

## Medicine

## Caught in a tight corner

By Hubert Filser



When a coronary artery is obstructed, time is of the essence: Ambulance and helicopter take the quickest route to the clinic.  
Photo: Hörhager/dpa

Unrestrained immune reactions promote atherosclerosis. Clinical researcher Christian Weber is studying the web of molecular decisions that lead to this misdirected and potentially fatal response.

It's almost 25 years ago now, but Christian Weber well remembers how he sprinted from the basement to the fifth floor of the Institute of Immunology in Munich, with blood dripping from the crook of his arm. Colleagues had taken a blood sample before he set off, and were waiting at the top to take another. "We wanted to measure the concentrations of different monocyte subtypes before and after stress, and find out where they came from," he recalls. "At the time we had no idea how important these immune cells are in the development of atherosclerosis." The pathogenesis of this serious disease remains a complex puzzle, but it is now clear that the recruitment and functions of various monocyte subtypes play a key role in the process. By the time this recognition dawned, Weber was already an established investigator in the field.

This flashback underlines how far atherosclerosis research has come in a quarter of a century, and how far biomedical science has penetrated into the underlying mechanisms in its attempts not only to understand the condition, but also how best to treat it. At one time, those interested in vascular biology regarded immune cells, including monocytes, as bystanders, as markers for the disease state. Now, like Christian Weber, they are engaged on teasing out the

details of all the processes in which these cells are involved. Weber can now describe in detail how fatty "plaques" form in the walls of major arteries, how they provoke chronic inflammation, and why the lipid-rich deposits sometimes rupture, releasing waste products and cell debris into the circulation. This material can in turn obstruct blood flow so severely that a stroke or a heart attack ensues. Indeed, this sequence of events accounts for a large fraction of premature deaths in industrialized countries. "An unrestrained immune response causes pathological deposits to form in the inner wall of the blood vessels, and the inflammatory reactions smolder on for years, stimulating further growth of the plaques", Weber explains.

### Chain reaction that leads to atherosclerosis

We owe the term "atherosclerosis" to the German pathologist Felix Jacob Marchand, who in 1904 derived it from the Greek words for "porridge" and "hardening", to refer to the texture of the fatty plaques and the loss of elasticity of the arteries. Christian Weber, now Director of the Institute for Prophylaxis and Epidemiology of Cardiovascular Diseases at LMU, analyzes the molecular mechanisms that give rise to these plaques. Using innovative methods and

a range of high-tech instrumentation, he focuses on the chronic changes that occur in the endothelium, the thin sheet of cells that lines the arteries. The integrity and function of this boundary layer between the bloodstream and the internal tissues is indispensable for normal physiology. It is also the gateway through which immune cells gain access to the underlying smooth muscle fibers and other tissues. In healthy subjects, the permeability of the endothelium is low, its surface is smooth, and blood flows in a largely undisturbed stream.

The endothelium's gateway function for immune cells in case of emergency is controlled by a battery of signal molecules that act as alarm bells to summon an armada of helpers – in particular white blood cells such as monocytes and neutrophils, whose role is to combat the perceived threat. "Several risk factors for atherosclerosis are known to activate the endothelium in this way. Among them are high levels of circulating fat molecules especially LDL-bound cholesterol – and reactive oxygen species (ROS), found in smokers," says Weber. Oxidation of LDL cholesterol by ROS is thought to initiate the degradation of endothelial function.

Weber is a mine of information on the intricate interactions that link these

## Research

factors and others to their long-term effects, from mechanisms known to promote atherosclerosis to the metabolism of fats in diverse tissues, from the many genes involved to how mutations hasten or slow down the whole complex process. "The interaction of all these factors determines how the drama plays out in each individual," he explains. "There are predisposing factors, as well as protective mechanisms, that operate in the body, and both are important." He produces micrographs showing how genetically labeled immune cells migrate between the cells of the artery wall, and diagrams that schematically depict the interactions of the different cell types, signal molecules and proteins. "The pathological changes primarily occur in segments of the vasculature where flow profiles undergo sudden change, for instance due to branching," he adds.

Weber's team employs sophisticated technology to monitor what happens to the endothelium at these critical branch-points and sharp bends in the arterial tree, such as the super-resolution microscopy techniques that won their inventors this year's Nobel Prize in Medicine, and multiphoton microscopy, which uses pulsed infrared laser light to visualize labeled molecules in living tissues. With these methods, researchers can follow monocytes or neutrophils as they migrate between endothelial cells, in real time. Weber focuses on what happens on the endothelial surface. The receptors CCR1 and CCR5 play a key role in the recruitment of monocytes, because they bind to the chemokine CCL5 on the endothelial surface. Indeed chemokines not only mediate monocyte recruitment, they make the arterial wall more permeable to other cells and activators of inflammation. "Agents that repress CCR1 and CCR5 or block the binding of their partner li-

gands may offer a new approach to the treatment of atherosclerosis," Weber remarks.

Damage to the boundary layer caused by exposure to noxious stimuli is what starts the chain reaction that leads to atherosclerosis. This attracts circulating immune cells that recognize damaged and necrotic cells. "These cells act as watchdogs in the bloodstream," says Weber, "they are a kind of early warning system. They express chemokines that attract other specialized immune cells to the site of damage, such as monocytes or neutrophils, whose job is to eliminate the harmful substances."

#### Provoking chronic inflammation

The monocytes differentiate into macrophages under the influence of cytokines like interleukin or interferon. Macrophages are "phagocytic" cells – they eat and digest dead cells, cell debris and excess fats. However, uptake of too much fat impairs their digestive function and can even kill them. As a result, distressed and dead cells, macrophages bloated with fat, accumulate, forming the plaque. The inflammation reaction eventually becomes chronic, as the growing plaque continues to send out distress calls in the form of biochemical signals, and the immune response goes into overdrive.

