

Jak-ing Up the Plaque's Lipid Core...and Even More

Peter Libby, Roberto Molinaro, Rob S. Sellar, Benjamin L. Ebert

Until recently, many viewed atherosclerosis as a lipid storage disease or a bland accumulation of proliferated smooth muscle cells. How the landscape has changed! Abundant evidence now implicates intertwining pathways of inflammation as crucial to atherogenesis and its clinical consequences.¹ Inflammatory mediators modulate the functions of vascular wall cells that provoke the disease. Multiple cell types beckoned to enter the arterial intima during atherogenesis can produce these inflammatory mediators. Actions of monocyte/macrophages and T lymphocytes implicated both adaptive and innate immunity in atherosclerosis. Indeed, many leukocyte lineages participate in chronic inflammation in the plaque. Recent evidence points to the participation of the acute inflammatory cell, the polymorphonuclear leukocytes, in plaque complication. An ongoing struggle between proinflammatory and anti-inflammatory and proresolving stimuli determine the evolution of the plaque, from its initiation through its thrombotic complications.

Article, see p e35

In addition to arterial disease, leukocytes and inflammation also modulate venous thromboembolism, heart failure, and the healing of myocardial infarction. Indeed, the brain, autonomic nervous system, and hematopoietic organs (the bone marrow and the spleen) engage in complex crosstalk with the heart and blood vessels.² We now recognize yet another link between leukocytes and atherothrombosis: clonal hematopoiesis of indeterminate potential (CHIP).³

With age, we commonly accumulate clones of leukocytes that circulate in peripheral blood derived from bone marrow stem cells that have acquired somatic mutations that confer a proliferative advantage (Figure [A]). Over 10% of septuagenarians harbor such clones. Unsurprisingly, individuals with these leukocyte clones have a markedly elevated risk of developing hematologic malignancies. But most bearers of these mutant clones of blood leukocytes will never develop

hematologic malignancies (hence the indeterminate in CHIP). Yet, total mortality in individuals with these clones by far exceeds the deaths attributable to hematologic malignancies. Unexpectedly, cardiovascular events account for this excess mortality. This cardiovascular risk seems independent of and at least as powerful as traditional risk factors. Thus, CHIP constitutes a previously unrecognized and potent cardiovascular risk factor.⁴

Remarkably, mutations in just 4 genes account for the majority of these clones. Three of these 4 mutations involve potential modulators of DNA methylation, and most likely act through epigenetic regulation of gene expression. Mouse experiments support this conjecture, as loss of function of one of the genes commonly mutated in CHIP (*Tet2*) augments expression of proinflammatory cytokines and chemokines, as well as accelerates atherogenesis in response to an atherogenic diet.^{3,5} These experiments also indicate a causal relationship between CHIP and atherothrombosis, rather than a mere association of 2 conditions that accompany aging.

A fourth common mutation in CHIP, a V617F mutation in Janus kinase 2 (*Jak2*^{V617F}), probably elevates atherothrombotic risk through distinct mechanisms. *JAK2*^{V617F} is the most common genetic lesion in myeloproliferative neoplasms, including polycythemia vera. These patients have a thrombotic diathesis, constituting a leading cause of their morbidity and mortality.

A new study by Wang et al⁶ provides pioneering insight into how *Jak2*^{V617F} influences atherothrombosis. *Jak2*^{V617F} mice developed atheromata that contain more neutrophils, have larger lipid cores, and demonstrate increased iron deposition (Figure [B]). Mechanistic studies identified ineffective efferocytosis, likely because of reduced MerTK expression, as a contributor to the increased lipid core. Plaques with larger lipid cores associate with rupture and thrombosis, probably predisposing to cardiovascular events in bearers of *Jak2*^{V617F}. This study also implicates products of the inflammasome in lesion complication in *Jak2*^{V617F} mice.

The iron excess seems to result from increased erythrophagocytosis. In mouse cells, this process associated with less CD47 on the surface of red blood cells, a signal that would normally deter phagocytosis (a do not eat me signal). In contrast, in human cells, the authors found increased erythrophagocytosis associated with increased levels of red blood cells of the prophagocytic signal calreticulin. The rich and often leaky microvasculature of human plaques provides a potential portal for erythrocyte entry. Plexi of plaque microvessels colocalize with regions of iron deposition, which can promote the local generation of •OH via the Fenton reaction (Figure).⁷ Recent work identified biliverdin reductase as one of the most dysregulated genes in human atheromata.⁸ Elevation in this enzyme, involved in heme catabolism, may reflect a response erythrocyte products within the plaque. These human observations

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From the Division of Cardiovascular, Department of Medicine (P.L., R.M.) and Division of Hematology, Department of Medicine (R.S.S.), Brigham and Women's Hospital, and Dana Farber Cancer Institute (B.L.E.), Harvard Medical School, Boston, MA; Broad Institute of the Massachusetts Institute of Technology and Harvard, Cambridge (R.S.S.); and Department of Haematology, UCL Cancer Institute, University College London, United Kingdom (R.S.S.).

