Mammalian primitive erythrocytes: neither fish nor fowl

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Mammalian primitive erythroblasts undergo enucleation in the circulation, thus refuting the long-standing perception that primitive erythroblasts remain nucleated and are more similar to nucleated avian, fish, and reptile red cells than to definitive red cells of mammals.

During mammalian embryogenesis, erythropoiesis progresses through distinct phases, each phase producing cells with dramatically different characteristics. When cardiac contractions begin in mice embryos at embryonic day 8.25 (E8.25), “primitive” erythroblasts, developed in yolk sac blood islands, enter the circulation.1 However, by E12.5 “definitive” erythrocytes, produced in the fetal liver, begin to circulate and quickly prevail as the dominant erythroid phenotype.3 While definitive erythroblasts synthesize adult hemoglobins and enucleate, primitive red cells are larger, contain embryonic and adult hemoglobins, and have been thought not to undergo enucleation during their life span. Although several earlier observations hinted that in mouse embryos a population of large, enucleated cells might be circulating,4 the longstanding perception has been that primitive mammalian erythrocytes retain their nuclei and are thus more similar to nucleated avian, fish, and reptile red cells5 than to definitive red cells of mammals.

However, in this issue, Kingsley and colleagues (page 19) report quantitative data showing that between E12.5 and E16.5 primitive erythroblasts progressively enucleate in circulation. Further, they observed that enucleated, primitive cells can be detected up to 5 days after birth. Using antibodies to specific regions of murine embryonic βH1-globin and adult β-major-globin, they were able to differentiate yolk sac–derived primitive red cells. These antibodies, in combination with nuclear staining, identified 3 distinct peripheral blood cell populations in E13.5 and E15.5 fetuses: a nucleated population expressing embryonic βH1-globin, an enucleated population lacking βH1-globin, and surprisingly, an enucleated population expressing βH1-globin. Small numbers of enucleated βH1-globin–expressing cells were initially detected at E12.5. By E16.5, all of the βH1-globin–expressing cells were enucleated. Morphometric analysis of cell area revealed that both nucleated and enucleated βH1-globin–expressing cells were 100 μm² in size and about 3-fold larger than definitive erythrocytes. Importantly, the disappearance of circulating nucleated primitive cells was due to their progressive enucleation and not loss from the bloodstream.

These findings provide a persuasive argument refuting the currently held view that primitive mammalian erythropoiesis resembles avian and reptilian erythropoiesis more than definitive mammalian erythropoiesis. Indeed, the authors delineate a number of important similarities between murine primitive and definitive erythropoiesis. Both differentiation programs exhibit maturation with enucleation. Additionally, prior to extrusion, nuclei condense and move to the plasma membrane, coincident with loss of intermediate filaments.6 Yet one striking difference in the differentiation programs is that primitive erythroblasts appear to undergo terminal differentiation in circulation, while definitive erythroblasts mature extravascularly within 3-dimensional erythroblastic islands, closely associated with macrophages and extracellular matrix proteins. A number of new questions can now be asked. Do circulating primitive erythroblasts require contact with macrophages of the reticuloendothelial system for enucleation? What is the trigger for enucleation? Are molecular mechanisms of chromatin condensation, cytoskeletal remodeling, and nuclear extrusion similar or
different in primitive and definitive erythroblasts? Do membrane mechanical properties of enucleated primitive cells differ from those of definitive cells, suggesting unique interactions among transmembrane and cytoskeletal components? The findings of Kingsley and colleagues open up many new avenues for exploration.

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6. Sanguori F, Woods CM, Lazarides E. Vimentin down-regulation in cytopenias.5 Neutropenia is considered to be an important factor in the pathogenesis of neutropenia in SLE. Approximately two thirds of patients with systemic lupus erythematosus (SLE) have antineutrophil autoantibodies (usually immunoglobulin G [IgG]).1 Neutropenia is more common in individuals with either anti-Ro autoantibodies.2 Several previous studies have implicated Fas (CD95)–mediated apoptosis of circulating neutrophils, monocytes, and lymphocytes of patients with SLE.3,4 Marrow hypoplasia and Fas-mediated apoptosis of CD34+ hematopoietic progenitor cells are additional factors contributing to SLE-associated cytopenias.5 The report by Matsuyama et al provides new evidence that accelerated apoptosis of neutrophils and their precursors is an important mechanism for neutropenia in systemic lupus erythematosus.