"The endothelium then summons the next wave," says Weber, and here new signals attract a different class of immune cells, so-called dendritic cells, to the plaques. These cells have more specific targets in view. Dendritic cells instruct another set of immune effectors – so-called T-cells – to attack cells that express certain proteins in their surface membranes. They do so by themselves displaying fragments on their surfaces

to which specific subsets of T cells bind. T-cell activation can also initiate antibody formation by so-called B cells. "Their function in the development of atherosclerosis is so far unclear", Weber says. His own research on this phase focuses on the chemokine CCL17, which is secreted by certain dendritic cells. CCL17 is essential for the activation and maintenance of T-cells, but in doing so it can inhibit regression and resolution of the inflammatory reaction. "As a consequence, the inflamed cells and cell debris can never be fully disposed of," says Weber. The plaques emulsify and cholesterol crystallizes, provoking a renewed burst of signaling.

However, there are some hopeful signs that this vicious circle can be broken. Weber and his colleagues have shown that the CCL17 produced by dendritic cells interferes with a self-regulating circuit that limits the local immune reaction – and thus enables the inflammation to persist. Weber believes that this mechanism offers a promising target for a therapeutic agent. "Using an antibody against CCL17, we have been able to arrest the progression of atherosclerosis in an animal model," he says.

Without such interventions, the maladaptive inflammatory response ultimately runs out of control completely. Around the core of each plaque – made up of cell debris – macrophages and T-cells, both recent immigrants and the products of local cell division, continue to accumulate. And a physiological response which normally protects the organism by rapidly eliminating noxious substances that manage to penetrate the body's natural barrier tissues, such as the skin or the mucous membranes of the gastrointestinal tract, threatens to destroy its host.



## Research



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The primary goal of clinical researchers like Christian Weber is therefore to find ways to arrest this pathological cycle of reactions and restructuring processes – as early as possible – by, for instance, blocking the action of the relevant receptors or their chemokine ligands, thus preventing transmission and amplification of the potentially fatal signals. Weber has meanwhile identified another possible point of attack for such an approach. His team has shown for the first time that different chemokines can interact to form complexes. Moreover, one such complex produced by platelets is responsible for allowing immune cells to squeeze between endothelial cells into inflamed tissue, and thus furthers the development of atherosclerosis.

In his “Atheroprotect” project, which has received 2.5 million euros in funding from the European Research Council (ERC), Weber is investigating the biological significance of such interactions

between chemokines further, hoping to uncover their possible roles in the fine-tuning of inflammation processes. “In my group, chemokines are our housepets,” he says. “They mediate the relevant intercellular communication and recruit the various cell types involved in the whole process. The interaction between chemokines is actually my favorite topic. It is like a language; a sentence is made up of different words, but it only makes sense when taken as a whole.” Or one can think of it as a long phone number, consisting of a country code, an area code and the extension number. By interrupting the dialing sequence, as it were, it should be possible to stop the whole inflammation process. So Weber’s aim is to discover strategies and substances that allow the different signal molecules to be selectively inhibited or reactivated as required.

But that is not all he has in mind. Together with his coworker Andreas

Schober, Weber demonstrated some years ago that so-called microRNAs in endothelial cells play an important role in the earliest phase of atherosclerosis. “MicroRNAs have a significant function in the regulation of gene activity”, Weber says. As the name implies, miRNAs are short fragments of single-stranded RNA. They are related to the longer messenger RNAs transcribed from the genomic DNA that direct protein synthesis, but they serve to inhibit the synthesis of proteins in a targeted fashion. Weber has shown that two of these RNA fragments, referred to as miR-126-3p and miR-126-5p, activate the repair of the endothelium following initial damage. In other words, they form part of a protective mechanism. In the absence of miR-126-5p, fatty deposits build up particularly in segments of the arterial tree where normal laminar flow is perturbed. As Weber and his colleagues recently reported in the journal “Nature Medicine”, a population of self-renewing endothelial cells normally resides at these sites, which acts as a reserve supply to replace damaged and dysfunctional ones. Lack of miR-126-5p leads to loss of these precursors, and this exacerbates the impact of known risk factors, such as high fat levels. In a mouse model, the researchers demonstrated that miR-126-5p, encapsulated in nanoparticles, could be successfully delivered to the endothelial cells at these endangered points in the arteries – and slowed the progression of atherosclerosis.

Of course, there are also relatively simple behavioral ways to reduce risk. Give up smoking, be careful what and how much you eat, and don’t sit around all day. Although the effects of sporting activities have yet to be confirmed at the molecular level, numerous epidemiological studies support the beneficial impact of physical exercise. One



## Research

of the latest shows that even periods of strenuous exercise seem to be more effective than half an hour of jogging," Weber says, "though we don't yet fully understand the underlying mechanism." Seen in this light, sprinting up five flights of stairs 25 years ago wasn't such a bad idea!



Prof. Dr. med. Christian Weber

Is the Director of the Institute for Prophylaxis and Epidemiology of Cardiovascular Diseases and holds a Chair in Preventive Vascular Medicine at the Munich University Medical Center. Born in 1967, he served as Professor of Cardiovascular Molecular Biology at the RWTH in Aachen, and holds a professorship at the Cardiovascular Research Institute at the Maastricht University in the Netherlands. In 2010, he was awarded an Advanced Grant by the European Research Council (ERC). In October he assumed the role of Spokesperson for the new Collaborative Research Center (SFB) 1123 funded by the German Research Foundation (DFG).

