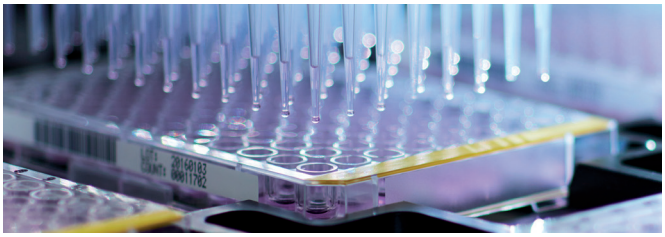
Immunology

Making fine distinctions

By Hubert Filser



Diverse molecular patterns, highly interconnected functional networks, complex signal relays: Robotic pipetting systems help find the critical checkpoints in the immune system.
Photo: Jan Greune

LMU immunologist Veit Hornung's research focuses on how the innate immune system differentiates between endogenous cellular structures and invasive agents.

Life is an unending struggle. At all events, when one considers the functions of the human immune system at the cellular level, terms such as 'attack' and 'defense' present themselves automatically. Our bodies are battlegrounds in which our cells are pitted against the nefarious designs of adversaries such as bacterial cells and viruses that threaten our very lives. Evolution has equipped us with two different defense systems against invasive pathogens – the so-called innate and adaptive immune systems. Both systems must solve what sounds like a simple problem but turns out to be exceedingly complicated: They must be able to distinguish between 'self' and 'non-self' structures, between molecules that are normal constituents of our cells and exogenous or otherwise atypical components that could possibly do us harm.

The innate immune system is our first bastion of defense. "Its significance was underestimated for a long time," says Veit Hornung, Professor of Immunobiochemistry at LMU's Gene Center. "The adaptive immune system, which learns from experience, has always held center stage. Its specially trained B cells and T cells produce specifically tailored antibodies and act as highly specific killer cells, respectively. But in fact, the two systems are functionally linked, and the innate immune system has an important regulatory role." However, many crucial details of how the two systems interact and of the molecular mechanisms that ensure that immune responses are properly directed remain obscure.

Hornung's research group focuses on the innate system and, in particular, on the strategies that enable it to promptly perceive potential dangers to the organism. As an immunologist and physician, he wants to understand how, in cooperation with the adaptive arm, the innate system organizes and coordinates the body's defenses at the molecular level. The details of the molecular interactions explain how the innate system detects foreign genetic material – and how it can sometimes be misled into classifying "self" molecules as dangerous intruders.

Tricked by putative allies

For it has become clear that the innate system not only plays a crucial role in the recognition of bacteria, viruses and deleterious substances, it is also involved in the pathology of widespread disorders such as gout, type 2 diabetes, Alzheimer's disease and atherosclerosis. These conditions are all associated with chronic inflammatory reactions, which are in part due to inappropriate activation of the innate immune system. Agents that inhibit the system could help to bring these reactions under control.

On the other hand, the immune system can be tricked into attacking the body's own tissues in a targeted fashion by "persuading" it that an infection is underway. Researchers hope that a better understanding of this attack mode will enable them to turn the innate system's attentions to certain types of tumors. Indeed recent advances have led to renewed interest in the use of immunotherapy against cancers. Following an initial phase of enthusiasm at the turn of the century – which ended in disappointment – the idea that one could use suitably modified vaccines to mobilize the body's own immunological resources against tumors largely went out of favor. Cancer cells turned out to be more wily than expected. They throw the attackers off the scent by sending out signals that identify them as allies and not foes. Since then, much more has been learned about signaling pathways, and it is now possible in the case of certain cancers to block the action of the signals that "camouflage" the nature of the tumor. This represents a significant step toward the goal of boosting our defenses against tumors.

Partly as a result of these encouraging findings, immunology is undergoing something of a boom. Much of the excitement concerns the innate system, and in particular a class of receptors that are specialized for the rapid recognition of molecules derived from foreign microorganisms. Thus immunologists Jules Hoffmann and Bruce Beutler won the Nobel Prize for Medicine 2011 for elucidating how the innate system is activated by so-called Toll-like Receptors (TLRs). "Among other things, they proved that the innate immune system actually does possess receptors that are capable of recognizing microbial products in the body," says Hornung.

Thanks to refined molecular biological methodologies, it is now easier to identify and characterize such pattern recognizing receptors and the signal pathways they control. Immunologists have in the meantime defined several families of these receptors. Indeed, most, if not all the relevant receptors have now been pinpointed. Hornung's team has discovered some, including AIM2, which recognizes foreign DNA in cells, and Hornung himself is now one of the most highly cited researchers in Germany.

