

Atherosclerotic Plaque Rupture Identifying the Straw That Breaks the Camel's Back

Yung-Chih Chen, Alex L. Huang, Tin S. Kyaw, Alex Bobik, Karlheinz Peter

The idiom “the straw that breaks the camel’s back” describes scenarios where seemingly minor incidents eventuate in a sudden, unexpected, and often detrimental downfall. In the case of atherosclerosis, over time multiple subclinical cellular events result in the development of unstable, vulnerable atherosclerotic lesions, which leads to the rupture of atherosclerotic plaques, culminating in the often catastrophic clinical manifestation of myocardial infarction or ischemic stroke. In this review, we first summarize lessons learned from autopsy studies, clinical investigations, and animal models mainly published in *ATVB*. We then present recent experimental studies published in *ATVB* that shed light on the underlying pathophysiological development of plaque instability and disruption, and how differential and sometimes maladaptive responses at cellular levels contribute to this complex process. These many publications in *ATVB* are a testament to the journal’s leading role in research on atherosclerotic plaque instability, an area of research with utmost translational relevance.

Characteristics of Plaque Instability (Vulnerability) in Humans

Our understanding of the pathology of unstable, vulnerable atherosclerotic plaques is mainly based on a limited number of postmortem examinations of human coronary arteries and the analysis of resected surgical specimens from patients who underwent carotid endarterectomy and performed for primary/secondary prevention of transient ischemia attack or stroke.^{1–3} The histopathologic definition of unstable plaques includes, as the most central features, thin fibrous caps (thickness <65 μm) with paucity of vascular smooth muscle cells, inflammatory cell infiltrates (eg, variable degrees of lymphocyte and particularly macrophage contents although the thin fibrous cap is most often infiltrated), localized proteolytic activity, and large lipid/necrotic cores (which typically account for >40% of the total lesion volume).^{4–7} Importantly, repeated subclinical plaque ruptures, histologically and potentially macroscopically visible as intraplaque hemorrhage, are described as an

additional cause of plaque instability.⁸ From an autopsy series of 800 cases of sudden coronary death, 3 independent pathogenic mechanisms were identified, namely, plaque rupture (55%–60%), plaque erosion (30%–35%), and calcified nodules (2%–7%).⁴ However, changes in risk factors (less smoking, more metabolic diseases) and widespread use of statins may result in a shift toward higher prevalence of plaque erosions.⁵

Although postmortem examinations provide invaluable information on histopathologic characteristics of vulnerable plaques, they are inherently limited by selection bias, and only lesions at the most advanced stage have been studied. Furthermore, they are snapshot in nature and cannot provide detailed information on the natural history of plaque progression. This deficiency is, in part, bridged by clinical studies that use intracoronary imaging in patients. In the PROSPECT study (The Providing Regional Observations to Study Predictors of Events in the Coronary Tree), 697 patients were followed up for 3.4 years, and multimodality intracoronary imaging was used to interrogate and characterize lesions in all 3 coronary vessels. Investigators have found that thin-cap fibroatheroma indeed confers higher risk of adverse outcomes (4.9% versus 1.3%; $P < 0.001$). However, the fact that only 4.9% of these lesions eventually give rise to clinical events limits the clinical utility of the criterion of plaque vulnerability, as used in PROSPECT, as a risk-stratifying tool. In addition, this further highlights the knowledge gap and the need for a more mechanistic understanding and better identification of characteristics reflecting plaque vulnerability.⁹

One direction of research is the development of intracoronary imaging methods toward the identification of unstable, rupture-prone coronary plaques. Several elegant examples of this approach have recently been published in *ATVB*. Near-infrared spectroscopy, as a means to determine the lipid content of a plaque, intravascular ultrasound, as a means to predominantly determine the size of the necrotic core and the thickness of the plaque cap, and optical coherence tomography as a means to predominantly determine the cap thickness and plaque surface microstructures (eg, thrombi on plaque microruptures), and these technologies in combination, have been investigated in regard to their ability to identify vulnerable plaques.^{10,11} Whether these measurements of individual morphological characteristics of plaque vulnerability have the prognostic power to be used for clinical risk stratification must be determined in future studies. However, these technologies have been shown to allow monitoring of morphological changes induced by plaque-stabilizing drugs and, as such, have strong potential as surrogate measures in clinical trials for initial proof of concept for plaque-stabilizing drugs.¹⁰

Noninvasive molecular imaging of inflammation, protease activity, or specific cell types is another highly promising approach toward the identification of vulnerable atherosclerotic

Received on: June 21, 2016; final version accepted on: June 24, 2016.

From the Atherothrombosis and Vascular Biology Laboratory (Y.-C.C., A.L.H., K.P.), and Vascular Biology and Atherosclerosis Laboratory (T.S.K., A.B.), Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; and Departments of Medicine and Immunology, Monash University, Melbourne, Victoria, Australia (A.L.H., A.B., K.P.).

Correspondence to Karlheinz Peter, MD, PhD, Atherothrombosis and Vascular Biology, Baker IDI Heart and Diabetes Institute, 75 Commercial Rd, Melbourne, Victoria 3004, Australia. E-mail karlheinz.peter@bakeridi.edu.au

(*Arterioscler Thromb Vasc Biol.* 2016;36:e63–e72.

DOI: 10.1161/ATVBAHA.116.307993.)

© 2016 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>
DOI: 10.1161/ATVBAHA.116.307993

plaques. Magnetic resonance imaging (MRI) and positron emission tomography, and in particular the combination of both, providing soft-tissue structural information and highly sensitive functional imaging, are the technologies with the greatest potential for vulnerable plaque imaging. Examples of this are the imaging of activated macrophages with the positron emission tomography tracer ^{64}Cu -DOTATATE (1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid)-*D*-Phe1,Tyr3-octreotate)¹² in carotid artery plaques in patients, the MRI imaging of ex vivo human carotid plaque-targeting activated platelets with MR contrast particles,¹³ and the combination of ^{68}Ga -Fucoidan imaging of P-selectin and high magnetic strength MRI in mouse plaques.¹⁴ Even less specific positron emission tomography tracers such as ^{18}F -sodium fluoride (NaF) and ^{18}F -fluoro-deoxyglucose are able to detect atherosclerotic plaque instability/rupture in patients.¹⁵ Overall, technical advances in imaging technologies, combined with advances in biotechnology, chemistry, and isotope production toward the generation of novel functional contrast reagents now offer the opportunity to assess whether the identification of vulnerable plaques can improve the management and treatment, and ultimately the outcomes, of patients with atherosclerotic disease.

Biomarkers of Plaque Instability

One of the most controversially discussed areas of atherosclerosis research is the ongoing quest for biomarkers of cardiovascular risk, which primarily aims toward identification of markers that can predict plaque rupture.¹⁶ To address this controversy, the American Heart Association has laid out a framework for the establishment of biomarkers of cardiovascular risk.¹⁷ Although there are several potential candidates, all of these still need to be further assessed to suit the criteria necessary to guide treatment decisions.

Inflammation is clearly a determinant of plaque instability,¹⁸ as seen in many studies demonstrating an association with a vulnerable plaque phenotype¹⁹ and the increased incidence of coronary events in patients with autoimmune diseases.^{20,21} Chronic inflammatory diseases such as asthma are also associated with an increased cardiovascular event rate.²⁰ It is, therefore, no surprise that the inflammation marker C-reactive protein (CRP) has been extensively investigated as a biomarker for cardiovascular risk. Indeed, large-scale epidemiological data have demonstrated that the inflammatory marker CRP predicts cardiovascular events, especially in intermediate-risk patients, and, most importantly, retains its risk-prediction value under therapy.²² However, the role of the high-sensitivity CRP assay for treatment decisions has not been fully established, and high-sensitivity CRP is not broadly applied as a risk marker for the prediction of plaque rupture.²²

Several potential new biomarkers of cardiovascular risk have recently been described in *ATVB*. Plasma levels of soluble interleukin-2 receptor α , which is a marker of T-lymphocyte activation, correlate with cardiovascular mortality.²³ Galectin-3 is a potential marker for cardiovascular mortality that, most interestingly, reflects plaque instability rather than overall atherosclerotic lesion growth.²⁴ A recent systematic assessment for potential correlations of adipokines with the histological criterion of carotid artery plaque

instability in patients reveals a strong association of chemerin with plaque instability.²⁵ Consistent with the role of matrix metalloproteinases in destabilizing atherosclerotic plaques, elevated plasma levels of matrix metalloproteinase-12, which can degrade elastin, fibronectin, laminin, and type IV collagen, correlate with an increased rate of coronary events in patients with type 2 diabetes mellitus.²⁶ However, there are also descriptions of plasma biomarkers that correlate with a more stable fibrous plaque phenotype and a lower incidence of cardiovascular events. Rattik et al²⁷ describe smooth muscle cell growth factors (eg, heparin-binding epidermal growth factor) as such markers.