I In this issue of Blood, Matsuyama and colleagues (page 184) report a novel mechanism for the pathogenesis of neutropenia in systemic lupus erythematosus (SLE). SLE is a relatively common disorder with a prevalence of 50 cases per 100 000 population and a female-to-male ratio of approximately 10:1. Neutropenia, defined as a blood neutrophil count less than 1.8 × 10⁹/L, occurs in about 50% of individuals with SLE. Although usually mild (ie, 1.0–1.8 × 10⁹/L), SLE-related neutropenia is considered to be an important factor predisposing these individuals to bacterial infections. Several mechanisms have been identified for the pathogenesis of neutropenia in SLE. Approximately two thirds of patients have antineutrophil autoantibodies (usually immunoglobulin G [IgG]).1 Neutropenia is more common in individuals with either anti-Ro autoantibodies.2 Several previous studies have implicated Fas (CD95)–mediated apoptosis of circulating neutrophils, monocytes, and lymphocytes of patients with SLE.3,4 Marrow hypoplasia and Fas-mediated apoptosis of CD34+ hematopoietic progenitor cells are additional factors contributing to SLE-associated cytopenias.5 The report by Matsuyama et al provides further insight into the pathogenesis of neutropenia in SLE by describing abnormalities in another mediator of apoptosis, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). In this study involving 28 patients with SLE and 8 healthy controls, serum TRAIL levels were inversely proportional to blood neutrophil counts. Expression of TRAIL receptor 3, a decoy receptor for TRAIL, was also lower in neutropenic patients than in patients or controls without neutropenia. In vitro, TRAIL induced apoptosis in neutrophils. Corticosteroid therapy reduced expression of TRAIL on T cells and enhanced expression of Fas-associating protein with death domain—like interleukin-1β-converting enzyme (FLICE)–inhibitory protein (FLIP), an antiapoptotic protein, by neutrophils. TRAIL may also play an important role in immunoregulation, and dysregulation of TRAIL or its cognate receptor may contribute to the development of systemic autoimmune diseases, such as SLE.6 For example, mice deficient in TRAIL have been previously shown to be susceptible to the development of accelerated autoimmune disease.

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**Atherogenic role of the type EIIIA fibronectin domain**

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Compared with “healthy” vessels, the extracellular matrix of atheromatous plaques includes increased content of type-EIIIA-domain-containing fibronectin. Apolipoprotein E-null mice engineered to produce fibronectin lacking this alternatively spliced exon exhibit reduced atherosclerosis. Diminished hypercholesterolemia and reduced macrophage foam cell formation may contribute to this phenotype.

Variations in the molecular composition of the extracellular matrix (ECM) play important roles during vascular remodeling in pathophysiologic processes (i.e., vasculogenesis and angiogenesis, wound healing, and vascular obstructive lesion formation). Fibronectin is a ligand for fibrin, heparin, collagen, and several integrins implicated in the recruitment of blood leukocytes to the arterial endothelium. While several splice-variant forms of fibronectin have been identified, the 2 major forms are plasma fibronectin (pFN), which is produced by hepatocytes, and cellular fibronectin (cFN), which is produced locally by different cell types and deposited and assembled into the ECM. A single gene encodes for distinct fibronectin moieties depending on alternative RNA splicing of exons encoding for the V/CS-1 segment (also known as the V exon), extra type III repeat segments (known as ED-A and ED-B in humans, or EIIIA and EIIIB in rodents), and the (V + C) region. pFN lacks extra type III repeat segments, whereas cFN contains variable amounts of these alternative domains.

Although a causal link between fibronectin and cardiovascular pathobiology has been difficult to establish because null mutations for fibronectin cause embryonic lethality, evidence exists implicating fibronectin in cardiovascular pathobiology: (1) EIIIA-positive fibronectin (EIIIA-FN) up-regulation is associated with clinical and experimental hypertension; (2) expression of EIIIA-FN, EIIIB-FN, and fibronectin lacking both alternative exons is rapidly induced after myocardial infarction in both humans and animal models; (3) EIIIA-FN and EIIIB-FN forms, which are not normally present in “healthy” vessels, are induced in neointimal lesions; (4) elevated plasma levels of circulating cFN have been described in clinical syndromes with vascular damage; and (5) in vivo studies using blocking antibodies have implicated the alternative fibronectin V/CS-1 segment in leukocyte recruitment during murine atherosclerosis.

