activated receptor 4 (PAR4) activating peptide, and the GPVI ligand, convulxin. SR-BI$^{-/-}$ platelets had a heightened response to PAR4-activating peptides, yet a blunted response to ADP and convulxin. This partial defect in platelet responsiveness to ADP and convulxin was not entirely unexpected, as SR-BI$^{-/-}$ mice are thrombocytopenic and their platelets have an altered morphology. Notably, this defect in platelet morphology and function is not due to platelet SR-BI deficiency per se, as SR-BI$^{-/-}$ mice transplanted with wild-type bone marrow (ie, platelets expressing normal levels of SR-BI) also exhibit a moderate thrombocytopenia and altered morphology. These findings suggest that the hyperlipidemic milieu associated with non–bone marrow–derived SR-BI deficiency is the principal cause of the thrombocytopenia and abnormal platelet morphology.

Using bone marrow reconstitution models, the authors elegantly demonstrate a dramatic shift in platelet reactivity as a function of SR-BI expression in bone marrow and non–bone marrow tissues. Their studies demonstrate that non–bone marrow SR-BI deficiency (presumably SR-BI in the liver) is the principal cause of platelet hyperreactivity, due to a profound increase in serum lipoproteins and a marked increase in platelet cholesterol content. However, enhanced platelet reactivity to all agonists was only observed with SR-BI expression in platelets. Thus, there appear to be 2 requirements for global platelet hyperactivity in SR-BI–deficient mice: (1) a marked dyslipidemia due to loss of the cholesterol scavenging function of SR-BI in non–marrow tissue and (2) unperturbed platelet expression of SR-BI, leading to increased platelet reactivity.

Ma et al demonstrate that SR-BI deficiency alone, under normolipidemic conditions, does not lead to a major defect in platelet aggregation, suggesting that this receptor is unlikely to play a major role in regulating the hemostatic function of platelets. In contrast, the authors convincingly demonstrate that platelet-expressed SR-BI plays an important role in promoting thrombosis in several distinct models of dyslipidemia, raising the interesting possibility that selectively targeting platelet SR-BI may represent a safe and effective approach to reduce platelet hyperactivity in dyslipidemic states.

These new findings by Ma et al provide further insight into the complex interplay between hyperlipidemia, platelet hyperreactivity, and a prothrombotic phenotype. Putative mechanisms for the modulation of platelet function by SR-BI have been proposed, including alterations in cholesterol organization required for the assembly of lipid rafts. However, the demonstration that the SR-BI receptor itself has an intrinsic role in modulating platelet responses to higher concentrations of physiologic agonists suggests that other mechanisms may also be involved. Future studies will be required to more clearly define the molecular mechanisms by which SR-BI modulates platelet function. In addition, a clearer understanding of the relationship between the prothrombotic effects of specific oxidized phospholipids through CD36 with the altered cholesterol loading through SR-BI are required to fully understand the approaches that are likely to be most effective at reducing increased platelet reactivity in dyslipidemic states.

Although these recent studies shed new light on the role of scavenger receptors in regulating platelet function, their importance to platelet hyperreactivity in humans remains to be determined as the mouse models do not accurately reflect the dyslipidemia that occurs in humans. Nonetheless, these recent studies by Ma et al offer potential new insights into the refractoriness of conventional antiplatelet therapies often encountered in disease states that accompany hyperlipidemia such as type II diabetes and the metabolic syndrome. Given the importance of CD36 and SR-BI in lipid scavenging in a range of cell types, the challenge remains to develop strategies that reduce the prothrombotic effects of plasma lipoproteins while minimizing the impact of these therapies on cholesterol uptake and steroidogenesis.

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Comment on Halene et al, page 1942

Megakaryocytes muscle in

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In this issue of *Blood*, Halene and colleagues reveal an essential function of the transcription factor SRF during megakaryocyte maturation and platelet formation. The spectrum of SRF-regulated genes overlaps with SRF targets in muscle cells, including those involved in actin cytoskeletal dynamics.

