protein–positive and –negative pre-HSC type I and II (Hes1 is a downstream target of Notch signaling). The Notch pathway was active in all pre-HSCs type I, but activity decreased on maturation of the cells into definitive HSCs (see also the model in figure 6 of Souilhol et al). To assess at which stage Notch is required, AGM explants were cultured in the presence of a chemical Notch inhibitor. This blocked HSC maturation from pre-HSC type I, but much less so from pre-HSC type II, revealing a critical requirement for Notch signaling in pre-HSC type I, but not pre-HSC type II. These results were in line with in vitro conditional deletion of the Notch transcriptional partner RBP-jk, which showed that Notch signaling is no longer required toward the end of AGM HSC maturation. In a set of converse experiments, AGM pre-HSCs were exposed to constitutive Notch signaling in culture. Pre-HSC type I exposed to these conditions no longer could develop into functional HSCs. Pre-HSC type II, in contrast, were not sensitive to constitutive Notch signaling. Together these experiments demonstrate that Notch signaling is indispensable in pre-HSC type I, but then needs to be downregulated to allow the cells to mature to pre-HSC type II.

Surprisingly, the authors found that not only the Notch1 receptor but also Notch2 is expressed by the HSC lineage. Interestingly, Notch1 and Notch2 show inverse expression patterns. Notch1 is expressed by all pre-HSCs type I and II, with the highest expression seen in type I, whereas Notch2 is not expressed on pre-HSC type I, but is on part of the pre-HSC type II. Addition of blocking antibodies to AGM explants confirmed a role for both Notch receptors in HSC development. The authors suggest that the weaker signaling strength reported for the Notch2 receptor may play a role in the downregulation of Notch signaling activity seen on maturation.

How does this compare with hematopoietic differentiation of human pluripotent cells? Also in that system, Notch activity is required for the endothelial–hematopoietic transition of hemogenic precursors, specifically in the early stages of the process. It will be interesting to see whether fine regulation of Notch signaling in the progenitors that emerge in these cultures affects the cell types generated and how these compare with the pre-HSCs type I and II of the embryo.

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Lymphoid Neoplasia

Comment on Huang et al, page 1578

EBV, an inhibited receptor kinase, and lymphoma

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In this issue of Blood, Huang et al investigate cellular gene expression profiles in Epstein-Barr virus (EBV)–immortalized B and normal B cells. They provide evidence that a viral protein inhibits a cellular receptor tyrosine kinase (RTK) and that this inhibition is important in lymphomagenesis.

The association of EBV with malignancy was first recognized with the discovery of the virus in association with African Burkitt lymphoma (BL). Shortly thereafter, the ability of the virus to immortalize B lymphocytes in vitro was appreciated. In those early days, it must have appeared that the link between viral infection and malignancy was straightforward. Immortalization of B lymphocytes was a model for tumorigenesis. Over the years, the spectrum of EBV–associated lymphoproliferative disease has continued to increase. In addition to African BL, the virus is associated with a subset of diffuse large B-cell lymphoma (DLBCL), posttransplant lymphoma, Hodgkin lymphoma (HL), and natural killer/T-cell lymphoma among others.

In the report by Huang et al, lymphocyte immortalization remains an important focus of investigation aimed at better understanding lymphomagenesis. Previous investigations of the immortalization process revealed that a half dozen viral proteins were required for immortalization. Prominent among them was the latent membrane protein 1 (LMP1). The protein is a tumor necrosis factor receptor family member that is constitutively activated. Expression is tumorigenic in some immortalized cell lines and in transgenic mouse models. However, EBV immortalization of B lymphocytes in vitro does not account for the viral gene expression programs found in most EBV–positive lymphomas or in lymphocytes in vivo following normal infection. Thus LMP1, although expressed in many EBV–associated tumors, including some posttransplant lymphomas, is not expressed in EBV–associated BL. Other EBV proteins required for immortalization including EBV nuclear antigen 2 are not expressed in EBV–BL, HL, and many DLBCLs. Thus, the details of
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 (Eph4A) 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. 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, the suggested terminology has been revised to acknowledge the occurrence in younger patients. “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. 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. In chronic lymphocytic leukemia, evidence has been presented that EphA4, including soluble EphA4, may contribute to nodal dissemination. 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. 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. The authors used adaptive bone marrow transplants to resolve this conundrum. They observed normal bleeding times when TSP2-null bone marrow is transplanted into
EBV, an inhibited receptor kinase, and lymphoma

Richard F. Ambinder