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Bleeding the laboratory mouse: Not all methods are equal

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The laboratory mouse is the model most frequently used in hematologic studies and assessment of blood parameters across a broad range of disciplines. Often, analysis of blood occurs in a nonterminal manner. However, the small body size of the mouse limits collection based on volume, frequency, and accessible sites. Commonly used sites in the mouse include the retro-orbital sinus, facial vein, tail vein, saphenous vein, and heart. The method of blood acquisition varies considerably across laboratories and is often not reported in detail. In this study, we report significant alterations in blood parameters, particularly of total white blood cells, specific populations of dendritic cells and myeloid-derived suppressor cells, and hematopoietic progenitor cells, as a result of site and manner of sampling. Intriguingly, warming of mice prior to tail bleeding was found to significantly alter blood values. Our findings suggest that the same method should be used across an entire study, that mice should be warmed prior to tail bleeds to make levels uniform, and that accurate description of bleeding methods in publications should be provided to allow for interpretation of comparative reports and inter- and intralaboratory experimental variability.

Identifying ways to decrease variability in measured parameters and standardize comparison among laboratories are important in minimizing animal usage and improving data evaluation. Age, gender, and strain can affect complete blood count (CBC) and blood chemistry values [1–3] and is the basis for including animal source and strain in study designs. Common sites for blood collection in mice include the retro-orbital sinus, facial vein (superficial temporal or submandibular veins), tail vein, saphenous vein, and heart (cardiac puncture) [2,4–6]. Classic studies have reported differences in CBC and chemistry values from limited sampling sites in rodents [2,4,7–13]. However, they did not comprehensively evaluate collection sites or use current anesthetics, automated cell counters. or flow cytometry. To our knowledge, the effects of warming or sampling method on phenotype-defined blood cells or the effects of bleeding method on blood cell progenitors have not been evaluated. We analyzed hematologic parameters using five common blood sampling sites/methods and compared differences in blood cell populations and function to assess the impact of bleeding method on acquired data.

Methods

Animals

All procedures were approved by the Indiana University School of Medicine and University of Illinois at Chicago animal care and use committees. *SPF* C57Bl/6J mice (8 weeks old, average 22 g) were used (Jackson Laboratories, Bar Harbor, ME). Mice were housed (five/cage) and maintained according to normal barrier rodent husbandry procedures.

Anesthesia

Isoflurane anesthesia was delivered in 100% oxygen. Induction was achieved using an induction box and 4% isoflurane until the mouse was nonresponsive, recumbent, and had a slowed, even respiratory pattern. Anesthesia was maintained using a nose cone and 1%–2% isoflurane.

Blood collection

Bleeding techniques and use of anesthetics and analgesics were consistent with current veterinary recommendations. All mice

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used in these studies were naïve to prior bleeding. For all procedures, approximately 100 μL of blood was collected, as measured using either marked capillary pipets or a syringe, and was immediately placed in EDTA-coated tubes (BD Microtainer No. 365974) and thoroughly mixed. The same two skilled researchers performed all bleeds in this study.

Retro-orbital. Mice were anesthetized with isoflurane, and a drop of 0.5% proparacaine hydrochloride ophthalmic solution (Bausch & Lomb, Tampa, FL) was applied 5 min prior to sampling. Blood was collected into EDTA capillary pipets (Drummound Scientific, Broomall, PA). Anesthetized mice were restrained in a fashion that created proptosis of the eye, a capillary tube was inserted at the lateral canthus, and the sinus was punctured with gentle pressure and twisting motion.

Facial vein. A small hairless area on the ventral jaw of unanesthetized mice was identified as a landmark as described [6]. A 5-mm lancet (Medipoint, Mineola, NY) was used to puncture the vessel slightly caudal and dorsal to the landmark, and blood was collected cutaneously using capillary pipettes.

