and then presented on surface MHC molecules on thymic MEC. Mature thymocytes percolate through the medulla, and, if their TCRs recognize a PTA/MHC complex in the appropriate affinity/avidity window, they will be overactivated and deleted from the repertoire. The results for MAGE by Goodyear et al[1] suggest that thymic expression of PTAs might be regulated by epigenetic modifiers. For instance, it is conceivable that pharmacologic approaches to up-regulating islet cell antigens to promote negative selection of autoreactive T cells in a still-functioning thymus could be tested to prevent diabetes mellitus or other autoimmune diseases.

The question of whether CTAs are differentially up-regulated in AML and leukemia stem cells compared with normal cells and hematopoietic stem cells remains unanswered. Furthermore, do the effects on antigen expression extend to other tumor antigens, such as LAA and mHA, and to what extent are other self-antigens up-regulated? Importantly, this study suggests a new strategy to identify tumor antigens by determining which genes are aberrantly expressed in cancer cells after exposure to agents that induce epigenetic modifications. Ultimately, the results reported by Goodyear and colleagues raise many additional questions and highlight the exciting possibility of future trials to test combinations of antigen-specific immune treatments with agents that epigenetically modify target antigen expression.

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

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● ● ● MYELOID NEOPLASIA

Comment on Badalian-Very et al, page 1919

BRAF, a piece of the LCH puzzle

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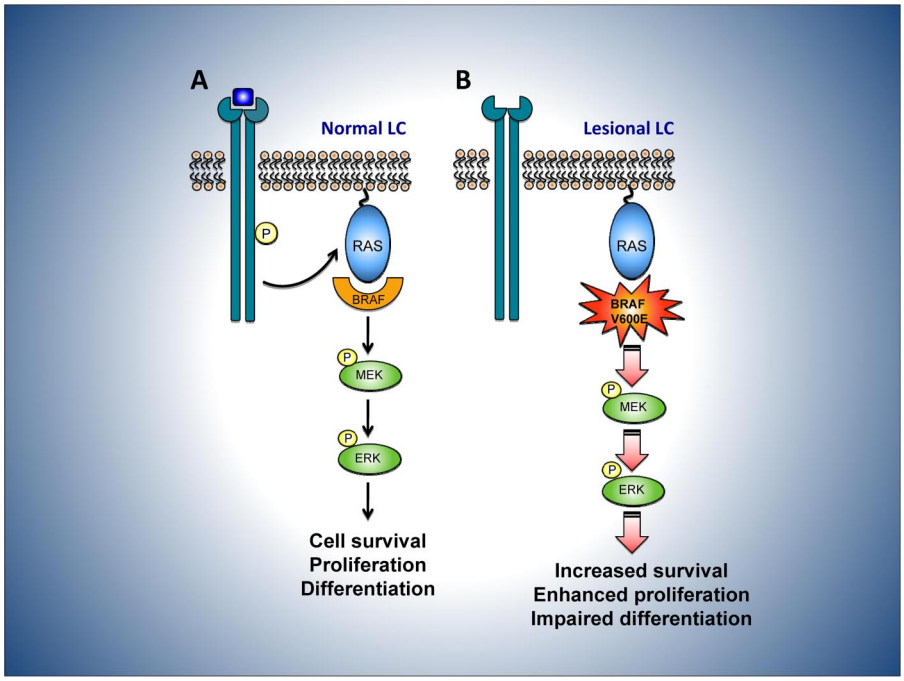
LCH is a rare disorder affecting patients of all ages that is characterized by the pathologic accumulation of immature LC and other inflammatory cells in organs such as skin, bone, liver, lungs, bone marrow, and brain. Although first described over a century ago, the etiology of LCH has remained elusive. In this issue of *Blood*, Badalian-Very and colleagues provide exciting new insights into LCH by demonstrating that a subset of cases exhibit somatic activating mutations in the proto-oncogene *BRAF*.[1]

The clinical manifestations of Langerhans cell (LC) histiocytosis (LCH) are remarkably variable with some patients exhibiting localized involvement of specific sites and others developing a disseminated form of disease that mirrors acute leukemia. Although the majority of the patients with localized LCH may be cured of their disease, the outcome for those with systemic involvement is suboptimal with 20% to 50% of patients dying despite the use of intensive multiagent chemotherapy and/or stem cell transplantation. To improve the outcome for patients with LCH, particularly those with high-risk disease, understanding the pathogenesis of this puzzling disorder is imperative.