Correspondence to Peter Libby, MD, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 77 Ave Louis Pasteur, NRB 7, Boston, MA 02115. Email plibby@bwh.harvard.edu

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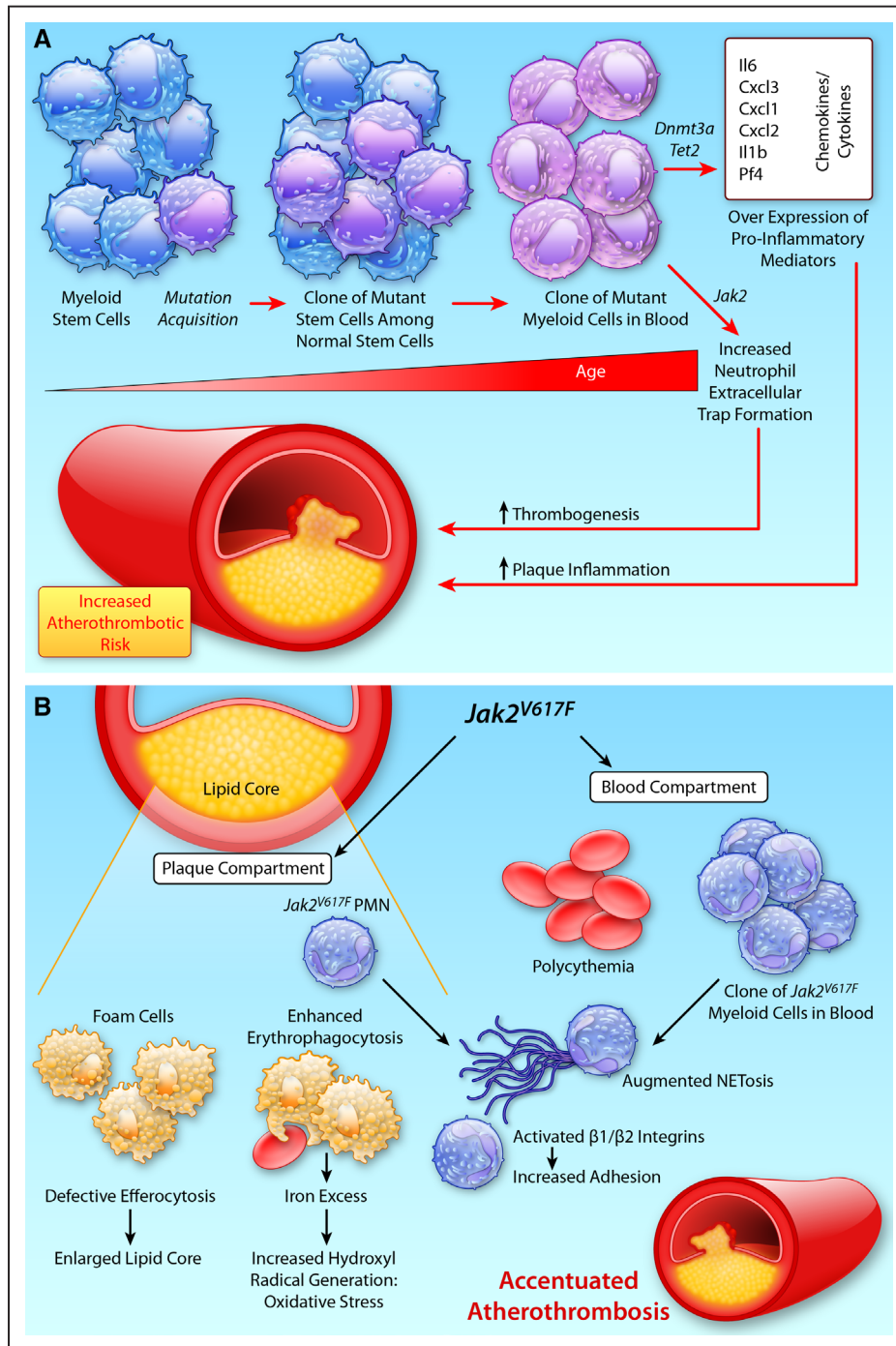