"We now know of six or seven families of pattern recognition receptors," he says. They can be differentiated on the basis of their subcellular localization, their overall structural organization or their modes of action. However, the signal pathways that they regulate are not all known in detail. Thus it is not always clear, for instance, how they activate the immune system and what role they play in the course of infectious or inflammatory diseases, he adds. In some cases, not even the nature of the structures they recognize is known. "What spurs me on is the desire to understand how these systems work at the molecular level and how they are functionally linked in the cell."

In principle, recognition of foreign structures follows a simple scheme. Each receptor possesses a dedicated binding site that acts as a template, which matches a specific molecular structure that is characteristic of an invasive pathogen. As a rule, these receptors bind to certain molecular components of the microbes that serve essential functions – and are thus evolutionarily conserved, i.e. unlikely to mutate. A typical example of such a conserved structure is the lipopolysaccharide coat found on the outer membrane of many bacteria.

Templates based on conserved traits

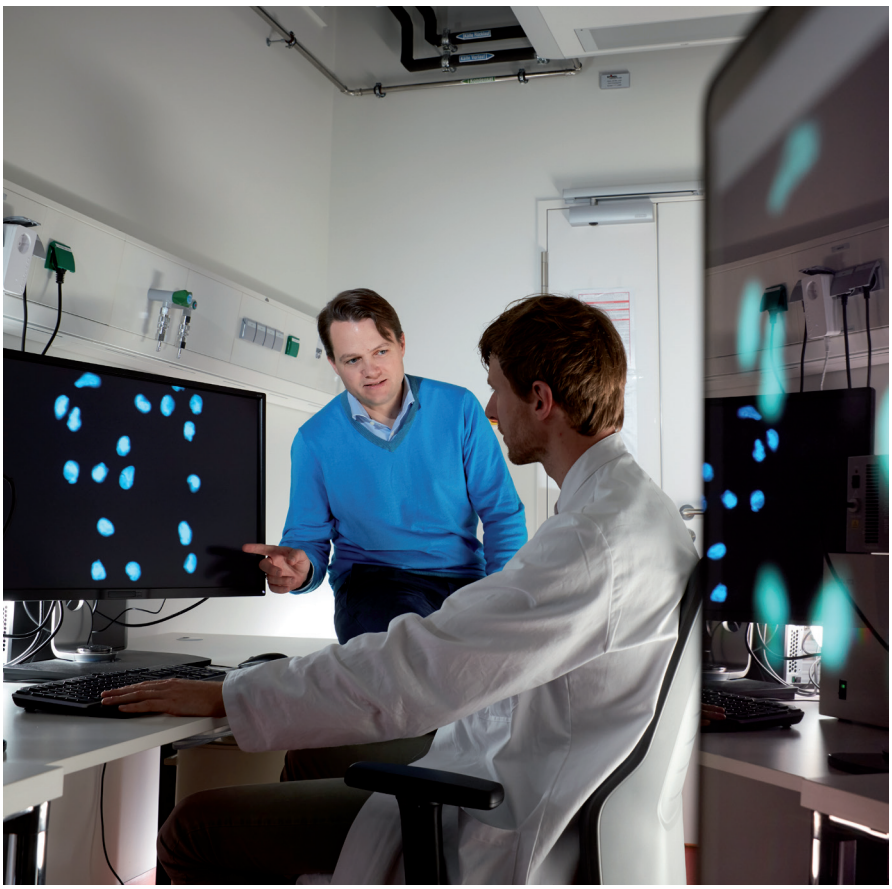
Even the simplest organisms possess such molecular templates for microbe recognition, and they have been refined over billions of years of evolution from single-celled organisms to mammals like ourselves. The genetic blueprints for the synthesis of these proteins are inherited, hence the term 'innate' for the system they serve. The mechanism activated when an invader has been recognized is likewise conserved. Binding of the receptor to the foreign mol-ecule rapidly triggers an alarm that activates an initial – albeit relatively non-specific – immune response, which alerts so-called phagocytic cells that attack and destroy the infected cells. If this response proves insufficient to eliminate the intruders, an inflammation reaction ensues, which results in the secretion of signal molecules that activate the adaptive immune system. In other words, the two systems cooperate closely with one another.

Over the past several years Hornung has focused on one striking feature of the immune system: it is not only capable of reacting to foreign invaders but also to the deleterious alterations they induce in host cells. "It's as if an investigator were able to identify a housebreaker not by virtue of his fingerprints but from the broken lock or the shattered window he has left behind," he says.

From an evolutionary point of view, this indirect mode of sensing danger has the advantage that it is more difficult for bacteria or viruses to subvert it. When a pathogen has gained entry to a cell, it must inevitably perturb the cell's operations and constituents, and this too can betray its presence to the innate immune system. The molecular nature of the pathogen itself is of secondary importance in this context, since the system does not need to recognize any foreign components. Because the principle of "non-self" recognition is at the heart of the adaptive system, many immunologists were skeptical of the idea that the innate system might utilize an indirect mechanism to detect invaders. However, the evidence for such a mechanism is now incontrovertible.

