4 Cell Differential Assessments of Peripheral Blood Films

ANY PERIPHERAL BLOOD NUCLEATED WHITE CELL differential analysis should be initiated with a cursory examination of the blood film from a global perspective. A remarkable amount of information may be gained from an initial overview of a blood film at lower powers of magnification (e.g., 10x objective with 10x oculars). The blood film should initially be screened for abnormal distributions, streaks, clumping, and other irregularities (Fig. 4). Streaks of purple material (DNA from disrupted leukocytes) may be expected in small measure. Similarly, indistinguishable denuded cells (nuclei without cytoplasm) may be encountered. However, if denuded cells comprise ≥5% of the cells in the blood film, something is likely to be wrong with the procedure or it may be pathognomic of a condition in the animal relating to cellular fragility. This is especially true if the observation is lineage specific. Likewise, the incidence of histiocytes, endothelial cells, or other tissue/vascular cells may be reflective of an "aggressive" preparation of the blood drop for the smear and may be a reason to reject the slide.

The cell differential is best performed within the feather edge of the blood film. The boundaries of the tip and side margins of the smear are not appropriate because the cellular distributions in these regions are often skewed. After beginning, the cell count should be continuous and across contiguous fields of view until at least 100, and preferably ≥200, cells are counted. This is done by scanning across the film, from side to side within the film margins, and then adjusting the slide to an adjacent (but not overlapping) field so as to avoid replicate counting of individual cells (Fig. 5).

The accompanying poster (Mouse Peripheral Blood Cells) and Figures 6–11 present a variety of high-power light microscopic views that are representative of each of the formed elements of the peripheral blood. We suggest using these as a quick reference guide to facilitate the cell differential analysis of nucleated white cells. The development of true expertise to perform cell differential assessments is based on repeated examination of blood films. We particularly suggest that investigators with little experience seek advice from hematology and/or comparative medicine colleagues to gain the necessary experience to distinguish among these cells
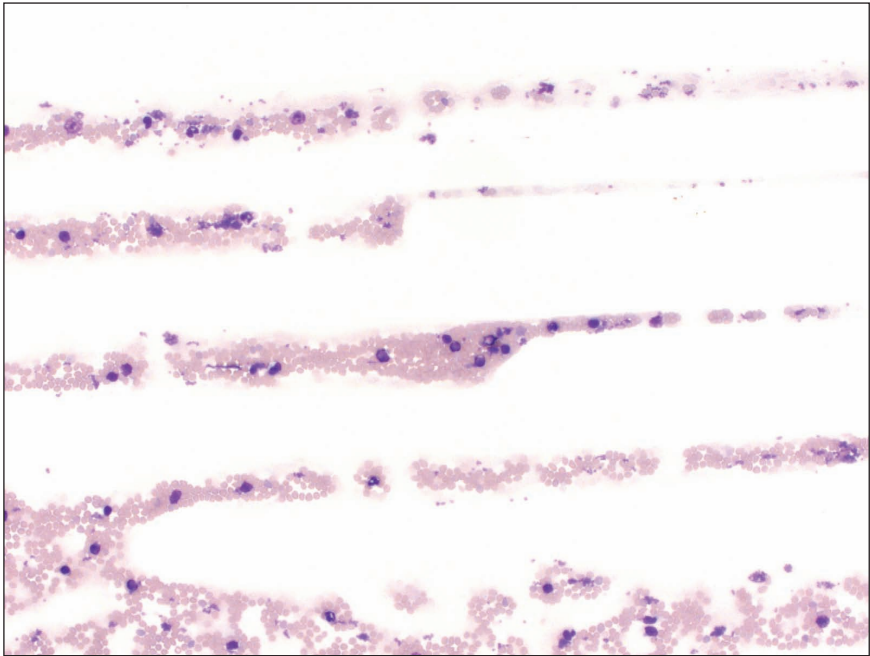
FIGURE 4. "Streaked" area of a film showing "fingers" of blood that will occasionally extend from the body of the spread blood. Although common and a potential source of many WBCs, it is not advisable nor good practice to use these areas for a differential assessment of the film because there may be a disproportionate retention of cells of some lineages.
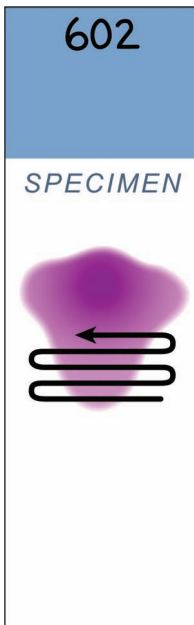

