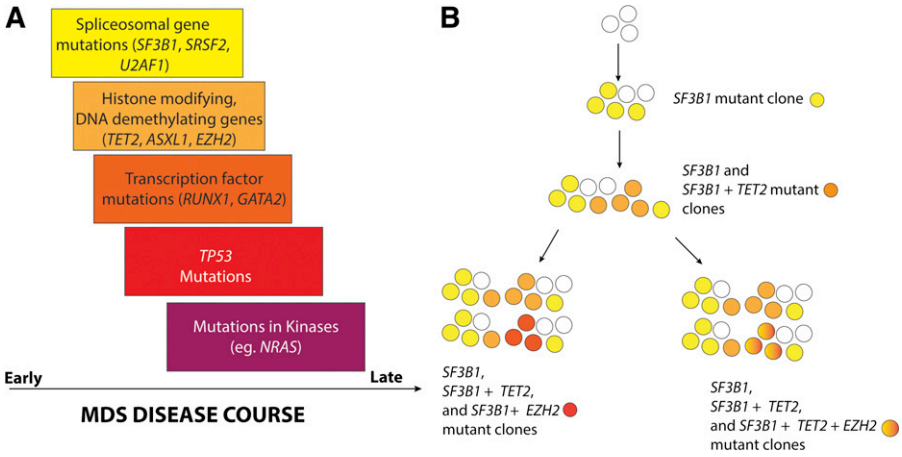

Temporal order of mutation acquisition in MDS based on data from Papaemmanuil et al. (A) Analysis of the variant allele fractions in 111 genes sequenced in 738 patients with MDS and related disorders yielded a common pattern of mutation acquisition in the natural history of MDS. Based on this work, it appears that mutations in genes encoding spliceosomal components occur early in the pathogenesis of MDS with mutations in epigenetic modifiers and transcripitional regulators occurring later. Mutations activating cytokine signaling appear to be among the latest clonal events in MDS. In the figure, the width of the box indicates the time frame in which mutations of genes described in the box occurs. The overlap of the boxes indicates the fact the patterns of mutation acquisition in MDS are not absolute. (B) The authors noted that nearly half of MDS patients had ⩾2 frequently mutated genes simultaneously. In these individuals, one-third had multiple subclones and an attempt to construct the clonal hierarchy of 1 such individual bearing *TET2, SF3B1,* and *EZH2* mutations from the study is shown.

individuals.[6] This may account for why the observations of mutational acquisition suggested from the data here are not absolute. Further work should be done via both longitudinal sequencing studies as well as functional studies to understand the potential importance, if any, on the order of acquisition of commonly occurring mutations in MDS.

The authors compared the predictive value of clinical prognostic measures, such as those considered by the International Prognostic Scoring System (IPSS), to the presence of mutations. In their global analysis, they found that the addition of mutation information did not significantly refine the predictive power of a model considering clinical variables alone, although it did improve on the IPSS. More careful analysis, potentially distinguishing different molecular subtypes of MDS, will be needed to determine how best to incorporate mutational information into the prediction of prognosis.

Finally, this study describe how extensive targeted sequencing provides reliable copy number data that completely coincides with clinical fluorescence in situ hybridization (FISH)/cytogenetic data in MDS. From a purely practical standpoint, this suggests that serial sequencing might simultaneously provide both mutational data and copy number data capable of supplanting recurrent FISH/cytogenetic testing in MDS where balanced translocations are rare.

Based on the above, it is very likely that longitudinal genetic characterization of MDS will add to our ability to predict clinical outcomes, as well as identify emerging clones with potentially actionable alterations. To fully take advantage of what we learn by sequencing, we will have to pair genetic results to specific treatments, something that will require more therapeutic options for patients with MDS than we have today. In the meantime, we can apply this data toward making better prognostic models and improving our understanding of biological processes that underlie these often morbid disorders. Further work to identify the effect of existing treatments, such as DNA hypomethylating agents and allogeneic transplantation, on the genetic characteristics of persistent and relapsed disease clones, is likely to be incredibly informative and of great value to patients.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● PLATELETS & THROMBOPOIESIS