Polymarker approaches using a pattern of protein or micro-RNA markers have been described and may indeed have a higher association with plaque instability than single marker approaches. Proteomic approaches are based on peptides that are released from unstable plaques with protease activity (eg, collagen peptides)²⁸ or on micro-RNAs that are potentially integral parts of the pathology of vulnerable plaques.²⁹

Contribution of Hemodynamics to Plaque Instability/Rupture

ATVB is the primary forum for original work and discussion of data and concepts on the role of hemodynamic parameters in atherogenesis and particularly plaque rupture, involving scientists of various disciplines. The observation that atherosclerosis, under the same systemic risk factors, preferentially develops in certain parts of the arterial tree where the vessel wall experiences low shear stress or turbulent flow, such as at branches and curvatures, is a long-appreciated indication of a direct role of hemodynamic characteristics in atherogenesis.^{30–32} Low shear stress has been identified as a major driver of plaque development and plaque instability, via mechanisms such as accumulation of lipids, macrophages, and other inflammatory cells; overexpression of adhesion molecules and proteases; reduction of stabilizing collagen fibers; increase in necrotic core volume; thinning of fibrous caps; and reduction of endothelial cell coverage.^{33,34} Endothelial cells are the major mechanosensors and a multitude of work demonstrates changes in numerous signaling pathways dependent on the hemodynamic settings experienced by endothelial cells. These changes include altered glucose uptake capacity,³⁵ gene expression,³⁶ epigenetic changes,^{37,38} and shear-dependent neutrophil adhesion.³⁹ Several recent articles in *ATVB* elegantly highlight the importance of the shear-sensing function of the endothelium.^{40–44}

However, low shear stress on its own does not seem to be sufficient to induce plaque rupture.^{45,46} Tensile (wall) stress, which is higher by several magnitudes than wall shear stress, seems to be an important determinant in causing a vulnerable atherosclerotic plaque to rupture.^{45,47} There is a high prevalence of plaque rupture in the proximal coronary arteries, where the tensile stress is higher than the periphery of the coronary artery system,³² indicating the importance of tensile stress over laminar shear stress. With the technical advances in flow measurements in the catheter laboratory, and also with flow measurements using noninvasive technologies, such as

computed tomography and MRI, hemodynamic considerations will increasingly become part of clinical decision making as well.

Animal Models of Plaque Rupture and Erosion

Animal models are crucial for research toward the understanding of atherosclerosis. They allow genetic and pharmacological manipulations, thereby elucidating the complex interplay between different genetic and environmental factors, and they offer the opportunity to develop and test novel therapeutic approaches. Ideally, animal models should recapitulate characteristic features of human plaque instability and rupture, and they should respond to clinically used pharmacotherapy displaying the same effects as seen in humans. In addition, they should be easy to apply and inexpensive, thereby allowing for statistically significant numbers of animals for experimental studies. Indeed, for the understanding of the initiation and progression of atherosclerosis, animal models such as ApoE^{-/-} and LDLR^{-/-} mice have been the basis of in-depth knowledge gain. *ATVB* gives testimony to the successful use of and important findings obtained by these mouse models. However, studies addressing the clinically most relevant pathology of plaque instability and rupture have been severely hampered by the paucity of animal models that reflect the pathology of plaque instability and rupture as seen in humans. *ATVB* is actively engaged in the development of novel animal models of plaque instability/rupture and provides an engaged forum of debate on the strengths, weaknesses, and translational potential of various models.

Sasaki et al⁴⁸ proposed a total ligation model in which the common carotid artery was ligated for 4 weeks and then a cuff was placed on top of the artery with no blood flow. Although this model has shown some of the features seen in human atherosclerosis, the pathogenesis of plaque rupture with artificial hemodynamics and cuff-induced inflammation is not widely accepted as a full reflection of human plaque rupture pathology. Williams et al⁴⁹ report the development of plaque instability and rupture in the brachiocephalic artery (innominate artery), describing the so-called buried fibrous caps as an indicator of previous and healed plaque ruptures. This model has created strong controversy, which is well captured in editorials and pro and con reviews in *ATVB*.⁵⁰⁻⁵²

Recently, several animal models have been designed based on the concept discussed above to create low shear stress and high tensile stress. Jin et al⁵³ describe a ligation and hypertension-induced model of plaque rupture. ApoE^{-/-} mice were subjected to partial ligations of the left renal and common carotid arteries. After 8 weeks, the authors report that all lesions in the left common carotid artery contained features of vulnerable plaques, including abundance of macrophages, high metalloprotease activities, and signs of collagen breakdown. Also, in 50% of these mice, there was evidence of intraluminal thrombosis.⁵³ Chen et al⁴⁷ used a tandem stenosis in the common carotid artery of ApoE^{-/-} mice to create a defined area that is exposed to low shear stress and high tensile stress. This model reflects human plaque instability, including the presence of ruptured thin fibrous caps, accumulation of lipids with large necrotic

cores, intraplaque hemorrhage, intraluminal thrombosis, and strong inflammatory cell infiltration. Importantly, it reflects pharmacological effects of drugs that are known to stabilize plaques in humans.⁴⁷

Recently, Li et al⁵⁴ have reported coronary plaque rupture/erosion in Watanabe Heritable Hyperlipidemia rabbits infused with angiotensin II. The authors provide strong histological evidence of plaque rupture, including fibrous cap break, luminal thrombosis, intraplaque hemorrhage, and a high percentage of mortality caused by myocardial infarction. Interestingly, the authors also describe some cases of plaque erosion as a cause of myocardial infarction. If shown that the response to pharmacological treatment mirrors what we see in humans, then the model described by Li et al⁵⁴ would constitute a compelling model for testing of new, potentially plaque-stabilizing drugs that could prevent myocardial infarction.

With any interpretation of data from the various animal models of atherosclerosis, we should bear in mind that differences between mouse models and human atherosclerosis are to be expected. A recent study in *ATVB* has shown that many genes found to be important for atherosclerotic lesion development in the classical mouse models of atherosclerosis, such as ApoE^{-/-} and LDLR^{-/-} mice, cannot be validated in human genome-wide association studies, highlighting the importance of confirming findings from murine experiments in human and clinical settings.⁵⁵ Also, the terms plaque instability, plaque vulnerability, and plaque rupture may need to be used more prudently in the various animal models of atherosclerosis. Overall, the potential limitation in translating findings from animal models to human atherosclerosis, and particularly plaque instability and rupture as seen in humans, needs to be acknowledged in our animal-based research on atherosclerosis.^{52,56,57}

Cholesterol and Other Lipids Boosting Plaque Instability

Clinical studies have established a close link between low-density lipoprotein cholesterol and plaque instability. Recent studies published in *ATVB* have identified novel contributors, provided novel mechanistic understandings of clinical observations, and also provided alternate avenues for novel therapies. Edsfeldt et al⁵⁸ explored the role of sphingolipids. By analyzing 200 carotid plaques, they have shown that sphingolipids, in particular glucosylceramide, are associated with plaque inflammation and instability and may represent a novel therapeutic target.⁵⁸ Ronsein et al⁵⁹ have found that niacin, although successfully raising high-density lipoprotein, fails to improve ABCA1 (ATP-binding cassette transporter A1)-specific efflux, the main cholesterol exporters in macrophages. This may explain why niacin is limited in its clinical benefits, as observed in the AIM-HIGH trial (The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes).⁶⁰ Melchior et al⁶¹ used an antisense approach to reduce cholesterol oleate packaging and have shown that hepatic knockdown of sterol-O-acyltransferase 2 not only arrests lesion growth but also remodels lesions into a more stable phenotype. This fits well

with clinical data reporting that the capacity for cholesterol efflux is inversely correlated with carotid stenosis and strongly associated with advanced carotid atherosclerotic lesion.⁶²

Intraplaque Hemorrhage as a Consequence and Amplifier of Plaque Instability

Intraplaque hemorrhage is one of the cardinal histological features of vulnerable plaques^{63–65} and is thought to result either from microruptures with intraplaque bleedings or from leaky neovascularization within the plaque.⁶⁶ Recent studies suggest that intraplaque hemorrhage could also be a trigger, rather than just a marker, of plaque instability. It plays a major role in plaque progression through accumulation of erythrocyte membranes, production and retention of cholesterol crystals, erythrophagocytosis, and enlargement of necrotic cores. In addition, it promotes oxidative and proteolytic activities, as well as leukocyte infiltration and inflammation in plaques.^{67–71} One of the key regulators of intraplaque hemorrhage and plaque instability may be hepcidin. Hepcidin degrades the iron transporter ferroportin 1, resulting in retention of iron, accumulation of intracellular lipids, and apoptosis of macrophages.⁷² Adenoviral-overexpressed hepcidin enhanced macrophage infiltration and destabilized plaques.⁷² Inhibition of hepcidin via the bone morphogenetic protein pathway in liver limits atherosclerosis progression by upregulating ABCA1 and ABCG1 (ATP-binding cassette subfamily G member 1).⁷³ These reports provide mechanistic insights into how erythrophagocytosis drives lesion vulnerability. Intraplaque hemorrhage may also release free heme, which is a strong activator for nicotinamide adenine dinucleotide phosphate oxidase and a major source of reactive oxygen species. Mehta et al⁷⁴ have shown that hemopexin, a ligand for heme, plays a protective role in heme elimination and modulates macrophage polarization. An interesting connection between blood pressure and prevalence of intraplaque hemorrhage in carotid plaques in patients (up to ≈22%) was recently reported.⁷⁵ The stiffer the arterial system, the more intraplaque hemorrhages were found.⁷⁶ The latter 2 reports indicate the relevance of intraplaque hemorrhage in human plaque instability.

