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References


To the editor:

Expression of ABH antigens on platelets

The report of Cooling and colleagues adds importantly to the understanding of ABH blood group antigens on platelets and their clinical significance.1 However, we wish to comment on several aspects of this report that we believe could be misleading.

First, the authors suggest that platelets from individuals whose red cell phenotype is A2 are “Bombay-like” in that they lack detectable quantities of A and H antigen. Second, it is claimed that H and A antigens are “proportionately coexpressed” on platelets even in persons with high expression of A. Both observations conflict with our previous report showing that A2 and O platelets carry comparable amounts of H antigen and that H expression on platelets, as on red cells, is inversely related to expression of A antigen.2 In our studies, we used an H-specific monoclonal antibody (MoAb) BRIC-198, whereas Cooling et al used FITC-labeled Ulex europaeus lectin to quantify platelet H antigen by flow cytometry. To check whether these 2 H-specific probes might yield different results, we obtained FITC-labeled Ulex from the same source as Cooling et al (Sigma, St Louis, MO) and examined its reactions against platelets using 2-color flow cytometry and washed platelets of different phenotypes isolated by differential centrifugation. Using a phycoerythrin-labeled MoAb against CD42b to gate on platelets, we found that FITC-Ulex binds equally well to O and A2 platelets and reacts only very weakly with platelets from an individual with type II high expression of A1 (Table 1). Similar reactions were obtained with MoAb BRIC-198. Findings made with both probes confirm our previous observations that groups A2 and O platelets carry about the same amount of H antigen and that overexpression of A is associated with markedly decreased levels of H.2

The alpha-2-fucosyltransferase responsible for the synthesis of H and the glycosyltransferases responsible for the synthesis of A and B antigens (including A2) are encoded at distinct loci on different chromosomes. It would be most unexpected for the H-transferase gene to be selectively inactivated in megakaryocytes of persons with the A2 red cell phenotype. Additionally, H antigen (the substrate for A-transferase) is eliminated when the “A” sugar is attached. Accordingly, it is to be expected that H substance will be decreased on platelets from individuals with high expression of A-transferase, rather than increased as found by Cooling et al.1 We suggest that the investigators need to re-examine their measurements of platelet H antigen expression made with FITC-Ulex.

Brian R. Curtis and Richard H. Aster

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References

2. Curtis BR, Edwards JT, Hessner MJ, Klein JP, Aster RH. Blood group A and B antigens are strongly expressed on platelets of some individuals. Blood. 2000;96:1574-1581. AQ1: Per style, we have included the article by Cooling et al in the references list as reference 1, and the original reference 1 was renumbered. Please check.

Response:

ABH expression on human blood platelets

It is unclear that Curtis and Aster's findings represent ABH on circulating platelets. In this report and other published studies, the authors have used centrifuged washed platelets. In contrast, we carefully avoided activating platelets by diluting platelet-rich plasma in buffer, followed by a one-step labeling procedure using reagents specific for both ABH and CD41. Early studies showed minimal platelet activation (CD62, < 3%), with exclusion of ABH-positive senescent RBCs, with this method. The need to avoid platelet activation to study circulating platelets cannot be overemphasized. Platelet activation can dramatically alter platelet
glycotype with physiologic consequences.\textsuperscript{2,3} Studies by Julmy et al have shown a 50% increase in ABH on A\textsubscript{1} platelets following platelet activation.\textsuperscript{2} The mechanism surrounding this phenomenon is poorly understood, but may involve a combination of altered glycoprotein expression following alpha granule translocation and microparticle shedding. Changes in glycosyltransferase could also play a role. The activity of many glycosyltransferases, including sialyltransferases in human platelets, can be regulated by posttranslational modification.\textsuperscript{3,4} At least one study has found evidence for posttranscriptional regulation of FUT1, the fucosyltransferase responsible for H-antigen synthesis.\textsuperscript{5} To be comparable with our study, the authors would need to repeat their studies avoiding centrifugation and activation of platelets.

We disagree with the authors that FUT1 may not play a regulatory role in platelets. H is a developmentally regulated antigen during megakaryocytogenesis and displays clonal variation.\textsuperscript{6,7} In colony agar assays, Dunstan observed clonal variation in A and H, with the highest A expression on colonies with high H expression. Based on their observations, Dunstan hypothesized that the ABH heterogeneity on circulating platelets may reflect, in part, clonal differences in megakaryocyte H-transferase (FUT1) activity.\textsuperscript{7} Our results support those findings.

