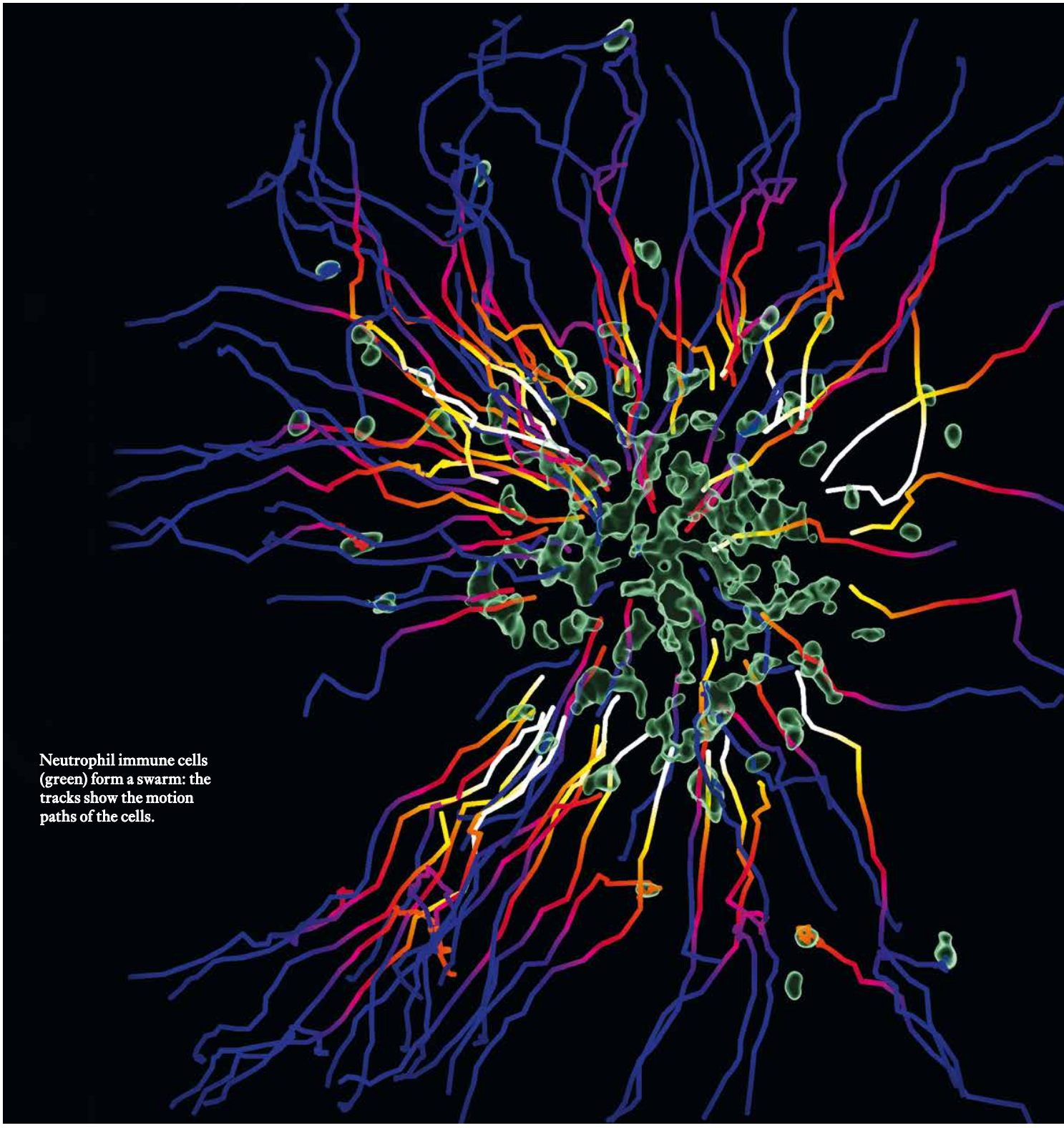

Neutrophil immune cells (green) form a swarm: the tracks show the motion paths of the cells.