Platelets as Therapeutic Targets in Plaque Instability?

Platelets play a pivotal role in atherogenesis. Their binding to the endothelium precedes the appearance of leukocytes in plaques and, as such, platelets are early players in atherogenesis.⁷⁷ Also infusion of activated platelets accelerates atherosclerosis.⁷⁸ Furthermore, platelets are clearly involved in the often fatal event of thrombotic vessel occlusion occurring with plaque rupture. However, there are only a few studies available that address the role of platelets in plaque instability. Platelets clearly adhere to inflamed endothelial cells that cover atherosclerotic plaques.¹³ They induce monocyte extravasation and foam cell formation, and thereby contribute indirectly to plaque destabilization.⁷⁹ Platelets are central players not only in thrombosis and hemostasis but also in inflammation and the innate immune system. The latter is also indicated by the finding that human platelets express all of the TLR1 (Toll-like receptor-1) to TLR10 transcripts.⁸⁰ Interestingly, women have higher expression levels of TLRs on platelets than men,⁸⁰ a

difference that might contribute to sex-dependent differences in the cardiovascular risk profile.⁸¹ Furthermore, aspirin intake is associated with decreased platelet TLR1, TLR3, and TLR4, and lipid treatment increased platelets TLR1, TLR2, TLR3, and TLR9, indicating a distinct association of cardiovascular risk factors with specific platelet TLR expression.⁸⁰ This study also demonstrates the association of TLR gene expression and the serum biomarkers of CRP, intercellular adhesion molecule-1, interleukin (IL)-6, MCP-1 (monocyte chemoattractant protein 1), tumor necrosis factor receptor, and P-selectin, which suggests a sex-specific link between thrombosis, immunity, infection, and cardiovascular disease.⁸⁰ Pathogens such as human cytomegalovirus also activate platelets in a TLR2-dependent manner and may ultimately contribute to atherosclerosis and plaque instability through this platelet-dependent mechanism.⁸² TLRs on platelets but also other cells may thus contribute to the association of infection and plaque instability.⁸³

In general, the cardiovascular risk reduction, as undoubtedly seen with antiplatelet therapy, is generally seen as an effect of platelet inhibition in the event of plaque rupture. However, as plaque rupture may be a repeated event resulting in microthrombi, which in turn increase plaque instability and the risk of further plaque rupture, platelets may well have direct involvement in plaque instability. It may be the inhibition of the repeated plaque ruptures or additional mechanisms of not yet identified plaque-stabilizing effects of platelets that also contribute to the reduction of cardiovascular events seen with antiplatelet therapy.

Monocytes/Macrophages as Central Players in Plaque Instability

The atherosclerotic lesion burden has been demonstrated to be positively correlated with the number of circulating monocytes in mice and the presence of coronary artery disease in patients.^{84,85} Vulnerable, rupture-prone plaques have abundant monocytes/macrophages, particularly in the shoulder region of thin caps, indicating their causative role in plaque rupture and also rendering macrophages good drug targets.⁸⁶ These activated macrophages release proinflammatory molecules such as IL-6 and tumor necrosis factor- α , proatherosclerotic chemokines, metalloproteinases, mi-RNAs, and reactive oxygen species.^{86,87} The role of monocytes in plaque destabilization is further supported by experimental inhibition of chemokine effects, such as the blockade of CCR-5 (C-C chemokine receptor 5), CX3CR1 (CX3C chemokine receptor 1), and CCL2 (chemokine C-C motif ligand 2), which are involved in the recruitment of both Ly6C⁺ and Ly6C⁻ monocytes. This inhibition almost fully abolishes atherosclerotic plaque development.^{88,89} In patients with chronic kidney disease, monocyte subtype blood counts, in particular of intermediate CD14⁺⁺CD16⁺, correlates with an increase in cardiovascular events, suggesting a role of specific monocyte subtypes in plaque instability.⁹⁰ This role of the intermediate monocyte subtype CD14⁺⁺CD16⁺ in plaque instability had also been suggested by the finding that this monocyte subset is a predictor of cardiovascular events in patients.⁹¹ In addition, previous imaging studies show an association of CD16⁺ monocytes with characteristics of plaque instability, as

visible in computed tomography or optical coherence tomography in patients, and reversal of this association under statin therapy.⁹² In a recent human autopsy study, another subtype, the M4 macrophage subtype, has been found to be associated with features of plaque instability.⁹³

Overall, monocytes/macrophages are clearly a driving force of plaque instability and, as such, represent a potent therapeutic target for plaque stabilization. However, further mechanistic studies must clearly define mechanisms of targeting and the subpopulation to be targeted. Depletion of circulating monocyte populations, inhibiting monocyte recruitment, and inhibiting macrophage proliferation within atherosclerotic plaques^{94,95} are potential therapeutic strategies.

Emerging Role of Lymphocytes in Plaque Instability

Multiple lymphocyte populations are found in vulnerable plaques.⁹⁶ Natural killer T (NKT) cells, which bridge the gap between innate and adaptive immunity, accumulate in human rupture-prone shoulder regions of vulnerable plaques, together with dendritic cells.⁹⁷ In ApoE^{-/-} mice, Li et al⁹⁸ showed that NKT cells, in particular CD4⁺ NKT cells, exert their effects on atherosclerotic lesions via cytotoxic mechanisms, mostly via perforin and granzyme B, stimulating apoptosis and large increases in necrotic cores. NKT cells are activated by many mechanisms, including lipid antigen presentation via CD1d expressed by dendritic cells,⁹⁹ which may explain their colocalization in lesions,⁹⁷ or via IgG antibodies activating NKT cell Fcγ3R¹⁰⁰; IgG auto-antibodies can be active mediators of atherosclerotic plaque instability.¹⁰¹ Selathurai et al¹⁰² have also shown that NK cells can contribute to lesion vulnerability by increasing necrotic core size via essentially similar cytotoxic mechanisms. In cytomegalovirus seropositive patients, the NKG2C⁺ NK cell subset has been associated with high-risk carotid atherosclerotic plaques.¹⁰³

Although relatively poorly studied, CD4⁺ T helper and cytotoxic CD8⁺ T cells are also increased in thin-cap fibroatheromas compared with stable lesions, with the largest increases in cytotoxic CD8⁺ T cells.⁹⁶ Kyaw et al¹⁰⁴ have shown that in atherosclerotic mice, CD8⁺ T cells contribute to apoptosis of key lesion cell types, including smooth muscle cells and macrophages, as well as increasing necrotic core size. Despite CD8⁺ T-cell-derived tumor necrosis factor- α being capable of inducing apoptosis,¹⁰⁵ only CD8⁺ T-cell-derived granzyme B and perforin stimulate apoptosis and so contribute to necrotic core size in developing vulnerable lesions.¹⁰⁴ The role of CD4⁺ helper T cells in vulnerable plaque development and rupture is less clear and may be indirect via cytotoxic CD8⁺ T cells, rather than direct, as the cytotoxic effects of CD8⁺ T cells are substantially diminished in the absence of CD4⁺ helper T cells. CD4⁺ helper T cells are required for both development of cytotoxic CD8⁺ T cells and also sustaining cytotoxic CD8⁺ T-cell responses.¹⁰⁶ Although cytotoxic CD8⁺ T cells clearly contribute to plaque instability, it is unclear whether these cells represent effector or memory cells, knowledge essential for effective therapeutic targeting of these cells for plaque stabilization. Several studies in humans also indicate that CD4⁺CD28⁻ T cells seem

to be important in acute coronary syndromes¹⁰⁷ and plaque instability.¹⁰⁸ Their development is associated with premature aging of the immune system, and they may be particularly important in promoting plaque instability and rupture in patients with other inflammatory disorders, such as rheumatoid arthritis, end-stage renal disease, and type 2 diabetes mellitus, where their numbers are increased. Functionally these cells infiltrate target tissues, are highly cytotoxic, expressing cytotoxins including granzymes and perforin, and are resistant to apoptosis and the suppressive effects of Foxp3⁺ regulatory T cells.¹⁰⁹ Unlike CD4⁺ helper T cells, these cells most likely directly contribute to plaque instability via cytotoxic mechanisms. Given the elevated numbers of circulating CD4⁺CD28⁻ T cells in patients with acute coronary syndromes, together with their high activation status¹¹⁰ and ability to kill endothelial cells,¹¹¹ these cells may also be critically important in plaque erosion. As the latter has attracted increased attention as a cause of myocardial infarction,¹¹² research addressing the roles of these cells in atherosclerosis might be of particular interest.