Recently, analysis of fibronectin conditional knock-out mice revealed a role of pFN in thrombus initiation, growth, and stability. Now, Tan and colleagues (page 11) directly test the function of EIIIA-FN in atherosclerosis by engineering EIIIA-null (EIIIA−/−) mice lacking the EIIIA exon. They crossed EIIIA−/− mice with atherosclerosis-prone apolipoprotein E-null (ApoE−/−) mice and found as much as a 67% reduction in aortic atherosclerosis in doubly deficient fat-fed EIIIA−/−/ApoE−/− mice compared with ApoE−/− controls. An intriguing observation that deserves further examination is that EIIIA−/−/ApoE−/− females display significant protection at all time points assayed (8, 12, and 16 weeks of fat feeding), while males are protected only after 16 weeks. Compared with ApoE−/− controls, both male and female EIIIA−/−/ApoE−/− mice displayed diminished total plasma cholesterol levels, a reduction that is specific to the very low density lipoprotein fraction. Increased EIIIA-FN expression was found in both the plasma and in endothelial cells and macrophages within atherosclerotic lesions of ApoE−/− mice. Moreover, in vitro foam cell formation by ApoE−/− macrophages was associated with increased EIIIA-FN mRNA expression, and lipid accumulation in EIIIA−/−/ApoE−/− macrophages was reduced by 31% compared with ApoE−/− controls.

Collectively, the study by Tan and colleagues convincingly demonstrates an atherogenic role of EIIIA-FN and suggests that this form of fibronectin is functional in both plasma lipoprotein metabolism and in macrophage foam cell formation. Future studies are warranted to elucidate additional systemic mechanisms and processes within the vessel wall by which EIIIA-FN may contribute to atherosclerosis. For example, the possibility that EIIIA-FN may interact with specific lipoprotein fractions and may affect leukocyte recruitment should be investigated. Moreover, because endothelial and smooth muscle cells cultured on different ECM components display significant differences in proliferation, migration, and apoptosis, comparing the phenotypic properties of vascular cells cultured on EIIIA-FN, EIIIB-FN, and fibronectin lacking cFN could be informative.

![EIIIA−/−/ApoE−/− mice are protected from atherosclerosis. See the complete figure in the article beginning on page 11.](image-url)
both EIII segments may shed significant insight into the role of distinct variants of fibronectin in atherosclerosis. ■

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VWF synthesized by endothelial cells either is constitutively secreted into the circulation in the form of low-molecular-weight multimers or is stored in Weibel-Palade bodies, and in response to endothelial cell agonists is released in the form of ultralarge multimers (ULVWF). ULVWF is the most adhesive and reactive form of VWF and may lead to spontaneous platelet aggregation if not further processed by the ADAMTS-13 metalloprotease. Lack of ULVWF cleavage by ADAMTS-13 is thought to be the primary defect underlying TTP.

In this study, Bernardo and colleagues investigated whether ULVWF release from endothelial cells and its subsequent cleavage by ADAMTS-13 are affected by the inflammatory cytokines TNF-α, IL-6, and IL-8, as these processes could represent potential links between inflammation and thrombosis. First, using an elegant system in which cultured endothelial cells are subjected to defined flow stress in vitro, the authors demonstrate that TNF-α and IL-8 (but not IL-6) stimulate the release of ULVWF strings from human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. As HUVECs lack an IL-6 receptor, the absence of response with IL-6 was not surprising; when the experiment was repeated with IL-6 precomplexed with soluble IL-6 receptor, ULVWF release was seen (although not to the level of the other cytokines).

The authors then turn their attention to whether these cytokines can alter the ability of ADAMTS-13 to cleave ULVWF. They show that IL-6 (but not TNF-α or IL-8) greatly abrogates the ability of ADAMTS-13 to cleave ULVWF strings under flowing, but not static, assay conditions. As this interesting effect is still observed when partially purified ADAMTS-13 is pretreated with IL-6, the authors speculate that IL-6 may physically impair binding of ADAMTS-13 to ULVWF under the shear stress of flowing conditions. As the authors point out, this observation is

IL-8 and TNF-α, but not IL-6, stimulated the release of ULVWF multimers. See the complete figure in the article beginning on page 100.
especially interesting in light of previous studies demonstrating elevated IL-6 levels in patients with TTP, and in coronary thrombi and atherosclerotic plaques.