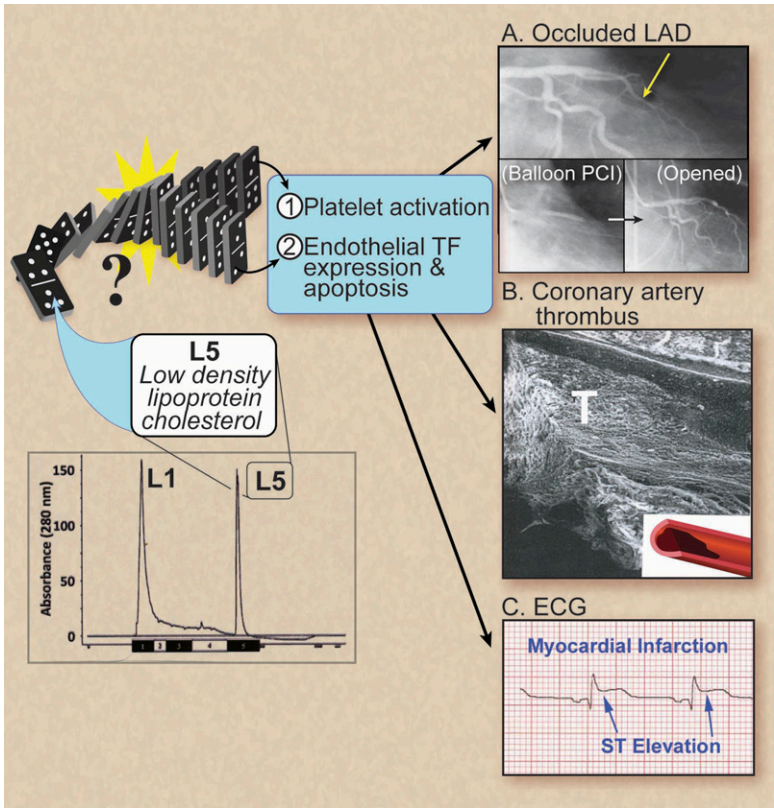
Comment on Chan et al, page 3632

Bad cholesterol breaking really bad

Timothy C. Nichols[1] [1]UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

In this issue of *Blood*, Chan et al have described mechanisms by which L5, the most electronegative of 5 recognized fractions of low density lipoprotein (LDL), activates both platelets and endothelium in a manner that supports thrombosis and could possibly produce ST-elevation myocardial infarctions (STEMI).[1]

Most importantly, L5 was significantly elevated in STEMI patients when compared with otherwise healthy control subjects. These changes in the fractional composition of LDL, often called "bad cholesterol" because of its role in



Can bad cholesterol get worse? L5, the most electronegative LDL fraction isolated chromatographically, was found to be significantly elevated in patients with STEMI compared with normal controls.[1] Ex vivo, L5 not only enhanced adenosine 5′-diphosphate-stimulated platelet aggregation, platelet P-selectin expression, and GP IIb/IIIa activation but also induced both endothelial tissue factor and P-selectin expression and apoptosis. Together, these data provide mechanistic details of the thrombophilic state created by L5 that could cause a domino effect and produce an occlusive coronary artery thrombosis resulting in an STEMI. (A) Coronary angiogram showing an occluded left anterior descending coronary artery (LAD) during STEMI and inserts showing balloon percutaneous coronary intervention (PCI) and the angiogram after opening the LAD. (B) Scanning electron micrograph of bisected LAD from a pig with an occlusive thrombosis (T) causing an STEMI. (C) Electrocardiogram showing a STEMI. Professional illustration by Debra T. Dartez.

atherogenesis, may add thrombophilic properties. Platelets that were exposed to clinically relevant doses of purified L5 exhibited enhanced adenosine 5′-diphosphate-stimulated aggregation, P-selectin expression, and GP IIb/IIIa activation with signaling through platelet-activating factor receptor and lectin-like oxidized LDL receptor-1. Endothelium exposed to L5 in a likewise manner expressed tissue factor and P-selectin that also supported platelet activation and aggregation. As previously shown, L5 also mediated endothelial apoptosis by reducing expression of the fibroblast growth factor-2 (*FGF2*) promoter via an epigenetic mechanism (CpG methylation). Injecting L5 into mice corroborated these ex vivo findings. Does elevated L5 cause a domino effect that is sufficient to activate platelets and endothelium, induce endothelial apoptosis, and produce occlusive coronary artery thrombosis and STEMI (see figure)?