Artery Tertiary Lymphoid Organs: A Future Focus of Plaque Instability

It has been known for >30 years that adventitial immune cell infiltrates are associated with advanced human atherosclerotic plaques, with structural organizations that suggest generation of local humoral responses.^{113,114} In ruptured plaques, the infiltrates seem to be greater in number than in nonruptured human atherosclerotic plaques, suggesting involvement in the pathology of plaque instability.¹¹⁵ More recent studies have identified similar adventitial infiltrates, now referred to as artery tertiary lymphoid organs (ATLO), associated with aortic atherosclerotic lesions in aged hyperlipidemic ApoE^{-/-} mice. In a recent *ATVB* publication, Srikakulapu et al¹¹⁶ focused on defining the B-cell populations within such structures, demonstrating the presence of effector B cells and plasma cells, as well as several immunosuppressive B-cell subtypes. B2 cells, which are proatherogenic, including transitional, follicular, and germinal center B cells, and multiple types of B1 cells were identified, including IgM⁺ cells. The identified plasma cells include IL-10-producing plasma cells, which may represent regulatory plasma cells. Follicular T cells have also been shown to be present,¹¹⁷ raising the possibility that artery tertiary lymphoid organs represent an effective T-follicular helper-germinal center B-cell axis that may drive local proatherogenic antibody production.¹¹⁷ CD8⁺ regulatory T cells are important in their regulation, as artery tertiary lymphoid organs development is increased when CD8⁺ regulatory T-cell function is impaired, and this is associated with enhanced disease development.¹¹⁷ The role of artery tertiary lymphoid organs in the development of advanced atherosclerotic lesions, plaque instability, and rupture promises to be a highly interesting area of research in the future.

Therapeutic Approaches for Plaque Stabilization

Despite a multitude of studies often based on gene deletion in mice, which has helped to provide in-depth knowledge of

mechanisms responsible for the initiation and progression of atherosclerosis, therapeutic antiatherosclerotic approaches are rarely described. The lack of novel therapies for plaque stabilization is even more apparent and at the same time worrisome, as this clearly reflects a major medical need.

Nevertheless, a few promising therapeutic approaches toward plaque stabilization are available and have been published in *ATVB*. A recent human study provides evidence that treating blood pressure has the potential to reduce intraplaque hemorrhage as seen in MRI in carotid plaques in patients.⁷⁵ DNA/RNA therapeutics hold great promise and initial promising approaches toward plaque stabilization have been published. These include micro-RNA,¹¹⁸ anti-micro-RNA,^{119,120} and a type B CpG oligodeoxynucleotide TLR9 agonist.¹²¹

With increasing knowledge of the specific cell types that mediate plaque rupture and advances in biotechnology providing cell-specific therapeutic approaches, selective plaque stabilization might be possible in patients. Preclinical approaches have been successful by targeting monocytes via the relative selective phagocytosis of drug-loaded nanoparticles.^{122,123} Another recent example of these promising developments is cell type-specific anticytokine effects achieved by a bifunctional antibody that combines cell-specific targeting against F4/80 surface molecules on myeloid cells and tumor necrosis factor inhibition.¹²⁴ Recombinant antibody technology will allow cell-specific blockade of secreted proteins and also receptors, and this represents a new direction in atherosclerotic therapy.

Despite the association of CRP with cardiovascular risk and potentially with plaque rupture, the pentameric form of CRP has not been associated with a direct causal role and thus the development of drugs that target pentameric form of CRP has not been pursued.¹²⁵ Nevertheless, newer data indicate that the dissociated conformation of pentameric form of CRP, the monomeric CRP, clearly possesses proinflammatory effects.¹²⁶ Indeed, the inhibition of monomeric CRP has recently been proven to exhibit a strong anti-inflammatory effect.¹²⁷ Therefore, monomeric CRP might be worthwhile to be assessed as a potential plaque-stabilizing therapeutic target. An alternative approach targeting inflammation, as a major force leading to plaque instability and rupture, is to choose targets that are upstream of CRP, such as IL-6 or IL-1. Several smaller clinical trials and 2 large-scale clinical trials are currently testing this hypothesis: the large trials are the CIRT (Cardiovascular Inflammation Reduction Trial), which is testing low-dose methotrexate as an inhibitor of the CRP/IL-6/IL-1 axis, and the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), testing the monoclonal antibody canakinumab and targeting IL-1 β . Overall, despite the current paucity of antiatherosclerotic therapy, novel targets and innovative biotechnological approaches justify the hope that plaque-stabilizing therapies are in reach.

Summary

It is easy to see the breaking of the camel's back, as it is typically an obvious, final, and dramatic scene that catches everyone by surprise. Yet it is difficult to see the straw itself and to understand all the strain that silently builds up and contributes to the final event. Similarly, the rupture of unstable

atherosclerotic plaques is a complex process ultimately representing a maladaptive response to hemodynamic conditions, lipid accumulation, arterial injury, necrosis, and inflammation. Growing understanding of the many facets of this pathological process offers multiple opportunities for early detection of unstable plaques with biomarkers and novel molecular imaging technologies, and hopefully will ultimately lead to the development of utterly needed plaque-stabilizing drugs.

Sources of Funding

Y.-C. Chen was supported by a National Heart Foundation (NHF) of Australia Postdoctoral Fellowship. A.L. Huang was supported by a NHF of Australia Postgraduate Scholarship. K. Peter was supported by a National Health and Medical Research Council (NHMRC) Principal Research Fellowship. The Bobik and Peter laboratories are funded by the NHMRC of Australia.

Disclosures

None.

References

- Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2000;20:1262–1275.
- Peeters W, Hellings WE, de Kleijn DP, de Vries JP, Moll FL, Vink A, Pasterkamp G. Carotid atherosclerotic plaques stabilize after stroke: insights into the natural process of atherosclerotic plaque stabilization. *Arterioscler Thromb Vasc Biol*. 2009;29:128–133. doi: 10.1161/ATVBAHA.108.173658.
- Chen YC, Peter K. Determining the characteristics of human atherosclerosis: A difficult but indispensable task providing the direction and proof of concept for pioneering atherosclerosis research in animal models. *Atherosclerosis*. 2015;241:595–596. doi: 10.1016/j.atherosclerosis.2015.06.009.
- Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. *Arterioscler Thromb Vasc Biol*. 2010;30:1282–1292. doi: 10.1161/ATVBAHA.108.179739.
- Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. *J Intern Med*. 2015;278:483–493. doi: 10.1111/joim.12406.
- Kolodgie FD, Burke AP, Farb A, Gold HK, Yuan J, Narula J, Finn AV, Virmani R. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. *Curr Opin Cardiol*. 2001;16:285–292.
- Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation*. 2003;108:1772–1778. doi: 10.1161/01.CIR.0000087481.55887.C9.
- Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. *Circulation*. 2001;103:934–940.
- Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW; PROSPECT Investigators. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011;364:226–235. doi: 10.1056/NEJMoa1002358.
- Puri R, Madder RD, Madden SP, et al. Near-Infrared Spectroscopy Enhances Intravascular Ultrasound Assessment of Vulnerable Coronary Plaque: A Combined Pathological and *In Vivo* Study. *Arterioscler Thromb Vasc Biol*. 2015;35:2423–2431. doi: 10.1161/ATVBAHA.115.306118.
- Madder RD, Puri R, Muller JE, Harnek J, Göteborg M, VanOosterhout S, Chi M, Wohns D, McNamara R, Wolski K, Madden S, Sidharta S, Andrews J, Nicholls SJ, Erlinge D. Confirmation of the intracoronary near-infrared spectroscopy threshold of lipid-rich plaques that underlie ST-segment-elevation myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2016;36:1010–1015. doi: 10.1161/ATVBAHA.115.306849.
- Pedersen SF, Sandholt BV, Keller SH, Hansen AE, Clemmensen AE, Sillesen H, Højgaard L, Ripa RS, Kjær A. ⁶⁴Cu-DOTATATE PET/MRI for Detection of activated macrophages in carotid atherosclerotic plaques:

