

It is still debatable whether *BHRF1*, a viral homologue and antiapoptotic member of the BCL-2 family, is expressed in BL cells.<sup>3</sup> The BHRF1 gene product has been found to interfere with the proapoptotic Bim protein, preventing apoptosis in newly infected human B cells, and is a likely viral contribution in all EBV-positive lymphomas.<sup>1</sup>

Interesting and promising candidates are EBV's miRNAs that may play a decisive role in lymphomagenesis. Presumably, they fine-tune the expression of many hundreds of cellular target genes with mostly unknown functions<sup>9</sup> but a recent report suggests that this virus' 44 miRNAs might directly contribute to cellular survival, promotion of cell-cycle entry, and proliferation of human B cells *in vitro*.<sup>10</sup>

The findings by Vereide and Sugden do not provide the ultimate explanations but the implication of their findings is clear. The induced loss of EBV from canonical BLs, which have progressed to depend on few viral genes only, will provide a promising assay to identify those genes that complement cellular survival and/or proliferation in the absence of viral functions. The smart approach by these authors has gone a long way in revealing this fundamental option, which will have important basic and clinical implications in the future.

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## ● ● ● THROMBOSIS & HEMOSTASIS

Comment on Lisman et al, page 2070

# Who controls the controllers?

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In a novel study of children who received livers transplanted from adult donors, Lisman and colleagues describe how plasma levels of coagulation proteins remain at pediatric levels posttransplantation, suggesting that control of the plasma levels is not primarily driven by the liver itself.<sup>1</sup> This study raises numerous important questions about the biology and regulation of the coagulation system, a key control system in our bodies, and should be the stimulus for much further research.

The concept that the coagulation system in children is quantitatively different from adults was introduced only 20 years ago, when Maureen Andrew coined the phrase “developmental hemostasis.”<sup>2</sup> Andrew's landmark studies, published in *Blood*, demonstrated that on functional testing (assays that use clot formation or chromogenic endpoints), the plasma levels of many coagulation proteins change with age, not reaching steady-state adult levels

until the late teenage years.<sup>2-4</sup> Subsequent studies of centenarians suggest that age-related changes continue through the spectrum of adult life.<sup>5</sup> Interestingly, there are no published studies comparing immunologic levels of most of these proteins. The possibility of qualitative differences in the relevant proteins has been raised recently.<sup>6</sup> Thus, while many questions remain about the true nature of the age-related differences in the proteins

themselves, how these differences are regulated has remained a total mystery. Perhaps even more important is the question, “Why?”

Possible mechanisms involved in controlling the plasma levels of coagulation proteins in children include: regulation at the gene level; post-translational modifications that affect protein function, delivery, or release; or differences in protein clearance. Given that the liver is the site of production for most coagulation proteins, many had been assumed that the liver was involved in this regulation. However, by demonstrating that even with a transplanted adult liver *in situ*, children maintain plasma levels of certain coagulation proteins at their expected age-related levels, Lisman and colleagues suggest the liver is not the primary regulator of plasma coagulation protein levels.<sup>1</sup> This should not really come as a surprise, as the body is full of remote sensor/regulator systems. Lisman et al propose explanations that include hormonal control, vascular endothelial control via an as yet unidentified mechanism, or control via variable clearance.<sup>1</sup> The vascular endothelium seems the most likely candidate. The endothelium is intimately involved with the function of the coagulation proteins,<sup>7</sup> and vascular endothelial dysfunction, as seen in disseminated intravascular coagulation, is usually measured by the degree of disturbance in coagulation proteins, even though it is not a primary disorder of coagulation.<sup>8</sup>

The fundamental question remains: why do the plasma levels of coagulation proteins differ with age? Potentially, this has nothing to do with coagulation. Coagulation proteins are examples of broad-acting proteins, such as serpins. Many of these proteins have been shown to have actions in multiple key biologic processes such as inflammation, wound repair, and angiogenesis.<sup>6,8</sup> Whether it is the requirements of one or many of these basic systems of survival that drive the plasma levels of these multifunctional proteins must still be determined. Regardless, the endothelium is a likely regulator for many of these systems. This question has real clinical relevance, as when we treat coagulopathic children with plasma proteins, we invariably use plasma collected from adults, or recombinant products that likely have subtle tertiary differences in structure from the native protein due to viral inactivation processes in manufacturing. Thus, the potential for these exogenous proteins to have adverse effects mediated by a biologic system outside of coagulation cannot be ignored.<sup>6</sup> By extension, the use of anticoagulant drugs in

children may also have implications for coagulation protein activity in other biologic systems. As yet, there is no research into these potential clinical implications. For example, neonatal and early childhood are times of active angiogenesis, especially for later developing organs such as the brain. Dramatically changing the level and subtype of proteins involved in angiogenesis (by the addition of an anticoagulant aimed at affecting the protein's role in coagulation) has the potential for subtle, but long-term, adverse effects on development.<sup>6</sup> The reported mechanisms for the protective effect of heparinoid anticoagulation in adults with cancer would support the concept that anticoagulation may affect processes such as angiogenesis.<sup>9</sup>

In vivo regulation of the coagulation system in humans is a technically difficult area to study. Most of our clinical testing of coagulation involves in vitro assays that do not represent physiologic function. Studying the interactions between the endothelium, which is

potentially different in diverse vascular territories within the body, and the coagulation proteins, in the context of these proteins being truly multifunctional and involved in multiple biologic systems, is even more complicated. In this issue of *Blood*, Lisman and colleagues have made the first step, through lateral thinking and accurate observation.<sup>1</sup> Novel approaches are likely to be required if we are going to increase our understanding of regulation of coagulation proteins and address the key questions of how and why they are regulated. Such research is essential for us to understand the fundamental biology of aging and to determine the true and broad clinical implications of therapies that impact on the coagulation proteins.

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