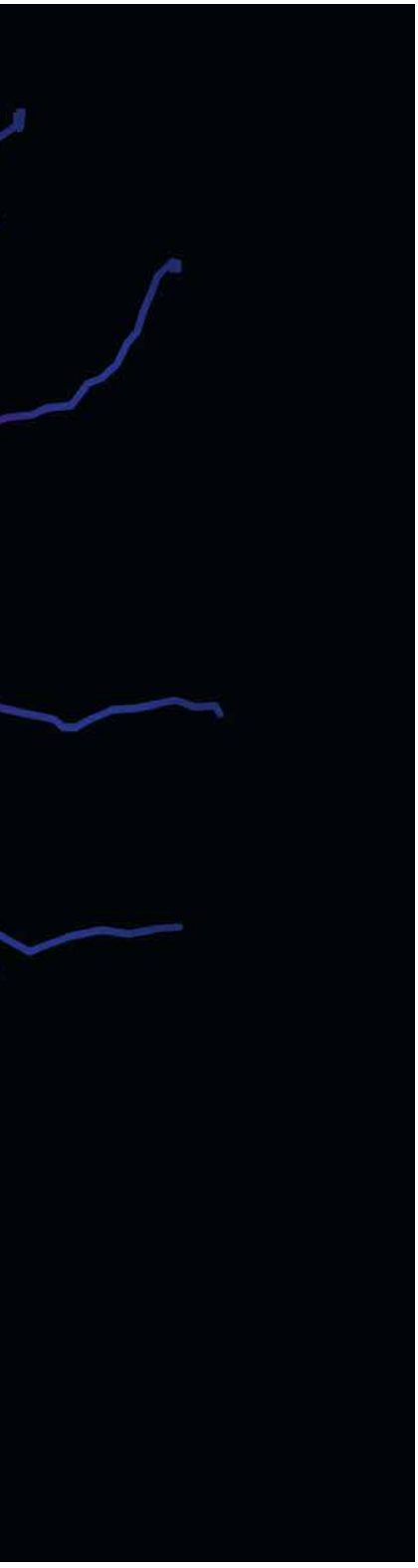

PHOTO: TIM LÄMMERMANN/MPI OF IMMUNOBIOLOGY AND EPIGENETICS

ATTRACTANTS FOR THE COLLECTIVE

TEXT: TIM LÄMMERMANN & HARALD RÖSCH

Max Planck scientist Tim Lämmermann is investigating how immune cells hunt pathogens in swarms. The cells exhibit a behavior that biologists will also be familiar with from an insect – the Asian honey bee.

PHOTO: LENA GIOVANAZZI FOR MPG

Katharina Glaser working on her doctoral thesis in Tim Lämmermann's lab. Here, she is isolating neutrophil granulocytes.

In a battle between females, the Asian honey bee doesn't stand a chance against a hornet. Yet the bee will be able to stand its ground against the intruder – if it calls upon its sisters for help. If an attacking hornet injures a bee, pheromones are released that alert the other female workers in the hive. Large numbers of them rush in and form an impenetrable ball around the hornet with their bodies. The temperature rises so much inside this ball that the hornet is literally "cooked" and dies from overheating.

Although at first glance, immune cells may seem to have little in common with bees, they behave quite similarly when defending. In particular, cells of the innate immune response use the same tactics when they encounter pathogens. Guided by various alert and messenger substances, they converge on the intruders from all directions. Unlike the bees, however, these cells do not kill the intruders by overheating them. Rather, they shield the pathogens off from the surrounding healthy tissue and render them harmless using their chemical weapons. "It is a fascinating finding that the swarm behavior of immune cells and insects follows similar rules to some extent – even though they are completely different entities of life," says Tim Lämmermann.

Hunters and guardians of the immune system

Lämmermann and his team at the Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany, are concentrating on so-called neutrophil granulocytes. These immune cells (which are also simply referred to as neutrophils) are created in the bone marrow and patrol throughout the entire body in the blood. As guardians and hunters, they form an important part of the innate immune system and are among the first to be on the scene of inflammation or an infection. Thanks to their flexible form, they move forward like amoebas, slipping through the walls of blood vessels and even dense tissue. By means of molecular sensors on their surface, the neutrophils detect alerts from injured or damaged cells. Intruders such as bacteria, parasites and fungi are killed off by means of antimicrobial substances.