- studies in patients undergoing endarterectomy. *Arterioscler Thromb Vasc Biol.* 2015;35:1696–1703. doi: 10.1161/ATVBAHA.114.305067.
13. von zur Muhlen C, von Elverfeldt D, Moeller JA, Choudhury RP, Paul D, Hagemeyer CE, Olschewski M, Becker A, Neudorfer I, Bassler N, Schwarz M, Bode C, Peter K. Magnetic resonance imaging contrast agent targeted toward activated platelets allows *in vivo* detection of thrombosis and monitoring of thrombolysis. *Circulation.* 2008;118:258–267. doi: 10.1161/CIRCULATIONAHA.107.753657.
 14. Li X, Bauer W, Israel I, Kreissl MC, Weirather J, Richter D, Bauer E, Herold V, Jakob P, Buck A, Frantz S, Samnick S. Targeting P-selectin by gallium-68-labeled fucoidan positron emission tomography for noninvasive characterization of vulnerable plaques: correlation with *in vivo* 17.6T MRI. *Arterioscler Thromb Vasc Biol.* 2014;34:1661–1667. doi: 10.1161/ATVBAHA.114.303485.
 15. Joshi NV, Vesey AT, Williams MC, et al. 18F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial. *Lancet.* 2014;383:705–713. doi: 10.1016/S0140-6736(13)61754-7.
 16. Libby P, King K. Biomarkers: a challenging conundrum in cardiovascular disease. *Arterioscler Thromb Vasc Biol.* 2015;35:2491–2495. doi: 10.1161/ATVBAHA.115.305233.
 17. Hlatky MA, Greenland P, Arnett DK, et al; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation.* 2009;119:2408–2416. doi: 10.1161/CIRCULATIONAHA.109.192278.
 18. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2012;32:2045–2051. doi: 10.1161/ATVBAHA.108.179705.
 19. Aubry MC, Maradit-Kremers H, Reinalda MS, Crowson CS, Edwards WD, Gabriel SE. Differences in atherosclerotic coronary heart disease between subjects with and without rheumatoid arthritis. *J Rheumatol.* 2007;34:937–942.
 20. Tattersall MC, Guo M, Korcarz CE, Gepner AD, Kaufman JD, Liu KJ, Barr RG, Donohue KM, McClelland RL, Delaney JA, Stein JH. Asthma predicts cardiovascular disease events: the multi-ethnic study of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2015;35:1520–1525. doi: 10.1161/ATVBAHA.115.305452.
 21. Schuett KA, Lehrke M, Marx N, Burgmaier M. High-risk cardiovascular patients: clinical features, comorbidities, and interconnecting mechanisms. *Front Immunol.* 2015;6:591. doi: 10.3389/fimmu.2015.00591.
 22. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, Blumenthal RS, Budoff MJ. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? *J Am Coll Cardiol.* 2013;62:397–408. doi: 10.1016/j.jacc.2013.05.016.
 23. Durda P, Sabourin J, Lange EM, et al. Plasma levels of soluble interleukin-2 receptor α : associations with clinical cardiovascular events and genome-wide association scan. *Arterioscler Thromb Vasc Biol.* 2015;35:2246–2253. doi: 10.1161/ATVBAHA.115.305289.
 24. Maiolino G, Rossitto G, Pedon L, Cesari M, Frigo AC, Azzolini M, Plebani M, Rossi GP. Galectin-3 predicts long-term cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol.* 2015;35:725–732. doi: 10.1161/ATVBAHA.114.304964.
 25. Gasbarrino K, Mantzoros C, Gorgui J, Veinot JP, Lai C, Daskalopoulou SS. Circulating chemerin is associated with carotid plaque instability, whereas resistin is related to cerebrovascular symptomatology. *Arterioscler Thromb Vasc Biol.* 2016;36:1670–1678. doi: 10.1161/ATVBAHA.115.306741.
 26. Goncalves I, Bengtsson E, Colhoun HM, et al; SUMMIT Consortium. Elevated plasma levels of MMP-12 are associated with atherosclerotic burden and symptomatic cardiovascular disease in subjects with type 2 diabetes. *Arterioscler Thromb Vasc Biol.* 2015;35:1723–1731. doi: 10.1161/ATVBAHA.115.305631.
 27. Rattik S, Wigren M, Björkbacka H, Fredrikson GN, Hedblad B, Siegbahn A, Bengtsson E, Schiopu A, Edsfieldt A, Dunér P, Grufman H, Gonçalves I, Nilsson J. High plasma levels of heparin-binding epidermal growth factor are associated with a more stable plaque phenotype and reduced incidence of coronary events. *Arterioscler Thromb Vasc Biol.* 2015;35:222–228. doi: 10.1161/ATVBAHA.114.304369.
 28. von Zur Muhlen C, Schiffer E, Zuerbig P, Kellmann M, Brasse M, Meert N, Vanholder RC, Dominiczak AF, Chen YC, Mischak H, Bode C, Peter K. Evaluation of urine proteome pattern analysis for its potential to reflect coronary artery atherosclerosis in symptomatic patients. *J Proteome Res.* 2009;8:335–345. doi: 10.1021/pr800615t.
 29. Bye A, Røsjø H, Nauman J, Silva GJ, Follstad T, Omland T, Wisløff U. Circulating microRNAs predict future fatal myocardial infarction in healthy individuals - The HUNT study. *J Mol Cell Cardiol.* 2016;97:162–168. doi: 10.1016/j.yjmcc.2016.05.009.
 30. Warboys CM, de Luca A, Amini N, et al. Disturbed flow promotes endothelial senescence via a p53-dependent pathway. *Arterioscler Thromb Vasc Biol.* 2014;34:985–995. doi: 10.1161/ATVBAHA.114.303415.
 31. Caro CG. Discovery of the role of wall shear in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2009;29:158–161. doi: 10.1161/ATVBAHA.108.166736.
 32. Cheruvu PK, Finn AV, Gardner C, Caplan J, Goldstein J, Stone GW, Virmani R, Muller JE. Frequency and distribution of thin-cap fibroatheroma and ruptured plaques in human coronary arteries: a pathologic study. *J Am Coll Cardiol.* 2007;50:940–949. doi: 10.1016/j.jacc.2007.04.086.
 33. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol.* 2007;49:2379–2393. doi: 10.1016/j.jacc.2007.02.059.
 34. Chatzizisis YS, Baker AB, Sukhova GK, Koskinas KC, Papafaklis MI, Beigel R, Jonas M, Coskun AU, Stone BV, Maynard C, Shi GP, Libby P, Feldman CL, Edelman ER, Stone PH. Augmented expression and activity of extracellular matrix-degrading enzymes in regions of low endothelial shear stress colocalize with coronary atheromata with thin fibrous caps in pigs. *Circulation.* 2011;123:621–630. doi: 10.1161/CIRCULATIONAHA.110.970038.
 35. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, Chen W, Zeiher AM, Potente M, Dimmeler S, Boon RA. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler Thromb Vasc Biol.* 2015;35:137–145. doi: 10.1161/ATVBAHA.114.304277.
 36. Cybulsky MI, Marsden PA. Effect of disturbed blood flow on endothelial cell gene expression: a role for changes in RNA processing. *Arterioscler Thromb Vasc Biol.* 2014;34:1806–1808. doi: 10.1161/ATVBAHA.114.304099.
 37. Jiang YZ, Manduchi E, Jiménez JM, Davies PF. Endothelial epigenetics in biomechanical stress: disturbed flow-mediated epigenomic plasticity *in vivo* and *in vitro*. *Arterioscler Thromb Vasc Biol.* 2015;35:1317–1326. doi: 10.1161/ATVBAHA.115.303427.
 38. Dunn J, Thabet S, Jo H. Flow-Dependent epigenetic DNA methylation in endothelial gene expression and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2015;35:1562–1569. doi: 10.1161/ATVBAHA.115.305042.
 39. Zhang X, Cheng R, Rowe D, Sethu P, Daugherty A, Yu G, Shin HY. Shear-sensitive regulation of neutrophil flow behavior and its potential impact on microvascular blood flow dysregulation in hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2014;34:587–593. doi: 10.1161/ATVBAHA.113.302868.
 40. Boulanger CM. Endothelium. *Arterioscler Thromb Vasc Biol.* 2016;36:e26–e31. doi: 10.1161/ATVBAHA.116.306940.
 41. Abe J, Berk BC. Novel mechanisms of endothelial mechanotransduction. *Arterioscler Thromb Vasc Biol.* 2014;34:2378–2386. doi: 10.1161/ATVBAHA.114.303428.
 42. Bryan MT, Duckles H, Feng S, Hsiao ST, Kim HR, Serbanovic-Canic J, Evans PC. Mechanoresponsive networks controlling vascular inflammation. *Arterioscler Thromb Vasc Biol.* 2014;34:2199–2205. doi: 10.1161/ATVBAHA.114.303424.
 43. Murphy PA, Hynes RO. Alternative splicing of endothelial fibronectin is induced by disturbed hemodynamics and protects against hemorrhage of the vessel wall. *Arterioscler Thromb Vasc Biol.* 2014;34:2042–2050. doi: 10.1161/ATVBAHA.114.303879.
 44. Zhou J, Li YS, Chien S. Shear stress-initiated signaling and its regulation of endothelial function. *Arterioscler Thromb Vasc Biol.* 2014;34:2191–2198. doi: 10.1161/ATVBAHA.114.303422.
 45. Slager CJ, Wentzel JJ, Gijssen FJ, Thury A, van der Wal AC, Schaar JA, Serruys PW. The role of shear stress in the destabilization of vulnerable plaques and related therapeutic implications. *Nat Clin Pract Cardiovasc Med.* 2005;2:456–464. doi: 10.1038/ncpcardio0298.
 46. Pedrigi RM, de Silva R, Bovens SM, Mehta VV, Petretto E, Krams R. Thin-cap fibroatheroma rupture is associated with a fine interplay of shear and wall stress. *Arterioscler Thromb Vasc Biol.* 2014;34:2224–2231. doi: 10.1161/ATVBAHA.114.303426.
 47. Chen YC, Bui AV, Diesch J, Manasseh R, Hausding C, Rivera J, Haviv I, Agrotis A, Htun NM, Jowett J, Hagemeyer CE, Hannan RD, Bobik A, Peter K. A novel mouse model of atherosclerotic plaque instability for drug testing and mechanistic/therapeutic discoveries using gene