Researchers have long debated whether LCH represents a true malignancy or a reactive immune condition. Resolving this issue is important as the answers are likely to have consequences in terms of diagnostic testing, outcome prediction, therapy, and patient counseling. Studies in favor of the notion that LCH is a malignancy include the demonstration that LC from nonpulmonary lesions are monoclonal based on a pattern of highly skewed X-chromosome inactivation.[2,3] Other supportive findings include the immature appearance of lesional LC, the existence of rare familial clusters, evidence of cell-cycle dysregulation within lesions, and the presence of significant telomere shortening of LCH cells compared with LC from other inflammatory lesions. Epidemiologic data also suggest a close association between LCH and cancer, particularly lymphoma and acute lymphoblastic leukemia. In support of a potential clonal relationship among the disorders are reports documenting identical molecular changes in the lymphoma or leukemia and the LCH.[4-6] In contrast, supporters of the opinion that LCH is a reactive process emphasize that clonal cell populations are commonly present within the immune system and that phenotypically immature LC often accumulate in areas of chronic inflammation (eg, dermatopathic lymphadenitis). The lesional expression of inflammatory chemokines and cytokines (most recently, interleukin 17, a key cytokine in several autoimmune disorders) has been reported, although conflicting data exist.[7,8] Finally, although high levels of p53 protein have been described in LCH, no mutations in the *TP53* gene have been reported, nor have recurrent chromosomal translocations or other genomic abnormalities been consistently described.[9]

In this study, Badalian-Very and colleagues demonstrate that 35 (57%) of 61 LCH specimens exhibit mutations in *BRAF* that encode a known oncogenic V600E form of the BRAF protein. In contrast, the related disorders



Potential consequences of mutant BRAF V600E in LCH. (A) In normal LC, mitogens such as growth factors bind to and activate cell-surface receptors, which signal through a complex consisting of adaptor proteins and exchange factors (not shown) to activate the small G-protein RAS on the inner surface of the plasma membrane. Once active, RAS binds to and activates the RAF family of proteins, comprising BRAF, ARAF, and CRAF. RAF then phosphorylates and activates MEK, which subsequently phosphorylates and activates ERK. ERK phosphorylates numerous substrates within the cytoplasm and nucleus, promoting cell division and enhancing survival, movement, and differentiation. (B) In LC from LCH lesions, constitutive activity of the mutant BRAF V600E protein is predicted to bypass the requirement for mitogen induced activation of RAF by RAS. This may lead to dysregulated signaling through the MEK-ERK pathway and thereby favor the survival and proliferation of lesional LCH cells while perturbing their differentiation.

dermatopathic lymphadenopathy and Rosai-Dorfman disease did not exhibit *BRAF* gene mutations. Although the BRAFV600E mutation was present more often in younger LCH patients, it was not associated with site or stage of disease.

BRAF is a member of the RAF family of serine threonine kinases, which are components of the RAS-RAF-MAPK signaling pathway (see figure). In normal cells, the activity of this pathway is controlled by mitogens, including growth factors, cytokines, and hormones that bind to cell-surface receptors. Ligand-bound receptors then activate RAS, which in turn binds and activates RAF and its downstream substrates, thereby enhancing cell survival, proliferation, motility, and differentiation. Somatic-activating *BRAF* gene mutations are observed in premalignant cutaneous nevi and a range of cancers, including melanoma, papillary thyroid cancer, colorectal and lung cancer, low-grade ovarian carcinoma, and pediatric low-grade glioma.[10] Among the mutations identified, V600E is by far the most common. This mutant version of BRAF is constitutively and highly active; it is proposed that unabated activation of the MEK-ERK pathway contributes to dysregulated cell proliferation, survival, and ultimate malignant progression.

