To the Editor:

Unusually low platelet counts were reported for control, untreated mice in two recent publications in Blood. In neither case was the discrepancy between the reported platelet counts and those traditionally found noted or explained. Our experience and that of others with platelet counts in blood from mice of several strains and different ages is that average values for groups of normal mice are almost invariably greater than 1,000,000 platelets/μL of blood. This value is obtained when phase contrast microscopy or automated counting in platelet-rich plasma is used to count the platelets. We believe it is unlikely that strain, age, or sex of the mice accounted for the marked differences between the higher values usually detected and the ones reported in the recent publications. We think that the likely explanation for the differences lies in methods used to collect blood and/or count platelets, and we respectfully request that the investigators respond to the comments below.

Longmore et al counted platelets in orbital plexus blood collected from 6-week-old female NIH/Swiss mice by using a Serono-Baker 9000 Cell Counter. Average values of 450,000 to 470,000/μL were reported for untreated mice in Table 1. They noted that platelet clumping was excluded by “concurrent examination of blood smears,” thus eliminating one source of error in automated platelet counts. However, we would also like to know what measures were taken to ensure that the automated cell counter accurately counted the entire population of mouse platelets. This is essential, because murine platelets are substantially smaller than the human platelets for which automated counters are typically calibrated. In at least three other reports, the Serono-Baker 9000 was used to count murine platelets. Control values varied from a low of 798,000 to a high of 965,000, all higher than in the report under question, but nonetheless lower than usual. Our own unpublished experience, separately obtained, is that counting murine platelets with automated cell counters can be problematic.

Harrison et al counted platelets “by routine methods” in female C57Bl/6J × DBA/2 F1-specific pathogen-free mice greater than 8 to 12 weeks of age. The average value for their controls was only 200,000 platelets/μL. In this case, it is also important to know the...
methods that were used to collect blood and count platelets and the quality controls that were applied to determine if the apparent thrombocytopenia was real or artificial. They found that administration of AZT resulted in a worsening of the thrombocytopenia. This contrasts with the report of Chow et al, who found that administration of a similar dose of AZT for a similar length of time to female C57Bl/6 mice of 6 to 8 weeks of age produced thrombocytosis. Chow et al also used an automated cell counter (ELT-8) without a statement about its calibration for murine platelets, and their control platelet counts (988,000/μL) were also low by traditional standards. Because of the variability in control values, one cannot know which, if either, of the results with AZT is correct. However, analysis of the effects of AZT on megakaryocytopoiesis in mice and on platelet production in human beings suggests that platelet production is stimulated by MT.

Clearly, it is advantageous to be able to quickly count murine platelets in samples of whole blood with automated cell counters. However, machines will essentially always produce a number whether it is the same as the actual platelet count or not. The variability in platelet counts in the reports cited and the uniform tendency for the values to be low suggest that uncritical adoption of new methodology for counting murine platelets has sacrificed accuracy. It is incumbent upon investigators and reviewers to be aware of standard values for murine platelet counts and to account for variances so that questions will not be raised about the integrity of the entire body of work.

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REFERENCES

1. Longmore GD, Pharr P, Neumann D, Lodish HF: Both megakaryocytopoiesis and erythropoiesis are induced in mice infected with a retrovirus expressing an oncogenic erythropoietin receptor. Blood 82:2386, 1993
11. Chow FR, Sutton PA, Hamburger AW: 3'-Azido-3'-deoxythymidine ameliorates the thrombocytopenia observed in a murine model of AIDS. Exp Hematol 18:1038, 1990

Response

Levin and Ebbe point out that the platelet counts in control, normal, adult mice reported in our study were unusually low, and suggest that possible explanations lie in the methods of blood collection and platelet counting. In control mice, as well as experimental mice, 200 μL of blood was collected from the orbital plexus into tubes containing 10 μL of EDTA. Whole blood, not platelet-rich plasma, was used for the determination of platelet numbers. Thus, the minor dilution effect of 1/20 was not taken into account. This adjustment would not significantly change the reported values. Platelets were counted using a Serono-Baker 9000 Cell Counter with a volume window setting of 1.8 to 20.2 μm². Concurrently, blood smears were examined to exclude platelet clumping and platelet counts were manually determined. Assuming a mouse platelet diameter of 1 to 3 μm (volume, 0.5 to 14 μm³), it is possible that, with the window settings used, small platelets may not have been counted on the automated counter. However, both manual and automated methods gave essentially the same result, with the manual count being slightly lower and exhibiting a greater standard deviation, thus having a higher likelihood of observer error.

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Response

We greatly appreciate the comments of Levin and Ebbe, who have succinctly highlighted important aspects of the literature regarding human and murine thrombopoiesis. Our method of obtaining blood from mice consisted of phlebotomy of 200 μL from the tail vein of the animal directly into 500-μL pediatric EDTA tubes (Microtainer EDTA Model 5961 tubes; Becton Dickinson Corp, Mountain View, CA); this required repeated "milking" of the tail vein to obtain the target volume. The specimen was then analyzed using a Coulter
Model STKR M26666429 (Coulter Corp, Hialeah, FL). Specimens were processed in an identical manner and run on the same machine to obtain results that were internally consistent. The machine was indeed calibrated for routine clinical specimens, and the explanation for the discrepancies between our values and those reported for other strains of mice may be caused by the gating calibration of the Coulter machine used in our study. At the time of the experiments, selected specimens were obtained by directly drawing tail vein blood into capillary tubes. The hematocrits determined by centrifugation were consistent with the Coulter readings; however, manual platelet counts were not performed.

The literature regarding the effects of AZT on megakaryopoiesis in mice yields conflicting findings. Gallicchio et al observed a dose-dependent reduction in colony-forming units-megakaryocyte (CFU-Meg) when murine hematopoietic cells were cultured in the presence of escalating doses of AZT; this contrasts with the findings of Chow et al that AZT stimulates splenic CFU-Meg as compared with untreated controls, in the report cited by Levin and Ebbe.

Indeed, the findings reported to date with respect to AZT's effect on murine and human megakaryopoiesis is confusing. The early clinical study of AZT in human immunodeficiency virus (HIV)-infected patients, published by Fischl et al, reported that several of their patients had depressed platelet counts after the administration of AZT. Conversely, other patients receiving AZT had elevated platelet counts. In murine samples, Gallicchio et al have shown in vivo and in vitro that AZT suppressed platelet counts and CFU-Meg in both normal and in immunodeficient mice. The same investigator has shown that, after the initial depression, there was enhancement of CFU-Meg, supporting the data of Chow et al.

AZT remains an important agent in the management of HIV-infected patients; our findings confirm other experimental models that show that erythropoietin is effective in ameliorating the hematopoietic toxicity of AZT, and demonstrate that heme potentiates the action of erythropoietin on hematopoiesis. It is our hope that clinical investigation of the use of heme in combination with erythropoietin will continue. Heme arginate has been shown to cause elevation of platelet and red blood cell counts in patients with myelodysplastic syndromes, and it may be that the combination of heme and erythropoietin will be beneficial to HIV-infected patients receiving AZT therapy.

REFERENCES

1. Longmore GD, Pharr P, Neumaun D, Lodish HF: Both megakaryopoiesis and erythropoiesis are induced in mice infected with a retrovirus expressing an oncogenic erythropoietin receptor. Blood 82:2386, 1993
Why are recently published platelet counts in normal mice so low? [letter; comment]

J Levin and S Ebbe