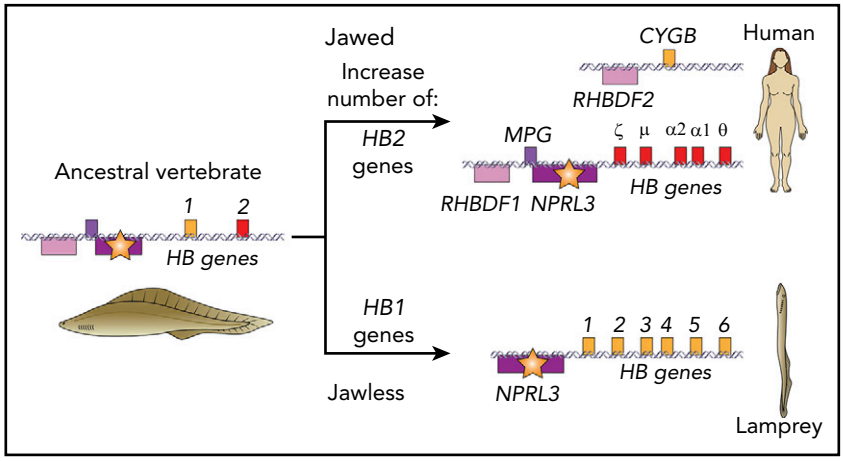

A globin gene regulatory element discovered in lampreys suggests an ancient origin in ancestral vertebrates. The Miyata et al study of lampreys showed that genes encoding globin polypeptides of the oxygen transporter hemoglobin (*HB* genes, light orange and red boxes) are adjacent to a ubiquitously expressed *NPRL3* gene (violet box) in both major branches of vertebrates, jawed and jawless, despite the separate, convergent evolution of *HB* genes in each branch, with *HB* genes in jawless vertebrates more related to *CYGB* (light orange boxes). Furthermore, an intron of lamprey *NPRL3* contains a major regulatory element for globin genes (star), as is the situation in humans. These maps in extant species suggest that the linkage of *NPRL3*, containing a strong regulatory element, to *HB* genes occurred in an ancestral vertebrate, represented as *Haikouichthys ercaicunensis*.[10] By hypothesizing multiple ancestral *HB* genes in the linkage group, one related to *CYGB* and another related to canonical vertebrate *HB* (red box), the model can explain convergent evolution of different oxygen-transporting globins as selective expansions of one or the other gene while maintaining strong regulation from the *NPRL3* intronic enhancer. Additional genes characteristic of this locus are also shown; boxes above the illustrative DNA helices are transcribed left to right, and those below the DNA are transcribed right to left. The *CYBG* gene and *HB* genes are on different chromosomes in humans.

NPRL3 gene with a strong regulatory element became linked to at least 2 different globin genes in the ancestor to vertebrates. Hints from gene arrangements in tunicates suggest that *NPRL3* was not linked to globin genes before this time. That strong regulatory element remained active, leading to high-level expression of the linked *HB* genes in both clades of vertebrates. However, in adapting to the need for oxygen transport, the ancestral globin gene corresponding to canonical hemoglobin genes expanded and diversified in jawed vertebrates, whereas the ancestral globin gene corresponding to *CYGB* had a similar fate in jawless vertebrates.

The new results show that gene regulatory elements can be deeply preserved over evolutionary time, long past the time frame illuminated by comparative genomics, but only careful biochemical and genetic analyses will reveal them. However, it is important to keep in mind that many regulatory elements are not strongly conserved; perhaps a majority of regulatory elements arose recently within specific lineages or have been repurposed for new functions.[8,9] The authors deduce a model that helps resolve a previously difficult evolutionary scenario. Still, many questions remain, including how the globin gene clusters arose within each clade and how various globins have been adapted to different functions across clades. It is likely that comparative biochemical and functional analyses of globin genes will remain fruitful for many future studies.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Holstein et al, page 279

Bleeding in acquired hemophilia: have we figured it out?

