This editorial refers to ‘Enhanced expression of haemoglobin scavenger receptor in accumulated macrophages of culprit lesions in acute coronary syndromes’, by K. Yunoki et al., on page 1844.

Atherogenesis is traditionally traced to endothelial dysfunction, secondary to the interaction between intravascular factors and the luminal vessel surface. Dyslipidaemia, hypertension, smoking, inflammatory cytokines, and advanced glycation endproducts are thought to negatively modify blood and, consequently, the vascular intima. Ensuing endothelial activation and dysfunction are characterized by reduced nitric oxide bioavailability, generation of reactive oxygen species (ROS), expression of prothrombotic and leukocyte adhesion molecules, subendothelial lipid, mononuclear and smooth muscle cell accumulation, and impaired oxygen diffusion from the bloodstream into a thickened and ailing intima. Plaque rupture is also traditionally linked to events within the vascular intima, including fibrous cap thinning, necrotic core expansion, weakened shoulder regions, and sudden bursts of proteases by resident inflammatory cells.

Yet, a large part of the vessel wall is represented by the media and adventitia, perfused by vasa vasorum that supply the outer layers. Proliferation of these neovessels has been recognized for some time to characterize atheromatous lesions. This is not surprising, since intramural hypoxia, inflammation, and apoptosis induce factors (such as vascular endothelial growth factor, platelet-derived growth factor, and transforming growth factor) that promote, within a local proangiogenic milieu, the sprouting of new vessels from existing capillaries. This predominantly ischaemia-driven process can be viewed as initially compensatory, aimed at restoring oxygen availability and at resolving inflammatory/necrotic foci (Figure 1).

Intraplaque haemorrhage is a well recognized feature of plaque vulnerability. Neoformed immature microvessels are devoid of basement membrane and easily leak lipids, proteins, and blood cells in the surrounding interstitium. In this setting, free haemoglobin (Hb) released from leaked red cells is promptly cleared by complexing with haptoglobin (Hp). Hp not only shields Hb from peroxidative modification and prevents Hb-induced oxidative toxicity, but also inhibits prostaglandin synthesis, leukocyte cell—cell and cell—matrix interactions, and proteolysis by cathepsin B, and promotes angiogenesis. A functional Hp 2-2 genotype, found in ~30% of European–North American individuals and in >50% of South-East Asians, determines a polymeric rather than a dimeric Hb–Hp complex that impairs Hb clearance, has weaker antioxidant and anti-inflammatory capacities, but stronger angiogenic effects. Interestingly, an interaction between diabetes, Hp 2-2 genotype, and atherothrombotic events has been described. The Hb–Hp complex is scavenged by high affinity CD163 receptors expressed on a specialized subpopulation of monocytes/macrophages. CD163 expression is reduced by hyperglycaemia, pro-inflammatory cytokines, and oxidative stress, and up-regulated by interleukin-10 (IL-10), glucocorticosteroids, and Hb–Hp complexes.

Yunoki et al. report immunohistochemical analyses performed on atherectomy specimens of culprit lesions from 39 stable and 35 unstable angina patients. The authors compare plaque area, in the two groups of specimens, occupied by macrophages, red cells, and microvessels. Iron content, expression of CD163 relative to total macrophage area, and lipid peroxidation, assessed as 4-hydroxy-2-nonenal (4-HNE) macrophage-positive area, are also compared. Demographics, traditional cardiovascular risk factors, and lumen diameter stenoses were not significantly different between the two patient groups, whereas prevalence of complex stenosis morphology and systemic levels of inflammation, as expected, were higher in unstable compared with stable patients. Foci of macrophages, microvessels, and neutrophils were present in 100, 83, and 57% of the ‘unstable’ culprit plaques, compared with 46, 31, and 5% of plaques from stable patients. Areas of red cell glycophorin A and iron deposition were found in 80 and 66% of unstable plaques, compared with 38 and 26% of stable plaques. Furthermore, CD163 expression and 4-HNE content were considerably higher in unstable than in stable plaques. Interestingly, in the combined patient population, linear relations were found among neovascularization, expression of CD163, and glycophorin A and 4-HNE content.

This study has the merit of elegantly showing a close association between intraplaque haemorrhage and unstable phenotype in patients undergoing coronary atherectomy. It also clearly correlates neovascularization with intraplaque haemorrhage, and the latter with lipid peroxidation and with the expression of...
CD163-positive macrophages. Notably, diabetes mellitus was present in 45% of stable and 37% of unstable patients. Comparison of lesions from diabetic vs. non-diabetic patients, and knowledge of the Hp genotype in stable and unstable patients would have been of interest. Moreover, a limit of the investigation is that the exact chain of events leading to coronary instability cannot be unravelled by the data provided.

