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Response:

Factor XIII genotypes and phenotypes

Drs Schroeder and Kohler raise an interesting point regarding the activation of factor XIII (FXIII) in whole blood concerning the Val34Leu polymorphism. They cite the work of Ariëns et al[1] in which a 2.2- to 2.5-fold increase in the catalytic efficiency of thrombin toward homogenous factor XIII Leu34 over Val34 was observed in an in vitro assay system using purified thrombin and fibrinogen. We have observed that FXIII activation in Leu34 carriers (12 heterozygotes, 2 homozygotes) is faster (~1.4 fold) than in Val34/homozygotes (23 individuals) in a Simplate (Organon Teknika, Durham, NC) bleeding-time blood model.[2] Ariëns et al[1] also observed that the Val34 release proceeded more slowly than fibrinopeptide A (FPA) release, whereas Leu34 release was at a rate similar to FPA. Drs Schroeder and Kohler seem to be implying that given the allelic frequency in the population (0.25) of Leu34 versus Val34 we should have observed, in a random population, a slower activation of FXIII than FPA release. Although it is conceivable that some of the 6 individuals studied are Leu34 variants (probability 0.32), the difference between the alleles with respect to FPA release and factor XIII activation would most likely fall within the error observed.[3] The contributions of Leu34 FXIII versus Val34 FXIII in whole blood is a complicated issue because the plasma contribution from each of the alleles and their turnover rates are not known. Thus, genotyping alone would not be sufficient to establish the plasma concentrations of the 2 products.

Caution must be taken when individual genetic factors are used without considering the entire environment in which the biologic reaction takes place. Heterogeneity in the healthy population is a consequence of many genetic and environmental confounders and these variations do not necessarily lead to hemostatic changes. In healthy individuals, alterations in plasma coagulation factor levels range over ± 50% of the mean plasma value.[4] The genotype and environment may alter both the qualitative and quantitative properties for every reactant in blood. This is illustrated by experiments in which FV Leiden is combined with reduced levels of tissue factor pathway inhibitor (TFPI). From in vitro measurements, van't Veer et al[5] hypothesized that reduced expression of TFPI would enhance the prothrombotic effect of FV Leiden. Eitzman et al[6] demonstrated that reduced expression of TFPI in FV Leiden transgenic mice is associated with thrombosis.

Predicting how coagulation should proceed in vivo from results obtained from purified systems has proven problematic. The order of events of fibrin formation in nonanticoagulated whole blood is not sequential, occurring as an integrated process involving FPA release, virtually simultaneous FXIII activation, and partial FPB release.[7] This result is not consistent with results obtained in anticoagulated plasmas[8,9] and purified protein systems.[10] Our study[3] involves the complex system of minimally altered whole blood and is thus a composite of all influences (genotype/phenotype and qualitative/quantitative). It is remarkable that healthy volunteers are, by and large, consistent in their response to tissue factor.

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To the editor:

Iron status indicators: hello new, goodbye old?

A recent Brief report[1] highlighted the clinical use of the reticulocyte hemoglobin content (CHr) in the assessment of iron status. It identified a strong correlation between CHr and an assessment of stainable bone marrow iron stores.

The conclusion that CHr could predict the degree of stainable iron perpetuates the misunderstanding that has dogged the assessment of "iron status" for many decades. It fails to recognize that this status can have different levels in different parts of the iron

metabolic process. Stainable iron stores give crude and imprecise estimates of the insoluble ferric hydroxide complexes deposited in the reticuloendothelial system. Whether or not this is a "store" or simply iron taken out of active metabolic employment is another matter. CHr, on the other hand, is a direct reflection of recent hemoglobin synthesis in developing red cells. It is a measure of the adequacy of the rate of iron supply and this, as has been amply shown in renal anemia, can be compromised even when there is iron on deposit in the stores. CHr is a new and valuable parameter in its own right.

The need to assess stainable iron owes more to tradition than to clinical diagnostic science. With the availability of parameters, such as CHr, that reflect a pathophysiologic reality, it is time to dispense with "Fool's Gold" standards (iron pyrites, a mineral deposit that looks like gold).

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To the editor:

Donor lymphocyte infusions as primary therapy for neoplasms

The recent article by Ballen et al[1] offers the opportunity for some considerations. The originality of this study resides in the infusion either of nonmobilized pheresed lymphocytes from HLA-identical siblings or of umbilical cord blood cells with various degrees of HLA mismatches in patients with refractory cancer or hematologic malignancies. The immunosuppression consisted only of 100-cGy total body irradiation. Not surprisingly, given the low level of immunosuppression, all cancer patients rejected the lymphocytes and all cord blood transplants did not engraft. The only patients evaluable for follow-up were the 11 patients who received lymphocytes from their sibling donor. Nine of them had some level of donor chimerism and 6 developed acute graft-versus-host disease (aGVHD). In this context, characterized by the infusion of allogeneic lymphocytes in an immunocompromised host without support of stem cells, the subsequent aGVHD may be compared with the transfusion-associated GVHD (TA-GVHD). TA-GVHD is a dramatic event with the clinical features of classical aGVHD and a later development of pancytopenia, with overwhelming infection as the most common cause of death.[2] In the Ballen et al[1] study, 6 patients experienced neutropenia, in some cases with Gram-negative sepsis. Unfortunately, Ballen et al do not clarify the correlation between neutropenia and aGVHD. However, it is noteworthy that 2 of the patients who developed GVHD later received an infusion of stem cells from their donors. It is reasonable to assume that, at least in part, the same patients who had aGVHD later developed neutropenia.

In a similar study, Porter et al[3] obtained the engraftment of allogeneic lymphocytes in 4 of 16 patients, and the only patient who developed grade 4 aGVHD subsequently had pancytopenia and died of infection. We believe that these findings are convincing enough to reconsider the rationale of clinical trials based on lymphocyte infusion without stem cell support, particularly in this era of "mini-transplants." Further, the Ballen et al[1] results highlight the unique susceptibility of hematopoietic tissue to graft-versus-host effect because, in the particular setting of this trial, the bone marrow damage is related only to the graft-versus-host effect. Otherwise, in the context of myeloablative and, at a lesser extent, nonmyeloablative transplantation, the graft-versus-host–induced aplasia is masked by the cytotoxic effect of the conditioning regimen and by the competition between recipient and donor stem cells. The high immunogenicity of hematopoietic tissue can explain, at least in part, the satisfactory clinical results obtained in the treatment of hematologic malignancies with allogeneic stem cell transplantation.[4]

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Response:

Low-dose TBI for malignancy: cellular immune therapy

The authors appreciate the communication from Dr Montefusco. However, we do not agree with the assertion that there was no stem cell support. This is not the case and it has been known for decades that nonmobilized peripheral blood contains stem cells. The procedure that we have carried out is perhaps the most "mini" of transplantations with only 100-cGy whole body irradiation and the infusion of relatively small numbers of stem cells with CD3 lymphocytes.[1] The latter point is critical. We have shown in extensive murine work in syngeneic systems that engraftment is determined by stem cell competition.[2] In the allogeneic setting either more ablation or some type of additional tolerization is necessary.[3] It is clear, however, that a major determinant of engraftment and chimerism is stem cell competition. A quite small number of stem cells will repopulate a host if the host has markedly depleted stem cells, as was undoubtedly the case with our patients. It has been shown in murine studies that a single stem cell can