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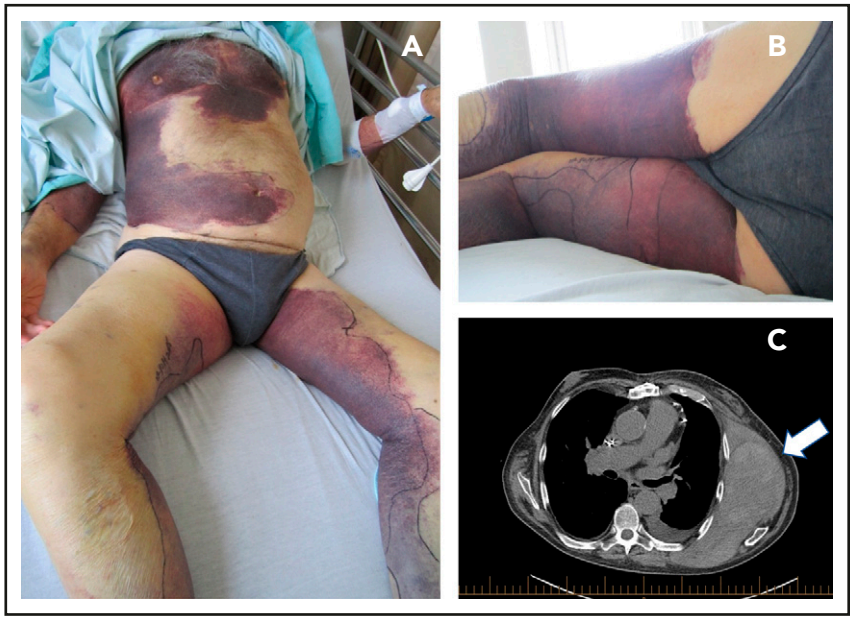
In this issue of *Blood*, Holstein et al show the extent to which the risk of bleeding after a diagnosis of acquired hemophilia A (AHA) remains significant until near-normal factor VIII (FVIII) level is attained.[1]

All consultant hematologists, whether from the benign or malignant side of our specialty, have a duty to recognize the cardinal signs of AHA, a rare but severe autoimmune condition where diagnosis is frequently and dangerously delayed.[2] Cardinal signs are unexplained excessive bleeding, usually in an elderly person



Common forms of bleeding in AHA. Extensive bruising (A), often accompanied by large subcutaneous hematomas (right thigh) (B) that are debilitating because they are associated with anemia, pain, and decreased mobility. (C) Large deeper hematoma of thoracic wall muscles (arrow). Iliopsoas muscle hematomas are also not uncommon.

who has an isolated newly prolonged activated partial thromboplastin time. Simple diagnostic algorithms have been published,[3] but the first crucial step in making the diagnosis is clinical suspicion. The type of bleeding can be very suggestive (see figure). Plasma FVIII level should be measured without delay. Few patients with AHA now die as a result of uncontrolled bleeding, but delayed diagnosis and treatment continue to lead to debilitating morbidity in these often-frail patients. However, long-term prognosis is good for patients who survive the initial acute episode.[4]

The prospective GTH-AH 01/2010 study has already greatly advanced the field with landmark reports on immunosuppression[5] and serologic predictors of remission in AHA.[6] In the accompanying report, our German and Austrian colleagues look more closely at bleeding events and response to hemostatic therapy in their cohort of 102 patients. Patients were recruited early during their treatment, with day 1 defined as the first day of immunosuppressive treatment (IST). They were monitored for all clinically relevant instances of bleeding, with the analysis focusing on the first 12 weeks of observation. A total of 148 bleeds in 80 patients were documented at presentation, but more importantly, 141 new bleeds occurred in 59% of the patients during the subsequent observation period at a mean rate of 0.27 bleeds per patient-week. Severe bleeds before day 1 or at presentation lasted a median of 9 days compared with 2 days for those occurring after day 1, emphasizing again the importance of early recognition and treatment.

Hemostatic treatments (mostly bypassing agents) were rated as effective in 96% of cases, confirming what other series and registries have previously reported. More interesting and unique to this study is the multivariate analysis of clinical features, including FVIII levels, predicting the risk of new bleeding after day 1. The pathophysiology of bleeding in AHA is puzzling in more ways than one. In contrast to congenital hemophilia, the measured plasma level of FVIII at diagnosis is not predictive of the risk or severity of bleeding in AHA.[2,7] Spontaneous, catastrophic bleeding may occur in patients with FVIII levels that would be considered of mild severity in congenital hemophilia. This phenomenon is generally attributed to the weak interaction of the inhibitory antibodies with FVIII in vitro (so called second-order kinetics) seen in AHA.[3] Presumably, this interaction is different and more powerful in vivo or in specific vascular beds, but to my knowledge, this has not been clearly elucidated. In this publication, the authors confirm that FVIII level and inhibitor titer at baseline do not predict the cumulative incidence of new bleeds after day 1. However, they do demonstrate that subsequent weekly FVIII levels are highly statistically associated with new bleeding during the 12 weeks after initiation of IST. It is important to point out that the differences in risk observed between FVIII levels of $<1\%$, 1% to $<5\%$, and 5% to 20% are minimal. Indeed, one starts feeling somewhat safer at levels $>20\%$, but the authors justifiably insist that only achieving an FVIII level $\geq 50\%$ abolishes the risk of bleeding. Again, this is consistent with the poorly understood physiopathology mentioned. Another intriguing finding is the marked decrease in the risk of new bleeding after 4 to 6 weeks of IST, irrespective of the residual plasma FVIII level. Might the antiinflammatory effects of corticosteroids mitigate bleeding risk or lead to other compensatory procoagulant effects?[8]

