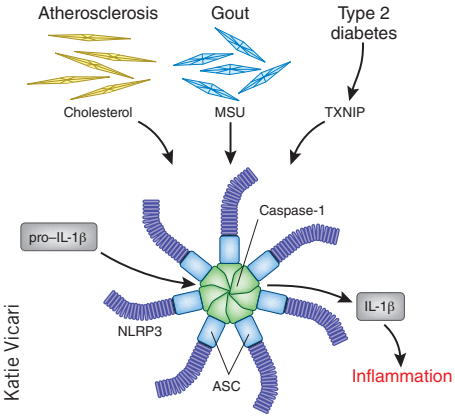

Figure 1 The NLRP3 inflammasome as common inflammatory node of complex diseases. The inflammasome is a heptamer formed by monomers containing the NLRP3 protein that serves as a receptor, the adaptor protein ASC and the enzyme caspase-1. When assembled in an inflammasome, NLRP3 ligation activates caspase-1, which processes pro–IL-1β into the bioactive IL-1β cytokine. Pathogen-associated molecular patterns, monosodium urate (MSU) crystals, which lead to gout, and cholesterol crystals, which are involved in atherosclerosis, can activate the NLRP3 inflammasome. In type 2 diabetes, a series of reactions involving translocation of TXNIP protein promotes NLRP3 activation. In all three conditions, increased production of IL-1β is a common trait that contributes to disease progression.

atherosclerosis than those reconstituted with wild-type bone marrow[13]. However, the crucial role of NLRP3 observed in this study could not be confirmed in a double-mutant experiment crossing atherosclerosis-prone (*Apoe*$^{-/-}$) mice with NLRP3-deficient mice[15]. The reason for this discrepancy is unclear and could be due to differences in cholesterol metabolism or in the role of IL-1 isoforms in the different models. It is also possible that minor differences between donor and recipient strains could cause alloresponses that contributed to the different outcome in the bone marrow transplantation experiment or that other genes carried over with the targeted ones could have a role in the compound knockout study.

That cholesterol leads to vascular inflammation and atherosclerosis by activating the inflammasome remains an attractive hypothesis, albeit one that requires more studies before it can be confirmed or rejected. It is evident from animal experiments that IL-1 is a major proatherosclerotic factor[16], and it is expressed in human atherosclerotic arteries[17]. Recently initiated clinical studies with the interleukin-1β–targeting antibody canakinumab, should clarify whether IL-1β blockade prevents recurrent clinical events in individuals with atherosclerotic cardiovascular disease. If so, it may become a new therapeutic approach in cardiovascular medicine.

In ten years, the inflammasome has gone all the way from its discovery to becoming a target for clinical therapy. It has turned out to be a cause of monogenic diseases and a contributor to complex syndromes (**Fig. 1**), and much more will probably be learnt from this little cellular machine. The story of the inflammasome thus provides an example of how basic research on molecular pathways, when performed in close contact with the world of clinical science and practice, can be transferred into major clinical progress in a very short time. A prerequisite is, as always, creative individuals with a broad vision and an ability to cross boundaries. Jürg Tschopp, who recently died at the peak of his scientific activity, provided us with one of the best recent examples of how to bring discoveries from bench to bedside.

COMPETING FINANCIAL INTERESTS
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■ BEDSIDE TO BENCH

Finding the culprit in your heart

Ira Tabas

Cardiovascular disease, the leading cause of death worldwide, is caused by atherothrombotic vascular occlusion, which leads to unstable angina (progressive ischemia of heart muscle), heart attacks, sudden cardiac death and stroke[1]. Atherothrombosis is a focal pathological process in medium-sized arteries, notably those that feed the heart and brain, and comprises two distinct stages. The first involves a decades-long accumulation of lipids, macrophages and other inflammatory cells, and extracellular matrix in the subendothelial space, or intima, of the blood vessel wall. This process alone does not cause acute cardiovascular events, because blood flow is preserved through outward remodeling of the arterial wall or, in the setting of gradual lumenal encroachment, new vessel formation[2].

However, in a small percentage of lesions, acute, occlusive lumenal thrombosis is triggered, leading to ischemia or death of distal organ tissue. When these disease-causing lesions are identified by imaging techniques or at autopsy, they are termed 'culprit lesions' because they are deemed responsible for causing the arterial occlusion. But what is different about the lesions that progress to the point that they cause these acute events and end-organ damage compared to those that do not? This question was addressed in a unique clinical study by Stone *et al.*[3], which reports findings that have important implications for basic research into culprit lesion formation.