FIGURE 5. Schematic representation of the method needed to scan a blood film for differential cell analyses. Once the feather edge is located, the cell differential assessment is accomplished by a steady progression across the smear; i.e., the investigator should move from one contiguous field to the next, making sure to classify each cell as it is encountered. On the return "sweep" of the slide, it is necessary to have moved the field of the objective so as not to overlap previously examined microscopic fields.

before systematic errors creep into cell differentials (i.e., practice without training and guidance does not make perfect—it makes permanent).

The nucleated cells (leukocytes) and other formed elements of peripheral blood normally exist within a limited range in healthy animals. It is thus possible to gain an initial impression of whether the blood values are relatively normal by comparing the cell counts to one another using the 40x objective and 10x oculars.

> *Technical Tip:* Generally, the red blood cell (RBC) count range for a mouse is 7×10^6 to $13 \times 10^6/mm^3$, whereas the platelet range is 3×10^5 to $10 \times 10^5/mm^3$. Thus, one can expect to find about one platelet for every 10 RBCs. However, platelets are only randomly disbursed if the blood sample has been treated with an anticoagulant. Otherwise, they will occur in clusters and clumps, making estimates of their numbers impossible. The cell count range of the remaining component of peripheral blood, the white blood cells (WBCs), is 5×10^3 to $12 \times 10^3/mm^3$ in a healthy mouse maintained in most pathogen-free environments. Thus, there should be about one nucleated WBC for every 10^2 platelets and every 10^3 RBCs. Although these values are based on ranges, the approximations can be valuable for recognizing one order of magnitude alteration in the ratios of the different peripheral blood components.

The formed elements of blood are mainly *erythrocytes* (i.e., RBCs, the classical hemoglobin-containing biconcave discs), which are all shaped approximately the same (isomorphic) (Fig. 6). Anisocytosis (i.e., red cells of varying shapes and sizes) is a prognostic indicator of pathology. RBCs that have been recently released from erythropoietic tissues are slightly larger and retain a bluish-gray tinge, reflective of residual RNA associated with hemoglobin synthesis; they are called *reticulocytes*. As reticulocytes mature, the slight basophilia owing to the presence of this RNA is lost and the cells acquire the normal red color of circulating RBCs.

> *Technical Tip:* The identification and quantification of reticulocytes in circulation provide a relatively quick and informative assessment of the subject mouse, including its age. In an otherwise healthy adult animal, reticulocytes comprise ~3% of total RBCs. However, this number is larger in younger animals (e.g., 7%–10% at weaning), and increased numbers of circulating reticulocytes (e.g., >10%) are a prognostic indicator of trauma (e.g., a sudden and significant loss of blood), diseases affecting erythopoiesis (e.g., renal tumors that release erythropoietin), and conditions resulting in hypoxia.