This important study provides insight into potential new mechanisms by which the inflammatory process is able to shift the hemostatic balance in favor of thrombosis. Cytokines present in a variety of pathologic conditions may influence both the release of ULVWF from endothelial cells and its subsequent processing by ADAMTS-13, thus allowing ULVWF to persist long enough to induce platelet adhesion and aggregation, and ultimately lead to thrombosis. New information regarding regulation of the interface between inflammation and thrombosis is always welcome as it eventually may lead to new points of therapeutic intervention for patients with inflammation-associated coagulopathies.

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Blood cells: excitable at last

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Evidence is growing that many types of ion channels and other molecules once thought to be restricted to the nervous system are expressed in hematopoietic cells, where they may function at various levels of differentiation.

In 1978, Miller et al inserted electrodes into a Guinea pig megakaryocyte and showed that when electric current was injected, the cell fired a biphasic action potential exactly as a neuron would do under the same conditions. Voltage-dependent calcium and potassium channels identical to those found in some nerve and muscle cells were later shown to underlie its action potential. Long distracted by the dazzling electric currents of neurons and myocytes, electrophysiologists had traditionally dismissed most other tissues—including blood—as “nonexcitable” and therefore unworthy of their attention. But the action potential of the Guinea pig megakaryocyte, which blurred this convenient distinction, was hard to ignore. Why did blood cells need ion channels? What business did they have generating electricity?

Inspired by the electronics of this curious bone marrow cell, and enabled by refinements in the patch-clamp technique that permitted characterization of single ion channels, electrophysiologists started around 1990 to tinker with other blood cells. They found to their great surprise that many of the same depolarizing (sodium and calcium) and hyperpolarizing (potassium and chloride) channels that had originally been considered the exclusive domain of neurons or muscle cells actually operated in hematopoietic cells as well.

We now recognize that blood cells, like neurons, generate and store electric potential energy. Through the cooperative behavior of a panoply of ion channels, they harness this energy to carry out a diverse range of hefty biophysical work, including volume regulation, cell movement, and degranulation. It is likely that whenever a blood cell like a platelet or a neutrophil does any “heavy lifting,” electric energy is spent through the operation of many of the same ion channels that exist in brain and muscle but that perform totally different tasks in those tissues.

In the current issue of Blood, Steidl and colleagues (page 81) take this concept a step further by reporting that human CD34+ cells express not only genes encoding many of the ion channels found in the brain, but also a variety of other proteins whose roles have been primarily defined in the nervous system. These include numerous neuromediators, receptors, kinases, phosphatases, and other proteins involved in the regulation of ion channels, neurotransmitter release, or other aspects of neuronal behavior. Of particular interest is their finding that many components of the exocytic machinery involved in neurotransmitter release at axon termini are expressed by CD34+ cells. This observation supports the view that agonist-mediated exocytosis in hematopoietic cells may share more similarities than differences at the molecular level with the highly specialized exocytosis that takes place in neurons. In their paper, Steidl et al go to great lengths to show that many neurobiologic genes are not only expressed at both the mRNA and protein level in
CD34+ cells, some of them exhibit their predicted functions in these cells. Since many of the ion channels that coexist in the brain and the blood carry out disparate tasks in these tissues, it is not surprising that many other “neurobiologic” proteins may have been adapted by nature for different roles in hematopoietic cells. Defining their functions in proliferative and mature blood cells is now the challenge. The blood–brain barrier is weakening.

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A new mechanism for inherited factor XI deficiency

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Two new mutations in the factor XI gene cause a moderate to severe factor XI deficiency and bleeding in heterozygotes by producing aberrant proteins that trap normal factor XI inside cells.

It is currently thought that factor XI is essential for propagation of coagulation after small amounts of thrombin are generated by the tissue factor pathway. Factor XI is activated by thrombin preferentially on platelet membranes, which leads through activation of factor X to further thrombin generation, even after the clot has formed. The additional amount of thrombin then activates thrombin-activatable fibrinolysis inhibitor, which results in stabilization of the clot.