Fifteen years ago, researchers used special microscopy to observe remarkable behavior of neutrophils in different organs of mice: as soon as the scientists induced inflammation or pathogens infected the tissue, these immune cells converged from every direction. They be-

"The swarm behavior of immune cells and insects follows similar rules to a certain extent."

TIM LÄMMERMANN

haved like a swarm and attacked the pathogens in a coordinated way. "Even then, it was obvious that the cells were coordinating with each other, although it was not yet known how they were doing it," recalls Lämmermann. Lämmermann arrived at the topic of swarming immune cells in a roundabout way. After earning his doctorate, the scientist, who carried out research at the National Institutes of Health in the USA initially focused on macrophages. These innate immune cells also hunt down pathogens and eliminate them. "It quickly became apparent that macrophages are 20 to 100 times slower than neutrophils, which made the experiments time-consuming and tedious. Given the snail's pace of the macrophages, my visa would have expired long before I had even presented the initial findings," relates Lämmermann with a wink. Looking through the microscope, however, something else caught the researcher's eye. Apart from the lame macrophages, he noticed that there were also neutrophils constantly flitting through the image. "Compared to the sluggish macrophages, it was a real seething mass. As a result, I quickly switched to neutrophils – a decision that's turned out to be dead right." Fascinated by this behavior, Lämmermann henceforth devoted himself to researching the swarming behavior of these immune cells. Since then, he and his team have gained important insights into how the cells congregate in a swarm and how this disperses again. The latter is crucial for ensuring that the immune response does not go too far.

Together with researchers from other institutions, Lämmermann has identified different phases of the swarm formation. Shortly after an injury, a few neutrophils in the immediate vicinity change their pattern of movement and migrate to the site of the inflammatory response in a targeted manner. This first wave is followed by a second wave of cells from more distant regions of the body. The neutrophils are attracted by a mixture of substances released by damaged or dying cells, which they sense with the aid of around thirty receptors on the cell surface. The researchers have discovered that just one single dying cell is often enough to summon the neutrophils. However, it is not yet understood in detail which alert substances from the location of the tissue damage or dead cells attract the neutrophils in this early phase.

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PHOTO: LENA GIOVANAZZI FOR MPG

With the aid of a stereoscopic microscope, which provides a three-dimensional view, researchers can prepare tissue samples for further examination.

According to Lämmermann's findings, a messenger substance is essential for the neutrophils to subsequently be able to get together in a swarm – that substance being leukotriene B4, or LTB4 for short. Activated neutrophils give off this substance externally, but can also sense it themselves thanks to special receptors. They register changes in the LTB4 concentration in the surroundings and migrate towards increasing concentrations. More neutrophils release more leukotriene B4, attracting even more cells. In this way, neutrophils form impressive swarms in which, in some cases, several hundred cells come together at a location where tissue is damaged. "So neutrophils are not complete loners. They communicate with each other when they form a swarm and thus act as a collective," explains Lämmermann. At the inflammation site, the neutrophils then literally "clutch" each other and form an impenetrable cluster. In doing so, they shield the inflammation site from the surroundings and can thus prevent bacteria, fungi and parasites from spreading in the body. They can then effectively take action against the encircled pathogens.

