area. (B) Neutrophils generate a coordinated response akin to that of bacteria, following the quorum-sensing paradigm. In response to tissue injury, pioneer neutrophils reach the necrotic area where ATP is being actively 5 released and spreads throughout incoming cells in a contact-dependent manner, via connexin 43 (Cx43) hemichannels. The increasing concentration of ATP

in the nascent cluster opens channels that enable propagation of calcium in the incoming cells, leading to the coordinated biosynthesis of leukotriene B4 (LTB4) and swarm formation.