Although important consensus has emerged in the management of active bleeding in patients with AHA,[3,9] the prophylactic use of bypassing agents in AHA patients is controversial, given the attendant thrombotic risk of these agents and the high prevalence of cardiovascular comorbidities in these patients. Promptly instituting IST to hasten inhibitor eradication has long been viewed as the best prophylaxis. The authors rightly point out that intensive IST presents its own risks and may also result in fatal complications. Defining the appropriate role of prophylactic hemostatic treatment is becoming even more important with the availability of new and emerging non–coagulation factor–based substitution therapies such as emicizumab.[10] These new agents are therapeutically active in patients with congenital hemophilia and inhibitors, but their risk-benefit profile has not been defined in patients with AHA. These new agents are likely to soon be the focus of exciting clinical trials in AHA. Although we have not yet completely determined why bleeding is more unpredictable in AHA, the findings of Holstein et al contribute significantly to quantifying the risk and lay the groundwork for future prophylaxis trials in AHA.

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LYMPHOID NEOPLASIA

Comment on Orvain et al, page 328

Thrombosis in ALL: a risk without clear mitigation

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In this issue of *Blood*, Orvain and colleagues, on behalf of the GRAALL, the French cooperative group leukemia study group, present data that help to define more clearly the incidence and risk factors for thromboembolism in adults receiving treatment on a pediatric-inspired regimen, GRAALL-2005.[1,2] In recent years, a number of prospective trials have demonstrated improved survival rates for young adults treated with pediatric-inspired regimens that contain frequent dosing of asparaginase and glucocorticoids, both known to be associated with an increased risk of thromboembolic events.[3-6] One of the challenges to further improving treatment outcomes in adult patients is to understand (and, one hopes, to modify) the toxicities that occur more frequently in the adult population treated with these pediatric-inspired regimens.

The GRAALL-2005 enrolled 813 adults with untreated acute lymphoblastic leukemia (ALL) from ages 18 to 60 years old between 2006 and 2014, and 784 of these patients were included in this analysis. Overall, the incidence rate of venous thromboembolism was 16% during the intensive part of chemotherapy, which is considerably higher than the cumulative rate reported recently of 7.9% by Rank et al in the NOPHO ALL2008 study that also employed an intensive pediatric regimen and included patients 1 to 45 years old.[7] Orvain et al identified several risk factors for thrombosis, including older age, female sex, obesity, and a higher platelet count at diagnosis as associated with increased thrombotic risk. The majority of these thrombotic events (84; 69%) occurred during induction therapy; importantly, the most serious of these events, cerebral venous thrombosis, occurred almost exclusively during induction therapy. Of note, neither oral contraceptive use prior to diagnosis nor history of smoking was associated with thrombotic rate.

These are interesting, valuable, and provocative data that identify for whom and when it may be most important to intervene with some kind of prophylaxis. The investigators in GRAALL-2005 provided guidelines (without prospective randomization) for treatment of coagulopathy and prevention of thromboembolism that included heparin (recommended unfractionated heparin), antithrombin supplementation to prevent thrombosis, and fibrinogen concentrates or plasma for patients at risk of bleeding. Although the investigators describe the results of these suggested interventions, this part of the study has some major limitations because these interventions were not prospectively randomized, not all patients received the recommended treatments, and many patients received a combination of therapies. The authors found some mitigation of thrombosis when a combination of antithrombin and heparin prophylaxis was used without risk of significant bleeding but found that fibrinogen concentrates increased the risk of thrombosis. Although these findings provide important hypothesis-generating data for a prospective study, they are clearly not definitive and are difficult to interpret, a fact acknowledged by the authors. An important prospective trial, THROMBOTECT, randomized pediatric patients ages 1 to 18 with ALL to low-molecular-weight heparin, antithrombin concentrates, or unfractionated heparin during induction therapy.[8] Overall, the thrombotic rate was 4.4% in this pediatric population, and patients randomized to antithrombin and low-molecular-weight heparin had significantly lower thrombotic risk than those randomized to unfractionated heparin (which was used primarily by patients enrolled in GRAALL-2005). The study demonstrated safety of the thromboprophylactic regimens (8/949 patients with major hemorrhage).

In conclusion, the study by Orvain and colleagues is a welcome step in defining and highlighting the problem of thrombosis in adults with ALL, identifying patients who might be at greatest risk, and alerting us to the highest risk period for thrombotic complications. The authors also provide hypothesis-generating