Figure. Clonal hematopoiesis, as explained in the text, arises from somatic mutations in bone marrow stem cells, associated with aging, that confers a proliferative advantage on the mutant clone. **A**, Mutations in *Tet2* and in *DNMT3A* likely augment expression of proinflammatory genes by epigenetic regulation. *Jak2* mutation can enhance neutrophil extracellular trap (NET) formation, implicated in thrombosis, among other effects shown in **B**. Modified from reference 4. (Illustration Credit: Ben Smith.) **B**, The *Jak2^{V617F}* mutation can influence atherothrombosis both by actions within the plaque and by effects on circulating leukocytes. The left side of this diagram depicts some of the alterations in aspects of plaques demonstrated in mice bearing the *Jak2^{V617F}* mutation. Impaired efferocytosis contributes to an enlarged lipid core. Increased erythrophagocytosis favors deposition of iron derived from heme that can generate the highly oxidant hydroxyl radical augmenting local oxidative stress. Mutant granulocytes in the blood compartment (right side) have a heightened tendency to form NETs, which may favor thrombosis and propagate vascular injury. They also have activation of $\beta1/\beta2$ integrins, that can enhance attachment to the endothelium, where they may contribute to endothelial damage and thus promote thrombosis. (Illustration Credit: Ben Smith.)

bolster the findings about abnormal erythrocyte trafficking and iron deposition described by Wang et al.⁶

Jak2^{V617F} neutrophils have a heightened tendency to form neutrophil extracellular traps, another contributor to thrombosis.⁹ In addition, *Jak2^{V617F}* polymorphonuclear leukocyte

translocate Rap1 to the surface membrane, activating $\beta1/\beta2$ integrins and enhancing leukocyte binding to endothelial adhesion molecules and augmenting experimental thrombosis.¹⁰ Thus, the *Jak2^{V617F}* mutation can alter atherosclerosis within the lesion itself, and also in the blood compartment,

sensitizing granulocytes to neutrophil extracellular trap formation (Figure [B]).

The study of Wang et al⁶ has some limitations. The promoter used directed not only expansion of myeloid cells, but also yielded polycythemia and thrombocytosis, and the mutant clone accounts for the vast majority of bone marrow-derived cells. Thus, while this pattern mimics myeloproliferative neoplasms, individuals with CHIP generally have smaller clones (a median variant allele fraction under 10%), and by definition do not have abnormalities in blood cell numbers.¹¹ These complexities render it difficult to dissect out the contributions of the quantitative elevations in the different lineages, as opposed to qualitative changes in cells, and the alterations in plaque character described. This circumstance, however, may have serendipitously permitted discovery of the altered red blood cell fates they found.

The involvement of granulocytes in human atherosclerosis has engendered controversy. Although mouse lesions incorporate polymorphonuclear leukocyte in all stages of atherogenesis, granulocytes in human atherosclerosis seem predominantly in lesions previously disrupted either from leakage from friable neovessels or by fibrous cap fissure followed by healing. Granulocyte involvement in the earlier phases of human atherosclerosis requires further study.

The extreme hypercholesterolemia generally produced in mouse atherosclerosis experiments presents another gap between the experimental conditions and the human disease. The increased use of ever more effective LDL (low-density lipoprotein)-lowering therapies has led to an almost 20-fold difference in the cholesterol concentrations in typical mouse experiments and what is currently encountered in many patients. As cholesterol crystals can coactivate the inflammatory, extreme hypercholesterolemia could exaggerate the contribution the inflammasome and its products mature IL (interleukin)-1 β and IL-18 in such mouse experiments.

Effective LDL lowering in the clinic may also lessen the prevalence of the so-called vulnerable plaque. Recent observations in mouse atherosclerosis that loss of function of IL-1 can augment characteristics of atheromata associated with the propensity to rupture¹² contrast starkly with studies of interruption of IL-1 β signaling in humans that show no structural changes in arteries and reduced risk of cardiovascular events.^{13,14} These directionally opposite findings in experimental atherosclerosis in mice and human studies inject a sobering note of caution in the extrapolation of results obtained in mice to humans.¹⁵ We should use mice as an invaluable tool to probe mechanisms, as done in the elegant study by Wang et al,⁶ rather than as a reliable predictor of translation to humans.

The recent recognition of the link between CHIP and worsened cardiovascular outcomes offers the community a cornucopia of opportunity to expand understanding of atherogenesis beyond traditional risk factors. Individuals in the CHIP demographic have often experienced decades of exposure to unnecessarily high concentrations of LDL, an indubitably causal risk factor. For such individuals, targeting pathways uncovered by the study of CHIP suggests treatments beyond the current standard of care (eg, JAK inhibitors, or blockers

of IL-1 β , IL-18, or IL-6), directed by CHIP status, that might address the remaining unacceptable burden of risk in a more personalized and precision manner than heretofore possible.

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Disclosures

R.S. Sellar and B.L. Ebert have filed a patent application covering the use of JAK-STAT inhibition with Ruxolitinib to inhibit NETosis. B.L. Ebert has consulted for Celgene. The other authors report no conflicts.

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