Factor XI is a homodimer of 80-kDa subunits linked by a disulfide bond. Thrombin cleaves in each subunit an Arg369-Ile370 bond yielding a heavy chain consisting of 4 “apple domains” and a light chain containing the catalytic site. The production of the homodimer is essential for the secretion of factor XI from producing cells, and for its function on the surface of platelets where one of the subunits was suggested to bind glycoprotein Ib embedded in lipid rafts and the other subunit to factor IX, its substrate.1,2

Factor XI deficiency, an injury-related bleeding tendency, was first described in 1953 by Rosenthal et al1 in 2 sisters and their maternal aunt and was considered to be transmitted in an autosomal dominant fashion. A later study distinguished between individuals with “major” and “minor” deficiencies represented by factor XI levels of less than 20 U/dL and 30 to 65 U/dL, respectively.4 This observation was consistent with an autosomal recessive pattern of inheritance. However, because some of the patients with a minor deficiency in this and other studies did bleed following injury, the designation “dominant” or “recessive” mode of inheritance has become less important. What remains important is that patients with a major deficiency are at a significantly greater risk of bleeding following injury than patients with a minor deficiency, specifically at sites where local fibrinolysis is present (oral mucosa, nose, urinary tract).5 The deficiency has been reported in sporadic cases from many parts of the world but was found to be particularly common in Jews of Ashkenazi (European) origin. There are 2 mutations, Glu117Stop and Phe283Leu, that predominate in this population with allele frequencies of 0.0217 and 0.0254, respectively. Altogether, 49 mutations have been published to date, among which dysfunctional (cross-reacting material–positive) deficiencies are extremely rare.

In this issue, Kravtsov and colleagues (page 128) describe 2 novel alterations in the factor XI gene that present a new mechanism for factor XI deficiency. The mutations, Gly400Val and Trp609Ser, abolish secretion of the mutant proteins from transfected fibroblasts, and in cotransfection experiments reduce the secretion of wild-type factor XI by 50%. Formation of heterodimers consisting of wild-type and mutant proteins was also demonstrable. The heterozygotes harboring these mutations had factor XI activity and antigenicity ranging between 10 to 58 U/dL and some had significant bleeding. These new data characterize for the first time a dominant-negative effect of 2 mutations that produce nonsecretable but dimerizable factor XI that can trap normal factor XI intracellularly, resulting in plasma factor XI levels below the range observed in heterozygotes and in bleeding.

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Quebec platelet syndrome: from the bench to the family

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Bleeding manifestations were quantitatively assessed in 23 affected and 104 unaffected members of a single family with the Quebec platelet disorder, an autosomal dominant trait caused by deficient platelet alpha granule procoagulant proteins.

McKay and colleagues (page 159) describe a careful, systematic evaluation of bleeding problems in a very large kindred with the Quebec platelet disorder (QPD). The pathophysiology of QPD is well defined. It is an autosomal dominant trait with increased megakaryocyte expression and storage of urokinase-type plasminogen activator (u-PA). The u-PA generates plasmin, causing degradation of platelet fibrinogen and other α-granule proteins important for hemostasis. Measurements of platelet u-PA and α-granule fibrinogen degradation products allow accurate identification of affected family members and clear distinction from unaffected family members. To assess the nature and severity of bleeding problems among family members, McKay and colleagues developed a questionnaire specific for QPD. Their study has important lessons for hematologists who investigate and manage patients with bleeding disorders.

First, the methodology is a model for clinical research. Too often clinical research is based on sound laboratory methods, but the patient observations are not quantitative and may not be reproducible. In this study the patient observations are made with rigorous attention to quantitative analysis.

Second, McKay and colleagues documented that some bleeding symptoms, such as very large bruises and bruises that tracked downward, occurred exclusively among affected family members, while the frequency of other bleeding symptoms, such as nosebleeds that lasted longer than 15 minutes, were not different between affected and unaffected family members. These observations remind us that healthy people do bleed, a simple but often overlooked fact, and that characterization of bleeding as abnormal may be difficult. Instruments such as this questionnaire provide the ability to define and measure abnormal bleeding. These instruments will allow quantitative estimates of the risk of bleeding with inherited disorders, comparable with our current ability to estimate risks for thrombosis in patients with inherited thrombophilia traits.

Third, the quantitative assessment by McKay and colleagues documented heterogeneity of bleeding manifestations among affected and unaffected family members. In such a large kindred, this may be expected because bleeding symptoms ultimately result from the interactions among multiple risk factors. For example, some unaffected family members who reported bleeding symptoms may have had undiagnosed von Willebrand disease type 1, a common risk factor for excessive bleeding. Some family members affected by QPD who reported less bleeding may have also inherited the factor V Leiden trait or another prothrombotic trait, which could diminish the risk for bleeding. The use of quantitative measures of bleeding symptoms will allow greater understanding of the interactions of multiple common inherited traits on the risks for excessive bleeding.