Autopsy studies of subjects that die of an acute atherothrombotic vascular event, such as a massive heart attack, have found that culprit lesions are distinguished not by lesion size but rather by several distinct morphological features, including collections of dead cells, often referred to as 'necrotic cores', and erosion or rupture of the scar tissue, or 'fibrous cap', that overlies the lesion[4]. But these studies are retrospective and sample only one point in time and thus cannot be used to draw firm conclusions on the process of culprit lesion formation. Prospective analysis would require serial 'sampling' of lesions during a period in which new atherothrombotic vascular events occur.

Although serial tissue sampling in humans is not possible, serial imaging of coronary arteries is feasible. In this context, Stone *et al.*[3] studied individuals who suffered an acute cardiac event and thus required a coronary artery catheterization procedure to reestab-

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lish lumen patency at the site of the culprit lesion. During this therapeutic procedure, the investigators imaged many nonculprit lesions in the coronary artery circulation by combining serial angiography with a technique called intravascular ultrasound (IVUS). After a median follow-up of ~3 years, an acute atherothrombotic clinical event occurred in 135 individuals, who then underwent repeat angiography that identified the sites of the new thrombosis. Through comparisons with the earlier images, the authors could identify the earlier lesions that gave rise to these new culprit lesions.

As expected, some of the new events were caused by recurrence at the sites of the original culprit lesions. But many events occurred at sites that several years previously were nonculprit lesions. The authors asked whether any features of these 'preculprit' lesions predicted clinical progression. Consistent with previous data, most lesions that progressed did not show marked lumenal narrowing at the earlier time point, but two features, large necrotic cores and thin fibrous caps—which together characterize 'thin-cap fibroatheroma'—were highly predictive of progression to culprit lesions and the only independent risk factor for major cardiovascular events (**Fig. 1**).

How do these findings open new avenues in basic research in this area and help us identify new molecular targets for potential therapeutic intervention? Atherosclerosis consists of numerous, heterogeneous cell biological processes, and different lesions in the same individual vary in terms of which processes are dominant. For example, most lesions have many cholesterol-loaded macrophage foam cells, others have fibrous tissue as the major feature and still others have the dangerous features identified in the above study. Initial lesion formation and progression of mostly early- to mid-stage lesions can be prevented, or even regressed, by lowering the concentration of plasma atherogenic lipoproteins[5]. But can more specific therapy be directed at potential time bombs such as the lesions with the highest potential to progress to the culprit stage in high-risk patients? Knowing which lesions among the heterogeneous mix are most likely to progress and the mechanisms involved is crucial, considering that, on average, less than 5% of lesions in individuals at risk progress to the culprit stage[4].

One key attribute of the lesions identified in the study, intimal necrosis, forms as a consequence of primary or secondary necrotic death of macrophages[6]. Primary necrosis refers to caspase-independent, nonapoptotic cell death[7], whereas secondary necrosis results from inefficient clearance, or 'efferocytosis', of apoptotic cells, which then become necrotic[6]. Most work has focused on the relevance and mechanisms of secondary necrosis of lesional macrophages. For example, evidence in humans and animal models has shown that prolonged endoplasmic reticulum (ER) stress can induce advanced lesional macrophage apoptosis[8] and that efferocytosis of apoptotic macrophages may go awry in advanced atherosclerosis[6].

But further work is needed to address crucial gaps in this field. Although *in vivo* causation studies have established a role for ER stress in advanced lesional macrophage death[8], it is not yet known whether molecules known to be present in advanced atheromata and to induce ER stress and apoptosis in cultured macrophages, such as saturated fatty acids or 7-ketocholesterol, are actually responsible for lesional cell death *in vivo*. Altering their accumulation in lesions and then measuring the effect on apoptosis will be needed to address this issue.

Another gap is the molecular basis of defective efferocytosis. One idea is that c-mer proto-oncogene tyrosine kinase (MERTK), a receptor that recognizes and engulfs apoptotic cells and shown to function in early atheroscle-