The history of establishing occlusive coronary artery thrombosis as the cause of STEMI has become an object lesson in investigative pathology and clinical trials. Between William Heberden's original description of angina in 1772[2] and the 1970s, the wide range of coronary thrombi found at autopsy of patients dying of suspected heart disease generated considerable debate as to its causative role. It was not until the pioneering work of DeWood,[3] Rentrop,[4] and several outstanding pathologists[5] in the 1970s that occlusive coronary artery thrombosis became accepted as the leading cause of STEMI. The key steps in resolving this debate were refinement in methods of detecting coronary thromboses, recognition that clot lysis occurs over time, and identification of coronary plaques that were vulnerable to rupture. A vulnerable plaque is characterized in part by a thin fibrous cap, high lipid content, inflammatory mediators, and extensive adventitial and intimal neovascularity. When vulnerable plaques rupture and expose subendothelium and plaque contents to flowing blood, coronary artery thrombosis develops and, if occlusive, can result in a STEMI. It is equally important to note that a subtler finding of plaque erosion is nonetheless also associated with coronary artery thrombosis.[6,7]

Identification of the precise mechanism(s) that causes vulnerable plaques to rupture or the sequence of events that precipitate coronary artery thrombosis and STEMI has been elusive. In a meta-regression analysis that included 36 studies to compute the population-attributable fraction, ie, cases that could be avoided if a risk factor were removed, the following hierarchy of suspected stimuli of myocardial infarction was established: air pollution, physical exertion, alcohol, coffee, negative emotions, anger, heavy meal, positive emotions, sexual activity, cocaine use, marijuana smoking, and respiratory infections.[8] The relatively low frequency of STEMI with each of these stimuli, however, suggests that other factors or cofactors are likely to be involved. Especially relevant to this argument is the consistent finding that small, "non-culprit" coronary plaques appear, over time, to be responsible for as many coronary events as larger "culprit lesions."[9,10] In contrast, the biological properties of L5 could produce STEMI by several mechanisms: first, initiating plaque erosion or rupture by supporting endothelial apoptosis and second, mediating coronary thrombogenesis via platelet and endothelial activation.

There are limitations to this study. A total of 30 patients is small for a STEMI study, but larger studies are underway that will address the somewhat wide variation in L5 levels in the patients included in this study. At present, the mechanism by which L5 becomes elevated in plasma is unknown as is the duration of elevation prior to STEMI. Such information would be important to propose therapeutic approaches to reduce L5 levels or modify it to be less thrombogenic. The association of elevated L5 levels with other traditional risk factors such as hyperlipidemia and diabetes suggests that risk factor reduction may be a first step. Likewise, low dose aspirin was shown to blunt L5-mediated endothelial apoptosis in vitro and it will be important to determine if this biological effect is also operative in vivo. The exact constituent

of L5 that confers the described biological properties has not been defined yet. Also, it is not clear if L5 is present in other populations beyond those reported or how gender or menopausal status might influence L5 levels. The procedures required for measuring L5 are rigorous and may limit its translation to general usage unless simpler methods can be developed. All of these limitations are surmountable.