Clusters of *thrombocytes* or platelets (small heterogeneously staining and irregularly shaped objects appearing more as cellular fragments than as intact cells) (Fig. 6), as well as cells of the various lineages of circulating

nucleated WBCs are also present. WBCs generally fall into two distinct subtypes: (1) polymorphonuclear leukocytes of granulocytic lineages, including *neutrophils* (Fig. 7), *eosinophils* (Fig. 8), and *basophils* (rare) (Fig. 9) and (2) lymphomononuclear cells such as *lymphocytes* (Fig. 10) and *monocytes* (Fig. 11). The WBCs in peripheral blood should all be mature. WBC numbers are scant in cases of leukopenia and abundant during leukemoid reactions and in some leukemias.
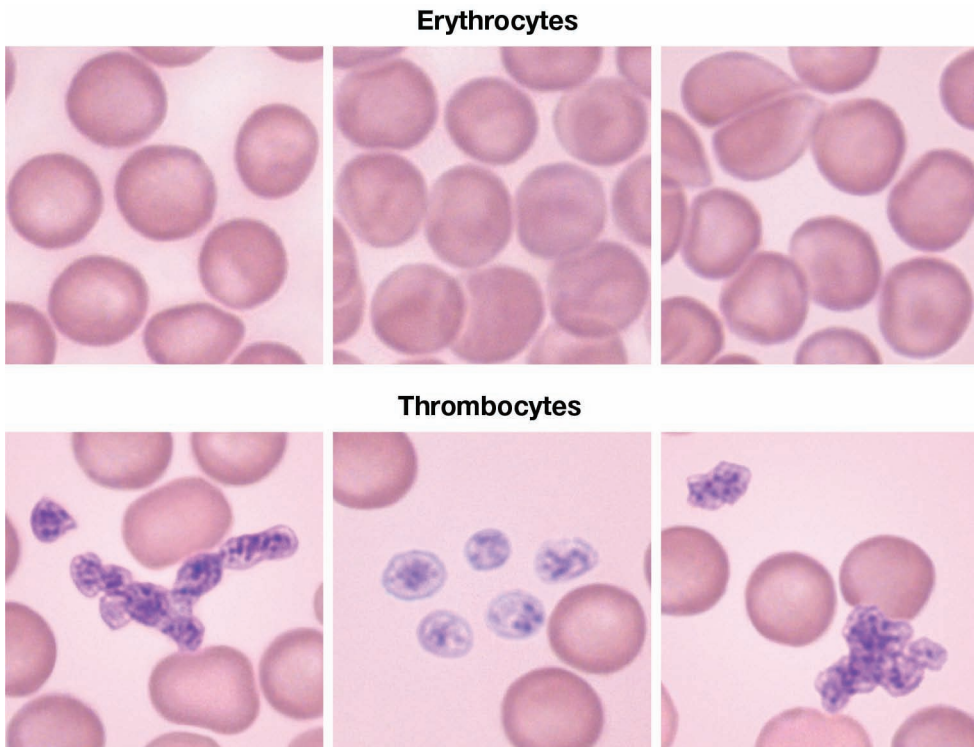
Erythrocytes

Thrombocytes

FIGURE 6. Variation observed in peripheral blood erythrocytes and thrombocytes. Erythrocytes (i.e, RBCs) and thrombocytes (i.e., platelets) comprise the majority of blood cells, at 0.7×10^7 to $1.3 \times 10^7/mm^3$ and 3.0×10^5 to $10.0 \times 10^5/mm^3$, respectively. In contrast, nucleated WBCs (i.e., leukocytes) comprise the smallest fraction at only 5.0×10^3 to $12.0 \times 10^3/mm^3$. Erythrocytes display little variation from their 6–8-μm diameter, concave disc-like appearance. The only variation of note is the occasional cell with a basophilic (i.e., blue-gray) hue, representing a recently formed cell from the marrow (i.e., a reticulocyte), whose residual cytoplasmic globin RNA results in the basophilic appearance. Thrombocytes are anucleate cytoplasmic fragments of marrow-resident megakaryocytes that are much smaller in appearance (1–2 μm) relative to either erythrocytes or leukocytes. This small size prevents visualization of significant variation among thrombocytes (regardless of the magnification), other than noting the appearance of single cells or clumps/groups of cells.