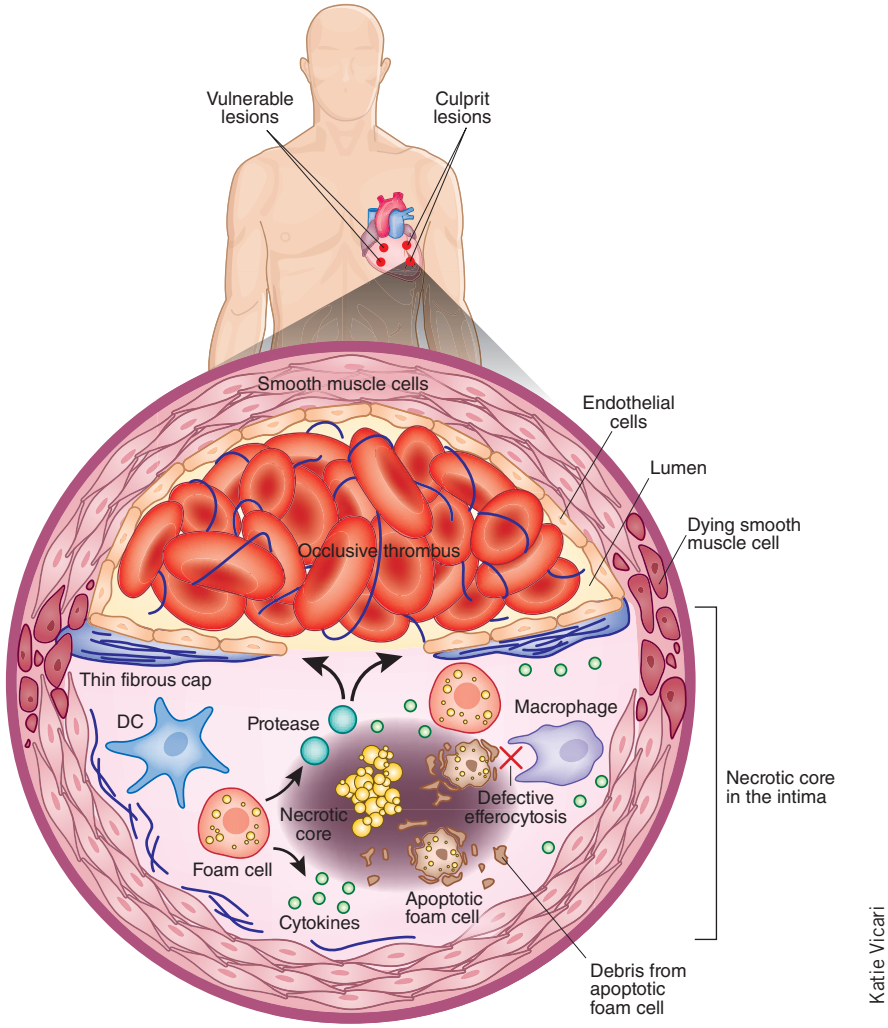

Figure 1 Features of a culprit atherosclerotic lesion. Lesions that are identified *post hoc* as having caused an acute atherothrombotic event have three key features—a large necrotic core, a ruptured or eroded fibrous cap and an overlying occlusive thrombus. The necrotic core is formed by progressive death of macrophage foam cells, accompanied by defective clearance (efferocytosis) of the dead cells by nearby living macrophages or dendritic cells (DCs). The necrotic core, together with inflammatory macrophages and possibly death of collagen-producing smooth muscle cells, leads to thinning and then rupture or erosion of the fibrous cap. The breach in the cap exposes lesional thrombogenic material to the lumen, which can cause acute, occlusive thrombosis and ischemia or infarction of distal myocardial tissue. Earlier, nonculprit lesions that have two key features—a large necrotic core and thinning of the fibrous cap—have a higher probability of progressing to the culprit stage compared with lesions without these features and thus are termed 'vulnerable plaques'.

rotic lesions[9,10], becomes disabled in advanced lesions by protease-mediated cleavage, a process shown to occur *in vitro* in response to inflammatory stimuli[11]. Additional ideas have focused on how other molecules involved in efferocytosis in advanced atherosclerosis might become depleted or dysfunctional[6]. Determining the relevance of these processes to advanced plaques will require additional mechanistic work and new *in vivo* models.

Regarding primary necrosis, *in vitro* studies have shown that macrophages treated with a caspase inhibitor and interferon-γ undergo primary necrosis mediated by the receptor-interacting protein 1 (RIP1)[12]. Testing the effect of RIP1 deletion on macrophage death and plaque necrosis in mouse models of advanced atherosclerosis will help identify whether this mechanism leads to primary necrosis in lesions.

Research on the other feature of preculprit lesions, thinning of the collagenous fibrous cap, has focused on the roles of intimal smooth muscle–like cells (SMCs) in collagen production[13] and macrophage-derived proteases in collagen degradation[14]. The death of SMCs in advanced lesions may lead to decreased collagen production, contributing to plaque thinning[13], but the effect of preventing SMC death on the fibrous cap remains unknown. Mouse models in which matrix metalloproteinases or cathepsins have been deleted or overexpressed have shown effects on plaque collagen or elastin content and integrity, but direct links with plaque rupture and acute thrombosis are not yet possible, as mouse lesions lack these two features[15].

The future challenges related to both lesional necrosis and fibrous cap thinning are substantial and will require development of improved animal models and new imaging techniques in humans[16]. Information emerging from these studies will continue to provide a strong foundation for basic research in how plaques progress, which may help identify new therapeutic targets to prevent the deadly minority of atherosclerotic lesions that trigger the leading cause of death worldwide.

COMPETING FINANCIAL INTERESTS
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