EBV-associated lymphoma pathogenesis and the relationship with EBV immortalization of lymphocytes remain elusive.

In previous investigations, these authors had studied differential expression of protein tyrosine kinases in primary B cells and EBV-immortalized lymphocytes, and identified 2 RTKs that were upregulated in EBV-immortalized lymphocytes. In the present report, they find an RTK that is downregulated in EBV-immortalized lymphocytes. The superfamily of ephrin receptors (Ephs) are classified into 2 subclasses, A and B, as a function of ligand binding specificity to Eph interacting proteins termed ephrins. Ephrin A molecules are glycosylphosphatidylinositol proteins anchored to the cell membrane, whereas ephrin B molecules have a single transmembrane domain. Eph/ephrin signaling is activated by cell-to-cell interactions and is bidirectional (ie, downstream signaling is activated in the Eph and ephrin-expressing cells).

The investigators present evidence that the EBV LMP1 inhibits erythropoietin-producing hepatocellular receptor A4 (EphA4) expression through the extracellular signal-regulated kinase-Sp1 pathway and show that EphA4 expression inhibits lymphocyte proliferation. They map domains of the LMP1 molecule and the EphA4 that mediate these interactions. Immunohistochemical assessment of normal tonsil and EBV-negative DLBCL showed expression of Eph4A, whereas expression was not detected in posttransplant lymphoproliferative disorder and EBV-positive DLBCL in most cases. LMP1 expression was inversely correlated with EphA4 expression. The investigators present an analysis of a public data set showing that lower EphA4 expression was correlated with a poor survival rate in DLBCL patients.

Some caution is warranted in generalizations about either the relationship between EBV LMP1 expression and EphA4 expression, or the prognostic value. There is great heterogeneity in EBV-associated posttransplant lymphoma and in DLBCL. Classification of both continues to evolve.[4,5] Originally described as "senile EBV-associated lymphoproliferative disorder," renamed "EBV-positive DLBCL of the elderly" for inclusion as a provisional entity in the 2008 World Health Organization classification,[6] the suggested terminology has been revised to acknowledge the occurrence in younger patients.[7] "Of the elderly" has been replaced by "not otherwise specified" (EBV$^+$ DLBCL, NOS). The viral gene expression pattern varies among these entities, and any relationship between expression LMP1 and inhibition of EphA4 may not be consistent.

However, the role of Eph/ephrin signaling in tumorigenesis is certainly of great interest, albeit multidimensional and complex. The present investigators present evidence that Eph4A expression inhibits lymphocyte proliferation. Some very different scenarios have recently emerged in other settings. EphA4 was expressed at higher levels in lung cancer compared with noncancer tissues, but EphA4 gene expression was associated with an improved outcome in patients with resected lung adenocarcinoma.[8] In a mouse model of breast cancer, primary tumor growth and metastasis of isografted breast cancer cells was inhibited in EphA4-knockout mice vs control wild-type littermates.[9] In chronic lymphocytic leukemia, evidence has been presented that EphA4, including soluble EphA4, may contribute to nodal dissemination.[10] As increasing attention focuses on the role of the tumor microenvironment in pathogenesis, it seems likely that important insights will be gained by study of the modulation of Eph/ephrin signaling by viral infection.

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Kristofik et al, page 1642

When is a thrombogenic matrix not thrombogenic?

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In this issue of *Blood*, Kristofik et al report that the subendothelial extracellular matrix (ECM) of thrombospondin-2–null (TSP2-null) mice is less able to support platelet adhesion than that of wild-type mice because of a defect in von Willebrand factor (vWF) recruitment.[1]