Definitive proof of a single mechanism mediating coronary artery thrombosis and STEMI seems unlikely to be forthcoming given the pleomorphic nature of atherosclerotic plaques and the myriad of potential interactions with the cellular and humoral thrombosis pathways. Consideration will need to be given to whether suspected stimuli are additive or synergistic, if there is a hierarchy among them, or if other cofactors are involved. The data presented by Chan et al[1] clearly document that elevations in L5 could initiate a domino effect that produces an occlusive coronary artery thrombosis and STEMI.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Comment on Wang et al, page 3642

Platelets using proteins creatively

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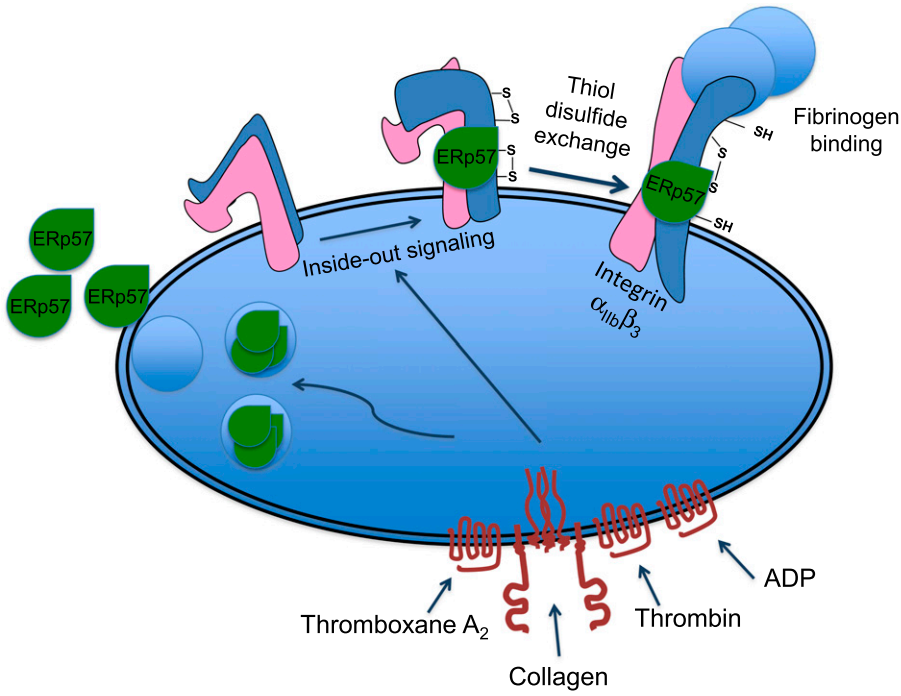
In this issue of *Blood*, Wang et al identify an important role for platelet-derived extracellular ERp57, a thiol isomerase enzyme, in platelet integrin regulation and recruitment into a growing thrombus.[1]

Platelets might seem like simple cells—but are they? They lack a nucleus and therefore do not have to worry about gene transcription, and as a consequence, they have only a limited life expectancy in the circulation, before being cleared and replaced by new platelets. Over recent years, however, the molecular processes that control the functions of platelets in hemostasis and thrombosis have begun to emerge and have revealed platelets to be perhaps more complex that may have been anticipated.

Curiously, platelets contain many proteins that one may not expect them to need, at least not if they use these proteins in a conventional manner. It seems, however, that platelets are creative and use some proteins in unexpected ways. A prime example of this is a family of enzymes known as thiol isomerases.[2] These proteins, the best characterized of which is protein disulfide isomerase (PDI), normally function within the endoplasmic reticulum of cells, where they perform an important role in ensuring the correct folding of proteins before they are secreted to the cell surface or beyond. They function to catalyze the reversible oxidation of thiols to disulfide bonds and the rearrangement of disulfide bonds. Together with other chaperone proteins, their location is normally restricted to a cell's secretory machinery where they enable proteins to



Platelet activation results in the relocation of the thiol isomerase enzyme ERp57 to the platelet surface where it binds to the β_3 integrin subunit. The absence of ERp57 protein, or inhibition of its activity, results in diminished platelet activation, aggregation, and recruitment into a growing thrombus. Because integrin $\alpha_{IIb}\beta_3$ activation, which is necessary for fibrinogen binding, is associated with disulfide rearrangement, this suggests ERp57 may contribute to its activation or stabilization in a conformation that is able to bind to fibrinogen.