Distal tail—cold. A modified tail-clip procedure was used [4]. Briefly, 2.5% Lidocaine and 2.5% Prilocaine (EMLA) cream (Hi-Tech Pharmacal, Amityville, NY) was applied topically to the distal 0.5 cm of tail 15 min prior to bleeding. A ≤1-mm portion was amputated with surgical scissors, and blood was collected using capillary pipets.

Distal tail—warm. Warming was achieved by evenly heating an empty cage to a floor temperature of 35°C–40°C. Mice were placed in the cage for 10 min or as described. Blood collection was as outlined above.

Cardiac. Mice were anesthetized with isoflurane, placed in dorsal recumbency, and swabbed with 70% alcohol. A 27-gauge needle was inserted into the abdominal wall just below the xyphoid process and slightly to the mouse's left side at a 10°–25° angle from the abdominal surface. Slight negative pressure was applied to the syringe until a splash of blood was visualized. One hundred microliters of blood was then collected.

Mobilization

Peripheral blood stem cell mobilization with granulocyte colony-stimulating factor (G-CSF, Neupogen) was performed and analyzed as we have previously described [14]. Briefly, mice were given subcutaneous treatments of G-CSF (50 μg/kg) twice a day for 4 days. On day 5, 100 μL of peripheral blood was acquired by various bleeding methods and lysed using RBC lysis buffer (StemCell Technologies, No. 20120) and defined volumes of blood plated in methylcellulose medium (StemCell Technologies, M3434) for 7 days, and the total number of colony-forming cells (CFCs) was determined.

Complete blood count, flow cytometry

Complete blood count analysis was performed using a Hemavet 950FS (Drew Scientific, Waterbury, CT). Flow cytometry was performed on an LSRII using BD Biosciences antibodies as we described [15].

Statistical analysis

Values are reported as means ± SEM. Statistical significance was determined by one-way analysis of variance with Bonferroni *post hoc* analysis and Student's *t* test where appropriate. Significance was set at $p < 0.05$.

Results

Effect of bleeding method on CBC values

To assess the effect of bleeding method on normal peripheral blood cell values, mice were bled by five different methods as described under Methods. With central cardiac bleeding as a baseline, comparison of the methods revealed that retro-orbital bleeding consistently resulted in the lowest absolute numbers of white blood cells (WBCs), neutrophils, lymphocytes, and monocytes in both male and female mice (Fig. 1). Facial bleeding yielded results comparable to those of cardiac bleeding. Both methods of tail bleeding produced significantly larger numbers of WBCs, neutrophils, and lymphocytes, but warming significantly reduced these increases compared with cold-tail bleeding. The percentages of different cell types were less affected by bleeding method, and no difference was noted between tail bleeding methods. In contrast to WBC parameters, total red blood cells and platelets, hematocrit, and hemoglobin concentration, were equivalent for cardiac and retro-orbital, but increased for facial and cold and warm tail. To evaluate whether CBC differences noted for facial, cold tail, and warm tail reflected bleeding method or were related to absence of anesthesia (cardiac and retro-orbital were performed under isoflurane), additional mice were bled by either facial, cold tail, or warm tail under isoflurane (Supplementary Figure E1, online only, available at www.exphem.org). There were no significant differences in CBC parameters, indicating that alterations are the result of site and manner of sampling.

Effect of bleeding method on blood cell populations

To evaluate lineage-defined population differences, T and B cells, myeloid and plasmacytoid dendritic cells (DCs), total myeloid cells, myeloid-derived suppressor cells (MDSCs) [16], and monocytes were evaluated by flow cytometry (Fig. 2A) for female and male (Fig. 2B, C) mice. A decreased proportion of DCs was observed for both warm- and cold-tail bleeding, suggesting this method is not ideal for studies evaluating/acquiring DCs. Intriguingly, warm-tail bleeding produced significantly more MDSCs than cardiac, retro-orbital, facial, and cold-tail bleeding. These results indicate that not only are total white blood cell values affected by bleeding method, but specific populations of cells can differ among sites.

Effect of mouse warming on number of WBCs

As discussed earlier, warming of the mouse prior to tail bleeding decreases CBC parameters (Fig. 1). Because