Serum response factor (SRF) is a highly conserved transcription factor that binds to a DNA motif known as serum response element.[1] Although ubiquitously expressed in adult tissues, SRF expression during embryonic development is largely restricted to cardiac and skeletal muscles.[2] SRF plays a critical role in cardiogenesis and is a major determinant of cardiomyocyte differentiation.[2] With low intrinsic transcriptional activity itself, SRF recruits cofactors to confer strong transactivation potential in a cell context-specific manner. SRF associates with 2 classes of coactivators, 1 of which is the myocardin family of proteins that includes myocardin, and myocardin-related transcription factors MRTF-A (also known as megakaryoblastic leukemia 1, or MKL1) and MRTF-B (or MKL2).[3] SRF-MKL target genes encode proteins involved in muscle-specific and contractile functions, and actin microfilament dynamics and cell motility.[3]

SRF regulates many cellular processes in diverse cell types but its role in hematopoiesis

is underexplored. Halene and colleagues examined in-depth the role of SRF in megakaryocyte maturation and platelet production through megakaryocyte-specific ablation of the *SRF* gene in mice.[4] Loss of SRF results in macrothrombocytopenia with prolonged bleeding times along with increased numbers of immature, dysmorphic megakaryocytes in the bone marrow and spleen. In vitro culture experiments attribute these defects in part to impaired adherence to fibronectin and reduced proplatelet formation in methylcellulose. Immunofluorescence studies showed that SRF-deficient megakaryocytes and platelets have intact tubulin bundles but abnormal stress fiber formation with disorganized filamental actin. Transmission electron microscopy further revealed an abnormal morphology of giant platelets, and lack of a uniform demarcation membrane system in the megakaryocytes. Furthermore, transcriptome analysis uncovered reduced expression in SRF-deficient megakaryocytes of numerous genes controlling actin cytoskeleton.

Among the most down-regulated genes in SRF-deficient megakaryocytes are the actin cross-linking protein Filamin A, the actin-binding protein Coronin 1a, and the actin filament-associated regulatory protein Calponin,[4] as well as genes involved in cell motility.[5,6] Notably, SRF also regulates many of these genes in other cell types, such as muscle cells.[3] This similarity suggests that SRF assumes similar functions in diverse cell lineages via similar mechanisms and also underscores the importance of actin cytoskeletal organization and dynamics in megakaryopoiesis and thrombopoiesis.[7] This study, along with several others, contrasts with the original view that SRF is a serum-stimulated transcription factor that regulates "immediate early" growth genes.[3,8] Rather, a large fraction of SRF target genes are in fact structural genes related to actin biogenesis and contractile functions.[3,8] Furthermore, mouse genetic studies in multiple cell types suggest that SRF is dispensable for cell proliferation, but is instead crucial for the actin cytoskeleton, cell migration and contraction, as well as differentiation.[3,8]

The phenotypic consequences of SRF loss in megakaryocytes are likely an underestimate due to the late and incomplete conditional excision of SRF in the megakaryocytic lineage. However, the intriguing results from this report warrant further studies in earlier megakaryocyte development and additional hematopoietic lineages using appropriate conditional transgenes.

A role for SRF during megakaryopoiesis is not unexpected in light of 2 previous reports showing that 1 of its cofactors, MKL1, plays important roles in megakaryopoiesis.[9] MKL1 overexpression promotes megakaryocytic differentiation of human $CD34^+$ cells cultured in thrombopoietin.[9] The effects of MKL1 overexpression are lost when SRF is depleted, suggesting that MKL1 function is dependent on SRF.[9] Moreover, knockdown of MKL1 in human progenitor cells and knockout of MKL1 in mice results in dysmorphic megakaryocytes with reduced proplatelet formation.[6,9] Not surprisingly, loss of MKL1 confers a less profound perturbation in megakaryocyte maturation compared with SRF deficiency. SRF recruits several cofactors, including MKL2 that might compensate for the loss of MKL1. In addition, SRF recruits members of the Ets family of coactivators, such as the ternary complex factors, to the serum response elements.[3] Assigning individual SRF cofactors to specific SRF targets requires further studies.

SRF activity is highly regulated and vital for many developmental processes.[10] Importantly, a growing number of human diseases is associated with changes in the activity of SRF and its cofactors.[3,8,10] The t(1;22) chromosomal translocation found in a subset of acute megakaryoblastic leukemia (AMKL) in children results in the in-frame fusion of the OTT transcription factor to MKL1 (also known as OTT-MAL). Expression of the oncogenic OTT-MKL1 gene from the endogenous OTT locus in mice leads to aberrant differentiation of hematopoietic stem/progenitor cells with a bias toward the megakaryocytic lineage.[11] Progression to full-blown AMKL requires the cooperativity with the activated thrombopoietin receptor.[11] The megakaryoblast proliferation also depends on the interaction between the OTT portion of the fusion protein and a component of the canonical Notch pathway.[11] However, whether the MKL1 portion of the oncogene plays a role in megakaryocyte differentiation and/or blast transformation and whether it is mediated by SRF during the pathogenesis of AMKL remains to be investigated.

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