The study by Badalian-Very and colleagues provides the first molecular insights into the pathogenesis of LCH and is important for several reasons. First, the identification of activating *BRAF* gene mutations strongly supports the hypothesis that LCH is a neoplastic process, at least in some cases. This observation has significant clinical implications as it suggests that alternative therapeutic approaches aimed at targeting active BRAF should be tested in LCH, particularly in patients with the systemic and aggressive form of disease. Furthermore, this mutation should provide a means to assess minimal residual disease status in a subset of LCH patients. Second, the authors observe that lesional LC stain strongly for phospho-MEK and -ERK, regardless of *BRAF* mutational status. This finding suggests that RAF-MEK-ERK pathway activation may be a general feature of LCH. Thus, mutations in other genes that regulate this pathway should be sought in LCH cases with normal *BRAF* alleles. Third, it should now be possible to generate mouse models for LCH by activating the RAS-RAF-MEK signaling pathway specifically in LC. Such models will provide insight into mechanisms of disease initiation and progression and facilitate examination of the effects of BRAF-directed therapies. Elucidation of the effects of oncogenic BRAF and the consequences of its inhibition are critical to optimize therapeutic efficacy and minimize adverse effects resulting from manipulation of the RAS-RAF-ERK pathway.

The question of whether LCH is a neoplasm or an immune dysregulation has stimulated many important investigations into this enigmatic disorder. Although the current work by Badalian-Very and colleagues has not solved the puzzle of LCH, it has provided us with critical information that moves us in the right direction. For example, the findings of this study suggest several possible and exciting areas of investigation, including the initiation of multicenter clinical trials examining BRAF as a therapeutic target and further assessing the association between *BRAF* gene mutations with disease site, stage, clinical response, and outcome. In addition, the current work opens the door to future basic research studies examining whether mutations in other members of the RAS-RAF-MEK pathway participate in disease initiation and identifying the additional molecular events that cooperate with mutant BRAF to promote disease progression.

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PLATELETS & THROMBOPOIESIS

Comment on Ma et al, page 1932

SR-BI and fatty platelets

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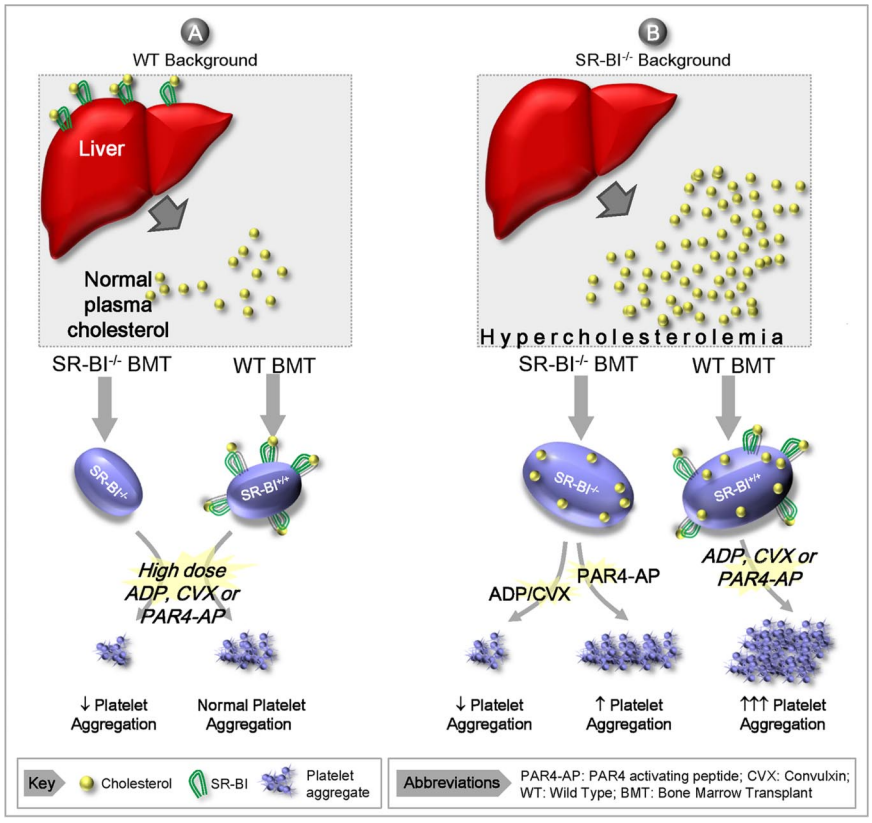
Dysregulated cholesterol transport and metabolism are important risk factors for atherothrombosis and are commonly associated with enhanced platelet reactivity; however, the molecular mechanisms linking hypercholesterolemia with increased platelet function remain unclear. In this issue of *Blood*, Ma et al define an important role for the platelet lipoprotein scavenger receptor SR-BI in regulating platelet hyperactivity and thrombosis in hyperlipidemic states.[1]