Hornung will in future devote more attention to the ability of the immune system to recognize and react to cell damage, for it is now clear that this mode of immune activation also plays a role in pathologies where microbial invaders play no part. They include some highly prevalent diseases, such as gout and diabetes. "These conditions are characterized by what are called sterile inflammation reactions," says Hornung. "The inflammation reaction triggered by the immune system need not be the primary factor in such illnesses, but it has a decisive and negative influence on their long-term course." The innate immune system is obviously able to detect the consequences of the underlying

metabolic perturbation, in the form of atypical modifications of cell components or other signs of cell damage. Immunologists refer to these characteristic signals as "damage-associated molecular patterns" or DAMPs for short, and they are recognized by the same basic types of receptors that are used to detect the presence of bacteria or viruses. "We would very much like to understand how such receptors recognize molecular patterns generated by specific types of cell damage and why the innate system sometimes overreacts to such signals," says Hornung.



Seeking clues to the workings of defense mechanisms: Veit Hornung (left) and doctoral student Thomas Ebert. *Photo: Jan Greune*

The damage identifies the culprit

The signaling pathways that mediate innate immune responses are usually highly complex. Quite often, several different receptors may contribute to the recognition of bacteria, viruses or cell stress. The proteins involved in signal transduction may generate intermediates which themselves have signaling functions, some more important than others. Researchers are always on the lookout for the central nodes in these functional networks. "One can spend years puzzling over a particular system in vain, simply because one of the elements has not yet been identified," says Hornung. "Once the missing piece has been found, everything falls into place."

Thanks to new technologies, it is now possible to view complex biological systems, even in human cells, in terms of interconnected subsystems. New methods of gene editing have made it much easier to introduce targeted modifications into mammalian genomes. Hornung's group has used the latter to knock out specific genes in cultured cells that are physiologically very similar to human monocytes. Since monocytes play a central role in controlling immune responses in the body, the modified cells provide an ideal model system in which to probe the effects of genetic changes on inflammation reactions and to identify the components that are crucial for signal transduction. And they can also be used to study how crucial receptors are activated. "So we are no longer dependent on the mouse, which has always been the classical model system in immunology."

Hornung and his team have recently used their monocyte system to analyze in detail how secretion of the messenger interleukin-1β (IL-1β) is regulated. IL-1β is a major protagonist in inflammatory reactions and also acts to induce fever in response to infections. To their surprise, the researchers discovered that an alternative and previously unknown pathway is involved in controlling the release of IL-1β by human monocytes. Here, a single stimulus was sufficient to activate the "inflammasome" (receptor complex) NLRP3, which plays a key role in sterile inflammation in gout, type 2 diabetes and atherosclerosis. In mouse monocytes, simultaneous application of two different stimuli is required to trigger NLRP3-mediated secretion of IL-1β. "We believe that this novel signal pathway is a critical element of inflammation processes in humans," says

Hornung. "Human cells behave very differently from murine cells in many respects. Our finding implies that some well-established mouse models used for the study of inflammatory processes may not reflect what happens in our own species."

Nodes in functional networks

Hornung hopes that his modifiable monocytes will facilitate a better understanding of the molecular bases of inflammatory diseases, and perhaps identify targets for the development of new therapies. The inflammasome NLRP3 is a possible candidate. "We are currently doing all we can to understand the function of this important node of the innate immune system. However, it is not yet clear precisely how NLRP3 is activated. All we know is that any kind of stress that results in an increase in the permeability of the cell membrane switches this inflammasome on. The targeted search for an inhibitor of its action can only begin when we know how the whole signal cascade works." In order to identify the components of this cascade, Hornung's group plans to knock out every gene in their human monocytes individually. "It is a huge task, but we believe the effort is worthwhile, in light of the central role we attribute to this mechanism in sterile inflammatory conditions." A better understanding of the process of damage recognition could have significant impact on the treatment of several classical diseases of affluence. Once the signal molecules are known and the architecture of the corresponding signal transduction pathways is clear, it ought to be possible to find new approaches to the therapy of chronic disorders such as gout, diabetes and atherosclerosis.

Prof. Dr. Veit Hornung
Chair of Immunobiochemistry at LMU's Gene Center. Born in 1976, Hornung studied Medicine at LMU, headed a junior research group in the Division of Clinical Pharmacology at the LMU Medical Center and did a postdoc at the University of Massachusetts Medical School in Worcester, USA. In 2008 Hornung was appointed Professor of Clinical Biochemistry at Bonn University Hospital, and later served as Director of the Institute for Molecular Medicine there. He took up his present position at LMU in 2015.

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