Let us try then to put the findings of Yunoki et al. in the context of atheroma growth and disruption that characterizes the natural history of cardiovascular diseases. Atherosclerosis is a chronic process with an important active and ongoing inflammatory component. Inflammation plays an important role not only in plaque growth, but also in promoting atherosclerotic plaque complications. While the inflammatory triggers of the early phases of atherogenesis are relatively well known, the more potent inflammatory triggers of the acute complications are still largely unknown. Indeed, it is still unclear why many patients with severe and extensive atherosclerosis remain stable for years without developing acute coronary syndromes, while others develop acute events as the first manifestation of ischaemic heart disease, frequently in spite of less severe coronary atherosclerosis.13

The observation by Yunoki et al. that microvessels, glycophorin A, and iron content are increased in unstable plaques suggests that haemorrhage may contribute importantly to the process of destabilization. Foci of macrophages and positivity to CD163 were also markedly increased in specimens from unstable patients. On the other hand, although much less represented, all these elements were seen in plaques from stable patients, suggesting partial overlap of a fundamental pathophysiological response to red cell leakage in both stable and unstable lesions (Figure 1). Haemorrhage-associated CD163-positive macrophages are considered, on balance, to perform important scavenging, immunomodulatory, and anti-inflammatory functions, not only by reducing oxidative stress but also by releasing the anti-inflammatory cytokine IL-10.14,15 Furthermore, 4-HNE in moderate concentrations exerts anti-inflammatory effects, by inhibiting the activation of nuclear factor-κB (NF-κB) and the transcription of inflammatory gene products.16 Thus, CD163 and 4-HNE may contribute to the maintenance of a critical balance between red blood cell leakage and ROS neutralization, thus promoting plaque stability.

In the unstable plaque, it is likely that still elusive inflammatory triggers lead to activation of resident inflammatory cells, thus breaking the balance that characterizes the stable plaque. Pro-inflammatory cytokines released by activated inflammatory cells, among other things, strongly promote neovascularization and permeability, which may result in enhanced red cell leakage and generation of free Hb.3,4 If the latter is not promptly bound by Hp and cleared by CD163-positive macrophages, the ensuing burst of oxidative stress leads to ROS generation, overcoming local scavenging capacities (Figure 1). At high concentrations, 4-HNE becomes pro-inflammatory and, in particular, chemotactic for neutrophils.17 Consistent with this, in the study by Yunoki et al.,11 neutrophils were rather specific for unstable plaques, suggesting a potentially unique role for neutrophils in breaking the ‘balance’. Accordingly, a recent study showed transient activation of telomerase in neutrophils from unstable plaques, but not in peripheral neutrophils from the same patients nor in those from stable plaques.18

In conclusion, the work of Yunoki et al.11 offers several valuable cues: it is an important reminder to keep a global view on the vessel wall, looking not only at luminal events but also at the outer layers, for the development of atherothrombosis. It focuses our attention on intraplaque haemorrhage as a potential precipitating factor for acute coronary syndromes, given its role in rapid plaque expansion and in fuelling inflammation. It also underscores the role of free Hb in producing oxidative stress, caused by excessive accumulation, reduced Hp binding, inefficient macrophage function, or a combination of these mechanisms. The challenge for the future is improving our understanding of

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**Figure 1** Hypothetical pathophysiological pathway in stable and unstable plaques. In stable plaques, intramural hypoxia and chronic inflammation induce sprouting of immature microvessels from vasa vasorum that leak blood into the interstitium. Haptoglobin–haemoglobin complexes are cleared by CD163-expressing macrophages. These cells exert antioxidant, anti-inflammatory, and scavenger functions that maintain a ‘stable balance’ between production and removal of debris and of reactive oxygen species. In unstable plaques, acute inflammation enhances hypoxia-induced neovascularization, vascular permeability, and blood extravasation. Antioxidant defences are saturated and macrophage scavenger function is engulfed. Oxidative stress in excess of antioxidant capacities fuels leukocyte activation, thus creating a vicious circle which tips the balance towards instability. Broken lines = inhibition. IL-10, interleukin-10.
the elusive triggers and mechanisms of inflammation that break the balance operating in stable plaques, leading to coronary instability. The demonstration of a profound perturbation in the T lymphocyte repertoire associated with acute coronary syndromes may help in this challenging enterprise.19.

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References