These data shed new light on the surprising observation reported in 1998 that TSP2 knockout (KO) mice have prolonged bleeding time when the tail is transected and placed in saline despite the fact that significant amounts of TSP2 are not found in platelets or plasma.[2] The authors used adaptive bone marrow transplants to resolve this conundrum. They observed normal bleeding times when TSP2-null bone marrow is transplanted into

wild-type mice. By contrast, prolonged bleeding times are observed when bone marrow from wild-type mice is transplanted into TSP2 KO mice, suggesting that the underlying defect lies in the vessel wall at the site of injury. To confirm this hypothesis, the authors performed an experiment in which denuded vascular grafts from TSP2 KO mice are placed in wild-type mice. They discovered that the TSP2-null grafts display a marked reduction in thrombus formation. Furthermore, as shown in figure 2 of Kristofik et al, TSP2-null vascular grafts exhibit very little immunostaining for vWF, whereas robust staining is seen in the wild-type grafts. The authors go on to show that vWF binding to the ECM produced by TSP2-null dermal fibroblasts is markedly reduced compared with its binding to ECM made by wild-type fibroblasts. Thus, the defect in thrombus formation observed in TSP2 KO mice is due to decreased vWF in the subendothelial ECM, which is essential for platelet adhesion under high shear. Interestingly, a very closely related member of the thrombospondin gene family, TSP1, also promotes vWF-mediated platelet adhesion, but through a different mechanism. TSP1 binds to vWF and protects it from cleavage by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif 13).[3] By contrast, as shown in their figure 6, Kristofik et al report that vWF does not bind directly to TSP2.

These observations beg the question, how does the absence of TSP2 result in decreased vWF in the subendothelial ECM? The authors posit that TSP2 affects the formation of collagen fibrils such that vWF binding sites are in some way more exposed and available. Most of the members of the thrombospondin gene family have been shown to bind to collagens and/or promote fibrilogenesis.[4] The collagen fibrils that form in the skin of TSP2-null mice are thicker and more uniform in diameter than those found in wild-type mice.[2] The collagen fibrils in the tendons of TSP4 KO mice are significantly larger.[5] Thrombospondin-5, also known as cartilage oligomeric matrix protein, binds to collagen II and IX and aggrecan to promote collagen fibril assembly and ECM formation.[6] Mutations in TSP5 lead to premature assembly of the ECM in the endoplasmic reticulum and cause the human dwarfing condition pseudoachondroplasia.[7] It will be important to determine whether or not the large morphologic changes in collagen fibril structure that are seen in TSP2 KO mice can be correlated with specific changes in ligand binding sites, such as those for vWF.

An alternative explanation is that a component of the ECM that is important for vWF binding is absent in the TSP2-deficient vessels. Perhaps TSP2 acts as a chaperone to facilitate incorporation of another protein or proteoglycan that binds vWF into the ECM. Muscle tissue in TSP4-null mice contains lower levels of proteoglycans, including glypican and β-glycan.[5] Alternatively, proteolysis may result in the loss of vWF binding sites from the ECM. TSP2 reportedly mediates the uptake and clearance of matrix metalloproteinase 2 by cells.[8] In the absence of TSP2, increased levels of extracellular matrix metalloproteinase 2 may degrade a component of the ECM that is involved in vWF binding. A detailed analysis of the composition of the subendothelial matrix in TSP2 KO mice may reveal that a key component is absent. Proteomic approaches have recently been developed to characterize the composition of the ECM, or matrisome.[9] This type of nonbiased approach would determine whether the absence of TSP2 results in few or many changes to the ECM. Whereas several recent studies have highlighted the importance of thrombospondins in the structure of the ECM, the study by Kristofik et al underscores their importance in the function of the ECM.

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● ● ● TRANSFUSION MEDICINE

Comment on Reisz et al, page e32

RBC storage lesions

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In this issue of *Blood*, Reisz et al present a proteomic and metabolomic analysis of red blood cells (RBCs) stored in Additive Solution 3 that focuses on the oxidative changes in the active sites of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The changes accumulate and become irreversible with time but are sufficiently infrequent that it is unclear that they matter for posttransfusion function.[1]

In 1916, Rous and Turner[2] showed that rabbit RBCs stored for 4 weeks in a solution of citrate and dextrose could be given back to the donor rabbits, raising their hematocrit without raising their reticulocyte count or causing bilirubinuria. The next year, Robertson[3] used this solution to build the first human blood bank. Anticoagulant citrate