Proposed model for the role of SR-BI in modulating platelet reactivity. (A) Normolipidemia. Under normolipidemic conditions the platelet count and cholesterol content remain normal irrespective of whether platelets express SR-BI. Note that SR-BI$^{-/-}$ platelets have reduced responsiveness to high concentrations of soluble agonists relative to wild-type (WT) controls. (B) Hyperlipidemia. In the absence of SR-BI expression by steroidogenic tissues (principally the liver), there is reduced cholesterol uptake from the circulation, leading to hyperlipidemia. The severe hyperlipidemia in SR-BI$^{-/-}$ results in thrombocytopenia and an increased platelet cholesterol content irrespective of whether platelets express SR-BI. Under hyperlipidemic conditions, SR-BI$^{-/-}$ platelets have increased responsiveness to PAR4 agonists, but a paradoxical hyporesponsive to other agonists. Note that WT platelets are hyperresponsive to all agonists in the presence of hyperlipidemia.

The exaggerated accumulation of platelets at sites of atherosclerotic plaque rupture is an important step in the development of arterial thrombi, and is the principal pathogenic mechanism underlying acute myocardial infarction and ischemic stroke. Heightened platelet reactivity is an important risk factor for arterial thrombosis and is commonly observed in patients with diabetes and hyperlipidemia. The mechanisms underlying increased platelet reactivity in hyperlipidemic states are complex and diverse, including the direct platelet-activating effects of oxidized low-density lipoprotein (LDL), changes in the intrinsic reactivity of platelets due to cholesterol loading of platelet membranes, as well as oxidative modification of membrane lipids and glycoproteins.[2-4] In a previous study, Podrez and colleagues demonstrated an important link between oxidative stress, dyslipidemia (increased serum LDL/triglycerides and decreased high-density lipoprotein [HDL]), and enhanced platelet reactivity.[4] These authors demonstrated that pathophysiologic plasma levels of oxidized choline glycerophospholipids (termed oxPCCD36) stimulate platelet activation through the lipid scavenger receptor, CD36. In the current study, Ma et al demonstrate an important role for a second scavenger receptor, SR-BI, in regulating platelet hyperactivity in the context of hyperlipidemia.

Scavenger receptor-BI plays a pivotal role in cholesterol metabolism. SR-BI is a multiligand receptor member of the CD36 superfamily. SR-BI functions to scavenge cholesterol esters from HDLs in tissues such as the liver for use in steroidogenesis.[5] Thus, the loss of SR-BI expression in such tissues results in excessive accumulation of lipoproteins in the circulation (see figure). The dyslipidemia associated with SR-BI deficiency is associated with increased unesterified cholesterol in the circulation as well as increased cholesterol loading in platelet membranes. Platelets have also recently been shown to express SR-BI on their surface; however, the role of this receptor in platelet function has not been well defined.

Using a series of mouse knockout and bone marrow transplantation models, Ma et al investigated the effects of SR-BI deficiency on platelet aggregation and thrombosis. Using platelets isolated from SR-BI$^{-/-}$ mice, they demonstrated differential responsiveness to adenosine diphosphate (ADP), protease-