Fourth, the types of bleeding symptoms manifested by patients with QPD are intriguing. We teach our students that clinical evaluation can distinguish patients who have abnormalities of primary hemostasis, such as platelet disorders, from patients who have abnormalities of coagulation, such as hemophilia. We say that abnormalities of primary hemostasis are manifested by the prompt occurrence of mucocutaneous bleeding, while abnormalities of coagulation are manifested by delayed bleeding with hemorrhathoses and large visceral hematomas. Patients with QPD had both types of bleeding. In addition to mucocutaneous bleeding, affected family members commonly reported joint bleeds and bleeding that began 12 hours or more after trauma. These clinical manifestations are consistent with the abnormalities of QPD, since it is not only a disorder of platelet function with abnormalities of aggregation but also a disorder of fibrin clot formation and fibrinolysis.

Therefore read this article not only to learn about the clinical manifestations of a rare inherited platelet abnormality, read it to learn how the application of quantitative and reproducible clinical assessments can reveal new insights into hematologic disorders.
Molecular monitoring of EBV-positive lymphoma

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Monitoring of Epstein-Barr virus DNA by quantitative real-time polymerase chain reaction may serve as an accurate surrogate marker of tumor load in patients with Hodgkin and non-Hodgkin lymphoma that were demonstrated to be EBV-positive.

Epstein Barr virus (EBV) is a widespread human herpesvirus that establishes lifelong asymptomatic infection of B cells. In 1997, EBV was classified by the World Health Organization–International Agency for Research on Cancer (WHO-IARC) as a group I human carcinogen because of its etiologic role in nasopharyngeal carcinoma and lymphomagenesis. EBV is associated with endemic Burkitt lymphoma, posttransplantation lymphoproliferative disease (PTLD), some T-cell and natural killer (NK) cell lymphomas, and Hodgkin disease. While the exact role of EBV in the pathogenesis of each type of lymphoma still needs to be elucidated,1 epidemiologic studies have shown strong associations between infectious mononucleosis and EBV-positive Hodgkin disease2 and also between posttransplantation EBV reactivation and the development of PTLD.3 Molecular monitoring of EBV DNA levels in patients with PTLD has been shown to accurately and sensitively reflect disease activity and is currently used to tailor treatment for the individual patient. Although viral infection is a presumed source of EBV DNA in patients with PTLD, release of EBV fragments from tumor cells, instead of fully assembled viral particles, is likely to play a major role in established PTLD.

The hypothesis that release of EBV DNA fragments from EBV-positive lymphomas might be a general phenomenon and might be used as a surrogate marker of disease activity was the starting point for the study of Au and colleagues reported in this issue of Blood (page 243). The authors show that molecular monitoring of EBV DNA by quantitative real-time polymerase chain reaction (PCR) closely mirrors the clinical picture of the individual patient with an EBV-positive lymphoma. In addition, patients with a high EBV DNA copy number before the start of treatment showed inferior outcome. The prognostic significance of quantified EBV DNA seemed independent from the type of lymphoma, but was especially evident in patients with NK cell lymphoma. These results compare well to earlier findings in patients with PTLD and suggest that patients with non–Hodgkin lymphoma as well as patients with Hodgkin disease should be evaluated for the presence of EBV at diagnosis and during follow-up if EBV positivity has been demonstrated. Such diagnostic evaluation may include in situ hybridization for EBV-encoded RNA (EBER) on histopathologic examination and the quantification of EBV DNA in plasma by PCR.

Au et al used a very sensitive real-time PCR and reported relatively high values of EBV DNA in the NK cell lymphoma and PTLD group. The copy numbers reported exceed those reported in PTLD by a number of other investigators. The different quantified levels of EBV DNA hamper the comparison and combined analysis of several studies. It strongly supports efforts aimed at standardization of recently used real-time technology, such as has been initiated by the Quality Control for Molecular Diagnostics (QCMD, www.qcmd.org). Furthermore, the data presented by Au et al emphasize the importance of studies aiming to elucidate the exact pathogenetic role of EBV in lymphomagenesis, as such studies may yield new targets for therapy in poor-risk EBV-positive lymphoma. So far, the interplay between the EBV gene program, type and developmental stage of infected lymphocyte, and putative additional oncogenic event has remained an open but intriguing question in most EBV-positive lymphomas.

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Mammalian primitive erythrocytes: neither fish nor fowl

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