Finally, the authors question the specificity of the UEA1 used in our study. Unlike previous reports,\textsuperscript{1} group A and group O RBCs were included in every experiment as internal controls. RBCs were stained with the same lots and working dilutions as platelets, allowing a direct comparison between RBCs and platelets. UEA1 staining to RBCs was consistent with serologic studies and differed dramatically from platelets. It is possible that the differences in UEA1 staining between the 2 studies may reflect working dilutions of reagents, since our reagents were titrated against RBCs to avoid hemagglutination. In unpublished experiments (L.C., 1996), we observed anti-H monoclonal antibody staining to platelets only at concentrations capable of agglutinating RBCs.

Among the reasons we chose FITC-UEA1 over monoclonal anti-H for labeling platelets is the ability to do one-step direct labeling, a high signal-noise ratio, and the ability of UEA1 to recognize H-antigen on N- and O-linked glycans. Commercial anti-H monoclonal antibodies are relatively weak and specific for type 2 chain H-antigen on N-glycans, which are capable of presenting a dense multivalent epitope. In contrast, UEA1 will recognize H-active structures on N-glycans, as well as branched, core 2 O-glycans like those on glycoprotein Ib. Unlike H on N-glycans, H-antigen on branched core 2 O-glycans may be sterically inaccessible substrates for A/B glycosyltransferases.

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References


To the editor:

Thiopurine methyltransferase in acute lymphoblastic leukemia

We recently reported that specific genetic polymorphisms, particularly polymorphisms in thymidylate synthase (\textit{TYMS}) and glutathione S-transferase M1 (\textit{GSTM1}) predicted the risk of relapse among children with acute lymphoblastic leukemia (ALL).\textsuperscript{1} An accompanying commentary noted surprise that the thiopurine methyltransferase (\textit{TPMT}) genotype was not predictive of relapse risk.\textsuperscript{2} The answer may lie in the fact that the starting dose of mercaptopurine (75 mg/m\textsuperscript{2} per day) in our study was higher than some groups use, and that therapy was individualized based on TPMT status.

\textit{TPMT} methylates and inactivates mercaptopurine. The approximately 10% of populations that inherit intermediate or absent \textit{TPMT} activity at a higher risk of myelosuppression and its attendant toxicity if prescribed “normal” doses of thiopurines;\textsuperscript{3} in addition, such patients appear at higher risk of secondary cancers.\textsuperscript{4,5} \textit{TPMT} activity is inherited as a monogenic autosomal codominant trait, with the vast majority of inactivating alleles accounted for by single nucleotide polymorphisms at amino acids 238, 460, and 719.\textsuperscript{6} TPMT activity can also be measured directly in erythrocytes.\textsuperscript{7} Thus, patients can be clinically screened for TPMT status and then prescribed doses of mercaptopurine that are tailored to their TPMT genotype or phenotype.\textsuperscript{5,6} At St Jude Children’s Research Hospital, since the early 1990s, we have used a combination of measurement of thiopurine metabolites, TPMT status, and clinical tolerance to continuation therapy to selectively decrease the dose of mercaptopurine (without decreasing the nonthiopurine therapy) in patients with low or intermediate TPMT activity, to counsel patients on compliance if thiopurine metabolites are low, and to increase doses of chemotherapy in patients demonstrating persistently high white blood cell counts. Because we have previously found that constant administration (ie, avoiding interruption) in thiopurine therapy resulted in fewer relapses,\textsuperscript{9} our goal has been to maintain the highest dose of daily mercaptopurine that is tolerable. Using this approach, \textit{TPMT} genotype was not predictive of hematologic relapse risk in our study Total XH1B (Figure 1), with 5-year cumulative incidences of 13.2% ± 2.3 versus 6.7% ± 6.7% among patients with the wild-type versus low-activity genotypes, respectively ($P = .46$).

As Zwann indicated, after 2 weeks of including a somewhat lower dose of mercaptopurine than we used (60 mg/m\textsuperscript{2} per day),...
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