- and microRNA expression profiling. *Circ Res.* 2013;113:252–265. doi: 10.1161/CIRCRESAHA.113.301562.
48. Sasaki T, Kuzuya M, Nakamura K, Cheng XW, Shibata T, Sato K, Iguchi A. A simple method of plaque rupture induction in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol.* 2006;26:1304–1309. doi: 10.1161/01.ATV.0000219687.71607.f7.
 49. Williams H, Johnson JL, Carson KG, Jackson CL. Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol.* 2002;22:788–792.
 50. Hansson GK, Heistad DD. Two views on plaque rupture. *Arterioscler Thromb Vasc Biol.* 2007;27:697. doi: 10.1161/01.ATV.0000261344.03489.0c.
 51. Falk E, Schwartz SM, Galis ZS and Rosenfeld ME. Neointimal cracks (plaque rupture?) and thrombosis in wrapped arteries without flow. *Arterioscler Thromb Vasc Biol.* 2007;27:248–249; author reply 250–252.
 52. Jackson CL. Defining and defending murine models of plaque rupture. *Arterioscler Thromb Vasc Biol.* 2007;27:973–977. doi: 10.1161/01.ATV.0000261545.53586.f0.
 53. Jin SX, Shen LH, Nie P, Yuan W, Hu LH, Li DD, Chen XJ, Zhang XK, He B. Endogenous renovascular hypertension combined with low shear stress induces plaque rupture in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol.* 2012;32:2372–2379. doi: 10.1161/ATVBAHA.111.236158.
 54. Li S, Wang YN, Niimi M, Ning B, Chen Y, Kang D, Waqar AB, Wang Z, Yu Q, Liu E, Zhang J, Shiomi M, Chen YE, Fan J. Angiotensin II destabilizes coronary plaques in watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol.* 2016;36:810–816. doi: 10.1161/ATVBAHA.115.306871.
 55. Pasterkamp G, van der Laan SW, Haitjema S, et al. Human validation of genes associated with a murine atherosclerotic phenotype. *Arterioscler Thromb Vasc Biol.* 2016;36:1240–1246. doi: 10.1161/ATVBAHA.115.306958.
 56. Schwartz SM, Galis ZS, Rosenfeld ME, Falk E. Plaque rupture in humans and mice. *Arterioscler Thromb Vasc Biol.* 2007;27:705–713. doi: 10.1161/01.ATV.0000261709.34878.20.
 57. Jackson CL, Bennett MR, Biessen EA, Johnson JL, Krams R. Assessment of unstable atherosclerosis in mice. *Arterioscler Thromb Vasc Biol.* 2007;27:714–720. doi: 10.1161/01.ATV.0000261873.86623.e1.
 58. Edsfeldt A, Dunér P, Ståhlman M, Mollet IG, Ascituro G, Grufman H, Nitulescu M, Persson AF, Fisher RM, Melander O, Orho-Melander M, Borén J, Nilsson J, Gonçalves I. Sphingolipids contribute to human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol.* 2016;36:1132–1140. doi: 10.1161/ATVBAHA.116.305675.
 59. Ronsein GE, Hutchins PM, Isquith D, Vaisar T, Zhao XQ, Heinecke JW. Niacin Therapy increases high-density lipoprotein particles and total cholesterol efflux capacity but not ABCA1-specific cholesterol efflux in statin-treated subjects. *Arterioscler Thromb Vasc Biol.* 2016;36:404–411. doi: 10.1161/ATVBAHA.115.306268.
 60. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W; AIM-HIGH Investigators. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med.* 2011;365:2255–2267. doi: 10.1056/NEJMoa1107579.
 61. Melchior JT, Olson JD, Kelley KL, Wilson MD, Sawyer JK, Link KM, Rudel LL. Targeted knockdown of hepatic SOAT2 with antisense oligonucleotides stabilizes atherosclerotic plaque in ApoB100-only LDLr^{-/-} mice. *Arterioscler Thromb Vasc Biol.* 2015;35:1920–1927. doi: 10.1161/ATVBAHA.115.305747.
 62. Doonan RJ, Hafiane A, Lai C, Veinot JP, Genest J, Daskalopoulou SS. Cholesterol efflux capacity, carotid atherosclerosis, and cerebrovascular symptomatology. *Arterioscler Thromb Vasc Biol.* 2014;34:921–926. doi: 10.1161/ATVBAHA.113.302590.
 63. Moreno PR. Vulnerable plaque: definition, diagnosis, and treatment. *Cardiol Clin.* 2010;28:1–30. doi: 10.1016/j.ccl.2009.09.008.
 64. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation.* 2003;108:1664–1672. doi: 10.1161/01.CIR.0000087480.94275.97.
 65. Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. *Eur Heart J.* 2013;34:719–728. doi: 10.1093/eurheartj/ehs411.
 66. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol.* 2005;25:2054–2061. doi: 10.1161/01.ATV.0000178991.71605.18.
 67. Michel JB, Virmani R, Arbustini E, Pasterkamp G. Intraplaque haemorrhages as the trigger of plaque vulnerability. *Eur Heart J.* 2011;32:1977–1985, 1985a, 1985b, 1985c. doi: 10.1093/eurheartj/ehr054.
 68. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, Farb A, Guerrero LJ, Hayase M, Kutys R, Narula J, Finn AV, Virmani R. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med.* 2003;349:2316–2325. doi: 10.1056/NEJMoa035655.
 69. Hellings WE, Peeters W, Moll FL, Piers SR, van Setten J, Van der Spek PJ, de Vries JP, Seldenrijk KA, De Bruin PC, Vink A, Velega E, de Kleijn DP, Pasterkamp G. Composition of carotid atherosclerotic plaque is associated with cardiovascular outcome: a prognostic study. *Circulation.* 2010;121:1941–1950. doi: 10.1161/CIRCULATIONAHA.109.887497.
 70. Di Stefano R, Felice F, Balbarini A. Angiogenesis as risk factor for plaque vulnerability. *Curr Pharm Des.* 2009;15:1095–1106.
 71. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res.* 2014;114:1852–1866. doi: 10.1161/CIRCRESAHA.114.302721.
 72. Li JJ, Meng X, Si HP, Zhang C, Lv HX, Zhao YX, Yang JM, Dong M, Zhang K, Liu SX, Zhao XQ, Gao F, Liu XL, Cui TX, Zhang Y. Hepcidin destabilizes atherosclerotic plaque via overactivating macrophages after erythropagocytosis. *Arterioscler Thromb Vasc Biol.* 2012;32:1158–1166. doi: 10.1161/ATVBAHA.112.246108.
 73. Saeed O, Otsuka F, Polavarapu R, et al. Pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2012;32:299–307. doi: 10.1161/ATVBAHA.111.240101.
 74. Mehta NU, Grijalva V, Hama S, Wagner A, Navab M, Fogelman AM, Reddy ST. Apolipoprotein E^{-/-} mice lacking hemopexin develop increased atherosclerosis via mechanisms that include oxidative stress and altered macrophage function. *Arterioscler Thromb Vasc Biol.* 2016;36:1152–1163. doi: 10.1161/ATVBAHA.115.306991.
 75. Sun J, Canton G, Balu N, Hippe DS, Xu D, Liu J, Hatsukami TS, Yuan C. Blood pressure is a major modifiable risk factor implicated in pathogenesis of intraplaque hemorrhage: an *In Vivo* Magnetic Resonance Imaging Study. *Arterioscler Thromb Vasc Biol.* 2016;36:743–749. doi: 10.1161/ATVBAHA.115.307043.
 76. Selwaness M, van den Bouwhuijsen Q, Mattace-Raso FU, Verwoert GC, Hofman A, Franco OH, Witteman JC, van der Lugt A, Vernooij MW, Wentzel JJ. Arterial stiffness is associated with carotid intraplaque hemorrhage in the general population: the Rotterdam study. *Arterioscler Thromb Vasc Biol.* 2014;34:927–932. doi: 10.1161/ATVBAHA.113.302603.
 77. Massberg S, Brand K, Grüner S, Page S, Müller E, Müller I, Bergmeier W, Richter T, Lorenz M, Konrad I, Nieswandt B, Gawaz M. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med.* 2002;196:887–896.
 78. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med.* 2003;9:61–67. doi: 10.1038/nm810.
 79. Badrnya S, Schrottmaier WC, Kral JB, Yaiw KC, Volf I, Schabbauer G, Söderberg-Nauclér C, Assinger A. Platelets mediate oxidized low-density lipoprotein-induced monocyte extravasation and foam cell formation. *Arterioscler Thromb Vasc Biol.* 2014;34:571–580. doi: 10.1161/ATVBAHA.113.302919.
 80. Koupenova M, Mick E, Mikhalev E, Benjamin EJ, Tanriverdi K, Freedman JE. Sex differences in platelet toll-like receptors and their association with cardiovascular risk factors. *Arterioscler Thromb Vasc Biol.* 2015;35:1030–1037. doi: 10.1161/ATVBAHA.114.304954.
 81. Mosca L, Manson JE, Sutherland SE, Langer RD, Manolio T, Barrett-Connor E. Cardiovascular disease in women: a statement for healthcare professionals from the American Heart Association. Writing Group. *Circulation.* 1997;96:2468–2482.
 82. Assinger A, Kral JB, Yaiw KC, Schrottmaier WC, Kurzejamska E, Wang Y, Mohammad AA, Religa P, Rahbar A, Schabbauer G, Butler LM, Söderberg-Naucler C. Human cytomagalovirus-platelet interaction triggers toll-like receptor 2-dependent proinflammatory and proangiogenic responses. *Arterioscler Thromb Vasc Biol.* 2014;34:801–809. doi: 10.1161/ATVBAHA.114.303287.
 83. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost.* 2011;106:858–867. doi: 10.1160/TH11-06-0392.