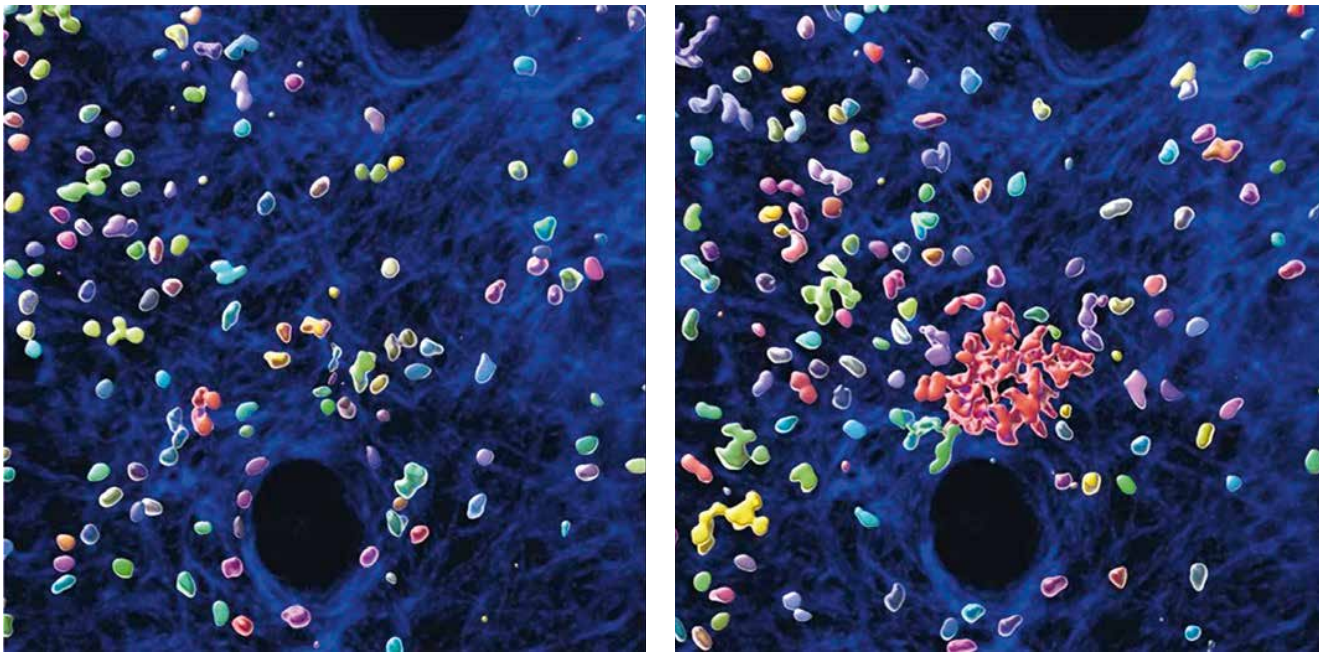
Always follow the attractants

In addition to LTB4, neutrophils also produce the messenger substance CXCL2. This substance, belonging to the chemokine family, also has a swarming effect for these cells. The attractants LTB4 and CXCL2 and the alert substances released by an inflammatory response are, therefore, among the key elements that hold the swarm together. "A swarm of neutrophils follows a simple positive feedback principle," says Lämmermann: "The individual cells move towards the increasing attractant concentration. In this way, even a small group can attract more and more cells and gradually become a swarm." If multiple places in the tissue are "burning", then several adjacent swarms form that sometimes compete for members. The larger ones are at an advantage and simply swallow the smaller collectives.

"The behavior of the neutrophils is reminiscent of the response of Asian honey bees to a hornet attack."

TIM LÄMMERMANN

Focus of inflammation in the skin of a mouse: individual neutrophils, illustrated here in different colors, attract more cells and thus trigger the formation of a swarm (red). There were 30 minutes between the photo on the left and the photo on the far right.



"Consequently, the neutrophils do not need a central authority to direct them; rather, they form a self-organizing system. In many respects, the neutrophil collectives perfectly resemble swarms of certain insect species or even the group behavior of slime molds," says Lämmermann. "According to our findings, however, individual neutrophils do not maintain distance from their neighbors in the swarm, like fish or birds," the scientist adds. After a certain time, the neutrophil swarms disperse again. This serves to prevent the cells from causing damage when they destroy scaffold proteins in their local cluster and literally "eat" holes in the tissue. An immune response that goes too far is often observed in cases of severe inflammation and could be one of the causes of lung damage with severe cases of COVID-19. So the body must balance out the activity of the neutrophils very carefully. In recent years, Tim Lämmermann and his team have, therefore, been increasingly preoccupied with the question of what actually stops the growth of a neutrophil swarm. The researchers' findings show that the cells can even control this behavior themselves and therefore create an optimal balance between the search and destroy phases in the battle against pathogens. "We have observed that, over time, swarming neutrophils become insensitive to their own attractants such as leukotriene B4 – that is, to those signals with which they originally initiated the swarm," Lämmermann explains.