84. Ley K, Miller YI, Hedrick CC. Monocyte and macrophage dynamics during atherogenesis. *Arterioscler Thromb Vasc Biol.* 2011;31:1506–1516. doi: 10.1161/ATVBAHA.110.221127.
85. Huang Y, Yin H, Wang J, Ma X, Zhang Y, Chen K. The significant increase of FcγRIIIA (CD16), a sensitive marker, in patients with coronary heart disease. *Gene.* 2012;504:284–287. doi: 10.1016/j.gene.2012.05.017.
86. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest.* 1994;94:2493–2503. doi: 10.1172/JCI117619.
87. Vendrov AE, Hakim ZS, Madamanchi NR, Rojas M, Madamanchi C, Runge MS. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler Thromb Vasc Biol.* 2007;27:2714–2721. doi: 10.1161/ATVBAHA.107.152629.
88. Combadière C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation.* 2008;117:1649–1657. doi: 10.1161/CIRCULATIONAHA.107.745091.
89. van der Vorst EP, Döring Y, Weber C. Chemokines. *Arterioscler Thromb Vasc Biol.* 2015;35:e52–e56. doi: 10.1161/ATVBAHA.115.306359.
90. Rogacev KS, Zawada AM, Emrich I, Seiler S, Böhm M, Fliser D, Woollard KJ, Heine GH. Lower Apo A-I and lower HDL-C levels are associated with higher intermediate CD14++CD16+ monocyte counts that predict cardiovascular events in chronic kidney disease. *Arterioscler Thromb Vasc Biol.* 2014;34:2120–2127. doi: 10.1161/ATVBAHA.114.304172.
91. Rogacev KS, Cremers B, Zawada AM, et al. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. *J Am Coll Cardiol.* 2012;60:1512–1520. doi: 10.1016/j.jacc.2012.07.019.
92. Kashiwagi M, Imanishi T, Tsujioka H, Ikejima H, Kuroi A, Ozaki Y, Ishibashi K, Komukai K, Tanimoto T, Ino Y, Kitabata H, Hirata K, Akasaka T. Association of monocyte subsets with vulnerability characteristics of coronary plaques as assessed by 64-slice multidetector computed tomography in patients with stable angina pectoris. *Atherosclerosis.* 2010;212:171–176. doi: 10.1016/j.atherosclerosis.2010.05.004.
93. Erbel C, Wolf A, Lasitschka F, Linden F, Domschke G, Akhavanpoor M, Doesch AO, Katus HA, Gleissner CA. Prevalence of M4 macrophages within human coronary atherosclerotic plaques is associated with features of plaque instability. *Int J Cardiol.* 2015;186:219–225. doi: 10.1016/j.ijcard.2015.03.151.
94. Robbins CS, Hilgendorf I, Weber GF, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat Med.* 2013;19:1166–1172. doi: 10.1038/nm.3258.
95. Tang J, Lobatto ME, Hassing L, et al. Inhibiting macrophage proliferation suppresses atherosclerotic plaque inflammation. *Sci Adv.* 2015 Apr;1(3). pii: e1400223.
96. Rohm I, Atiskova Y, Drobniak S, Fritzenwanger M, Kretzschmar D, Pistulli R, Zanow J, Krönert T, Mall G, Figulla HR, Yilmaz A. Decreased regulatory T cells in vulnerable atherosclerotic lesions: imbalance between pro- and anti-inflammatory cells in atherosclerosis. *Mediators Inflamm.* 2015;2015:364710. doi: 10.1155/2015/364710.
97. Bobryshev YV, Lord RS. Co-accumulation of dendritic cells and natural killer T cells within rupture-prone regions in human atherosclerotic plaques. *J Histochem Cytochem.* 2005;53:781–785. doi: 10.1369/jhc.4B6570.2005.
98. Li Y, To K, Kanellakis P, Hosseini H, Deswaerte V, Tipping P, Smyth MJ, Toh BH, Bobik A, Kyaw T. CD4+ natural killer T cells potentially augment aortic root atherosclerosis by perforin- and granzyme B-dependent cytotoxicity. *Circ Res.* 2015;116:245–254. doi: 10.1161/CIRCRESAHA.116.304734.
99. Godfrey DI, Rossjohn J. New ways to turn on NKT cells. *J Exp Med.* 2011;208:1121–1125. doi: 10.1084/jem.20110983.
100. Kim HY, Kim S, Chung DH. FcγRIII engagement provides activating signals to NKT cells in antibody-induced joint inflammation. *J Clin Invest.* 2006;116:2484–2492. doi: 10.1172/JCI27219.
101. Montecucco F, Vuilleumier N, Pagano S, Lenglet S, Bertolotto M, Braunerreuther V, Pelli G, Kovari E, Pane B, Spinella G, Pende A, Palombo D, Dallegri F, Mach F, Roux-Lombard P. Anti-Apolipoprotein A-I auto-antibodies are active drivers of atherosclerotic plaque vulnerability. *Eur Heart J.* 2011;32:412–421. doi: 10.1093/eurheartj/ehq521.
102. Selathurai A, Deswaerte V, Kanellakis P, Tipping P, Toh BH, Bobik A, Kyaw T. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. *Cardiovasc Res.* 2014;102:128–137. doi: 10.1093/cvr/cvu016.
103. Martínez-Rodríguez JE, Munné-Collado J, Rasal R, Cuadrado E, Roig L, Ois A, Muntasell A, Baro T, Alameda F, Roquer J, López-Botet M. Expansion of the NKG2C+ natural killer-cell subset is associated with high-risk carotid atherosclerotic plaques in seropositive patients for human cytomegalovirus. *Arterioscler Thromb Vasc Biol.* 2013;33:2653–2659. doi: 10.1161/ATVBAHA.113.302163.
104. Kyaw T, Winship A, Tay C, Kanellakis P, Hosseini H, Cao A, Li P, Tipping P, Bobik A, Toh BH. Cytotoxic and proinflammatory CD8+ T lymphocytes promote development of vulnerable atherosclerotic plaques in apoE-deficient mice. *Circulation.* 2013;127:1028–1039. doi: 10.1161/CIRCULATIONAHA.112.001347.
105. Liu AN, Mohammed AZ, Rice WR, Fiedelley DT, Liebermann JS, Whitsett JA, Braciale TJ, Enelow RI. Perforin-independent CD8(+) T-cell-mediated cytotoxicity of alveolar epithelial cells is preferentially mediated by tumor necrosis factor-α: relative insensitivity to Fas ligand. *Am J Respir Cell Mol Biol.* 1999;20:849–858. doi: 10.1165/ajrcmb.20.5.3585.
106. Serbina NV, Lazarevic V, Flynn JL. CD4(+) T cells are required for the development of cytotoxic CD8(+) T cells during *Mycobacterium tuberculosis* infection. *J Immunol.* 2001;167:6991–7000.
107. Dumitriu IE, Baruah P, Finlayson CJ, Loftus IM, Antunes RF, Lim P, Bunce N, Kaski JC. High levels of costimulatory receptors OX40 and 4-1BB characterize CD4+CD28null T cells in patients with acute coronary syndrome. *Circ Res.* 2012;110:857–869. doi: 10.1161/CIRCRESAHA.111.261933.
108. Liuzzo G, Goronzy JJ, Yang H, Kopecky SL, Holmes DR, Frye RL, Weyand CM. Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes. *Circulation.* 2000;101:2883–2888.
109. Broux B, Markovic-Plese S, Stinissen P, Hellings N. Pathogenic features of CD4+CD28- T cells in immune disorders. *Trends Mol Med.* 2012;18:446–453. doi: 10.1016/j.molmed.2012.06.003.
110. Baruah P, Lee M, Odutoye T, Williamson P, Hyde N, Kaski JC, Dumitriu IE. Decreased levels of alternative co-stimulatory receptors OX40 and 4-1BB characterise T cells from head and neck cancer patients. *Immunobiology.* 2012;217:669–675. doi: 10.1016/j.imbio.2011.11.005.
111. Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation.* 2002;105:570–575.
112. White SJ, Newby AC, Johnson TW. Endothelial erosion of plaques as a substrate for coronary thrombosis. *Thromb Haemost.* 2016;115:509–519. doi: 10.1160/TH15-09-0765.
113. Houtkamp MA, de Boer OJ, van der Loos CM, van der Wal AC, Becker AE. Adventitial infiltrates associated with advanced atherosclerotic plaques: structural organization suggests generation of local humoral immune responses. *J Pathol.* 2001;193:263–269. doi: 10.1002/1096-9896(2000)9999:9999<::AID-PATH774>3.0.CO;2-N.
114. Moreno PR, Purushothaman KR, Fuster V, O'Connor WN. Intimomedial interface damage and adventitial inflammation is increased beneath disrupted atherosclerosis in the aorta: implications for plaque vulnerability. *Circulation.* 2002;105:2504–2511.
115. Kohchi K, Takebayashi S, Hiroki T, Nobuyoshi M. Significance of adventitial inflammation of the coronary artery in patients with unstable angina: results at autopsy. *Circulation.* 1985;71:709–716.
116. Srikakulapu P, Hu D, Yin C, Mohanta SK, Bontha SV, Peng L, Beer M, Weber C, McNamara CA, Grassia G, Maffia P, Manz RA, Habenicht AJ. Artery tertiary lymphoid organs control multilayered territorialized atherosclerosis B-cell responses in aged ApoE-/- mice. *Arterioscler Thromb Vasc Biol.* 2016;36:1174–1185. doi: 10.1161/ATVBAHA.115.306983.
117. Clement M, Guedj K, Andreatta F, et al. Control of the T follicular helper-germinal center B-cell axis by CD8+ regulatory T cells limits atherosclerosis and tertiary lymphoid organ development. *Circulation.* 2015;131:560–570. doi: 10.1161/CIRCULATIONAHA.114.010988.
118. Di Gregoli K, Jenkins N, Salter R, White S, Newby AC, Johnson JL. MicroRNA-24 regulates macrophage behavior and retards atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2014;34:1990–2000. doi: 10.1161/ATVBAHA.114.304088.
119. Du F, Yu F, Wang Y, Hui Y, Carnevale K, Fu M, Lu H, Fan D. MicroRNA-155 deficiency results in decreased macrophage inflammation and attenuated atherogenesis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol.* 2014;34:759–767. doi: 10.1161/ATVBAHA.113.302701.

120. Karunakaran D, Richards L, Geoffrion M, Barrette D, Gotfrit RJ, Harper ME, Rayner KJ. Therapeutic inhibition of miR-33 promotes fatty acid oxidation but does not ameliorate metabolic dysfunction in diet-induced obesity. *Arterioscler Thromb Vasc Biol*. 2015;35:2536–2543. doi: 10.1161/ATVBAHA.115.306404.
121. Koulis C, Chen YC, Hausding C, Ahrens I, Kyaw TS, Tay C, Allen T, Jandeleit-Dahm K, Sweet MJ, Akira S, Bobik A, Peter K, Agrotis A. Protective role for Toll-like receptor-9 in the development of atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2014;34:516–525. doi: 10.1161/ATVBAHA.113.302407.
122. Nakashiro S, Matoba T, Umezu R, Koga J, Tokutome M, Katsuki S, Nakano K, Sunagawa K, Egashira K. Pioglitazone-incorporated nanoparticles prevent plaque destabilization and rupture by regulating monocyte/macrophage differentiation in ApoE^{-/-} mice. *Arterioscler Thromb Vasc Biol*. 2016;36:491–500. doi: 10.1161/ATVBAHA.115.307057.
123. Katsuki S, Matoba T, Nakashiro S, Sato K, Koga J, Nakano K, Nakano Y, Egusa S, Sunagawa K, Egashira K. Nanoparticle-mediated delivery of pitavastatin inhibits atherosclerotic plaque destabilization/rupture in mice by regulating the recruitment of inflammatory monocytes. *Circulation*. 2014;129:896–906. doi: 10.1161/CIRCULATIONAHA.113.002870.
124. Efimov GA, Kruglov AA, Khlopchatnikova ZV, Rozov FN, Mokhonov VV, Rose-John S, Scheller J, Gordon S, Stacey M, Drutskaya MS, Tillib SV, Nedospasov SA. Cell-type-restricted anti-cytokine therapy: TNF inhibition from one pathogenic source. *Proc Natl Acad Sci U S A*. 2016;113:3006–3011. doi: 10.1073/pnas.1520175113.
125. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. *Circ Res*. 2016;118:145–156. doi: 10.1161/CIRCRESAHA.115.306656.
126. Eisenhardt SU, Habersberger J, Murphy A, Chen YC, Woollard KJ, Bassler N, Qian H, von Zur Muhlen C, Hagemeyer CE, Ahrens I, Chin-Dusting J, Bobik A, Peter K. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. *Circ Res*. 2009;105:128–137. doi: 10.1161/CIRCRESAHA.108.190611.
127. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, Bannasch H, Scheichl A, Woollard KJ, von Dobschütz E, Kolodgie F, Virmani R, Stark GB, Peter K, Eisenhardt SU. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: *in vivo* proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation*. 2014;130:35–50. doi: 10.1161/CIRCULATIONAHA.113.007124.

KEY WORDS: atherosclerosis ■ atherosclerotic plaque ■ biomarkers ■ C-reactive protein ■ interleukins

Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

Atherosclerotic Plaque Rupture: Identifying the Straw That Breaks the Camel's Back Yung-Chih Chen, Alex L. Huang, Tin S. Kyaw, Alex Bobik and Karlheinz Peter

Arterioscler Thromb Vasc Biol. 2016;36:e63-e72

doi: 10.1161/ATVBAHA.116.307993

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231

Copyright © 2016 American Heart Association, Inc. All rights reserved.

Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

<http://atvb.ahajournals.org/content/36/8/e63>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
<http://atvb.ahajournals.org/subscriptions/>