SUMMARY

Some immune cells form swarms of several hundred cells on the hunt for pathogens. In doing so, the cells release substances that attract more cells.

The cell swarms are self-organizing systems that follow a positive feedback principle: the higher the concentration of the attractant, the stronger the attraction.

Over time, the sensitivity of the immune cells to their own attractant decreases. Thus, the swarm can disperse again as soon as the immune response is finished.

This finding came as a surprise because it had previously been believed that messenger substances and signals from other cell types in the tissue would inactivate the neutrophils again and disperse the swarm. Instead, the neutrophil scavenger cells possess a molecular brake that they themselves use to stop their movement as soon as they sense very high concentrations of the accumulating attractants. The brake goes by the name of "G protein-coupled receptor kinase 2" – a protein that ensures that the cells no longer react to the attractant at high concentrations. "This protein causes the attractant signals sensed by the receptors on the cell surface to no longer be transmitted to the cell interior after a certain point," explains Lämmermann. Therefore, neutrophils without this brake continue to react to the attractants, rush around relentlessly in the tissue and thus search an excessively large area for inflammation. As a result, they are unable to combat the pathogens that usually grow at single locations effectively. "Instead of searching around tirelessly, it is far more effective to surround and seal off a focus of infection in the swarm," says Lämmermann.

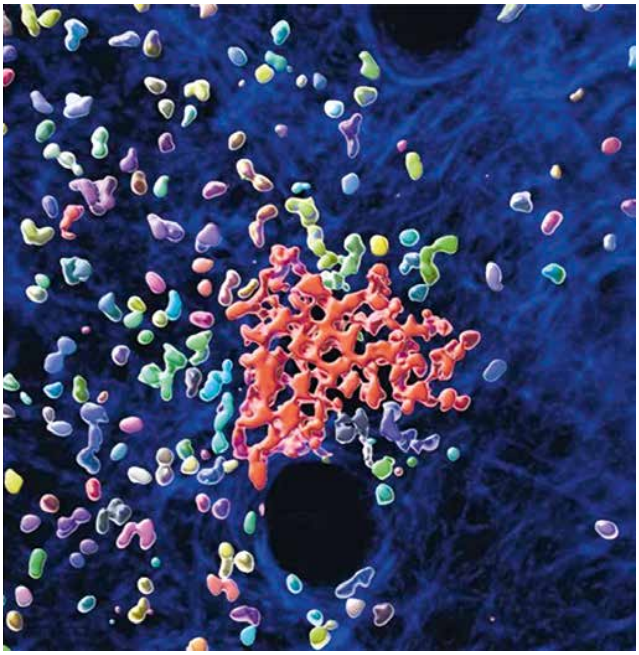


IMAGE: TIM LÄMMERMANN/MPI OF IMMUNOBIOLOGY AND EPIGENETICS

The researchers have thus uncovered an important aspect of the immune defense against bacteria. Their findings provide important stimuli for research into the collective behavior of other cell groups right up to the swarm behavior of higher organisms. "The behavior of neutrophils is actually reminiscent of the response of Asian honey bees to a hornet attack. These bees also behave in a swarm-like way as soon as they smell the substances given off by an injured member of the same species," says Lämmermann. Pheromones emitted by the workers alert other creatures, which then follow the increasing concentration of pheromones and surround the intruder. The hot bee ball resembles the clusters of neutrophils that bring together the antibacterial arsenal of many individual cells in one place. And like the immune cells, the bees do not need a leader; they smell what needs to be done and then simply organize themselves. *www.mpg.de/podcasts/schwarm (in German)*