Incorporating Mouse Genome
Mammalian Genome
From Genotype to Phenotype

Sex and strain-related differences in the peripheral blood cell values of inbred mouse strains

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With the sequencing of the mouse genome nearing completion, experimental biology is entering a new era. The mouse is the primary experimental organism used to define mammalian gene function and model human disease. Besides the advantages it offers with regard to size, generation time, and evolutionary proximity to humans, the availability of inbred strains makes the mouse highly amenable to genetic investigation. Inbred strains enable researchers not only to eliminate genetic variation as a consideration when measuring the effect of genetic or environmental manipulations, but to exploit it in classical genetic studies designed to identify genes involved in specific biological processes. There is, however, a lack of baseline phenotypic data pertaining to most commonly used inbred strains, and it is only recently that an attempt to generate such information has begun.

Initiated in 1999, the Mouse Phenome Project (MPP) is an international collaboration designed to coordinate the phenotypic characterization of inbred mouse strains (Paigen and Eppig 2000). The MPP maintains a universally accessible repository for protocols and raw data which are available for on-screen viewing and downloading, in addition to a catalog of other published studies examining particular phenotypic differences between inbred strains. Key areas for which raw data sets can already be downloaded include behavior, cardiology and circulatory physiology, disease susceptibility, obesity, consumption, gastrointestinal and liver physiology, and neurology. The database can be found at http://www.jax.org/phenome.

In the present study, clinical hematology parameters were measured for 16 commonly used strains of inbred mice by performing complete blood cell counts (CBCs) on peripheral blood samples. All mice were obtained at 8 weeks of age from The Jackson Laboratory (Bar Harbor, Me.) with the exception of 129S6/SvEv mice, which were obtained from Taconic (Germantown, N.Y.), and housed in a specific pathogen-free (SPF) environment.

Between the ages of 12 and 16 weeks, 150 μl of peripheral blood was drawn by retro-orbital puncture with a Pasteur pipette. Although it has been suggested that CBCs obtained via this method should not differ significantly from counts performed on samples acquired by cardiac puncture (Sluiter et al. 1985), in our hands retro-orbital bleeding gave far more consistent results (Fig. 1A). Blood was collected in Microtainer brand tubes containing K_2EDTA (Becton Dickinson, Franklin Lakes, N.J.), and samples were analyzed with a Cell-Dyn 3500R automated veterinary hematology analyzer (Abbott Diagnostics, Santa Clara, CA) according to the manufacturer's instructions. EDTA is preferable to heparin as an anticoagulant, largely owing to the tendency of heparin-treated blood samples to undergo platelet aggregation and hence produce false platelet number and mean platelet volume readouts (Kawamoto et al. 2000; Muriithi et al. 2000). In addition, heparin is unsuitable for leukocyte counts or blood smears. Although previous studies have established the veracity of the Cell-Dyn 3000 white blood cell differential counts performed on human peripheral blood (Van Leeuwen et al. 1991; Cornbleet et al. 1992), the accuracy of the 3500R veterinary analyzer differentials was confirmed here through manual inspection of peripheral blood smears obtained from male and female C57BL/6J mice (Fig. 1B).

The results of the automated CBCs are presented in Table 1 and Fig. 2; the raw data set is available from the MPP online database. Nine commonly used inbred strains (129S1/SvImJ, A/J, BALB/cByJ, C3H/HeJ, C57BL/6J, CAST/Ei, DBA/2J, FVB/NJ, and SJL/J) were initially recommended as high priority for

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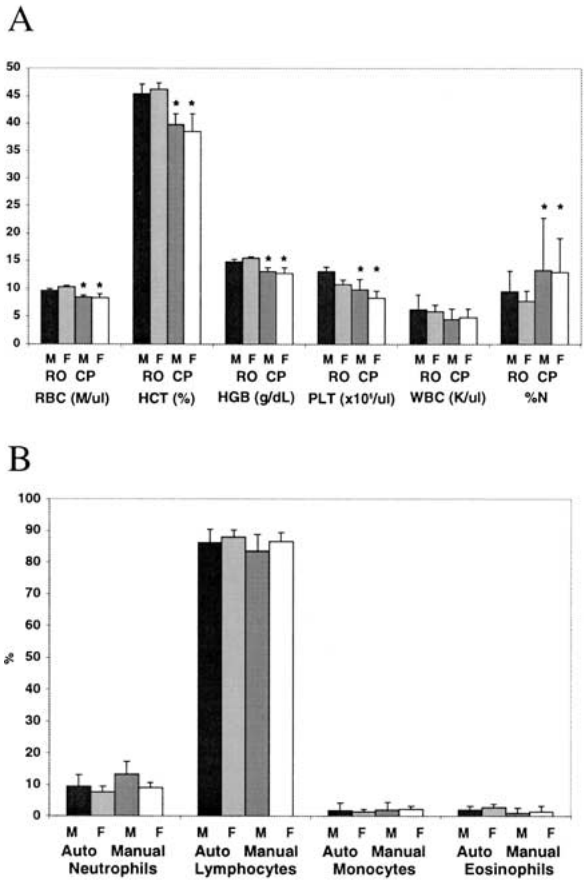

Fig. 1. A. Comparison of the impact of retro-orbital and cardiac puncture bleed techniques on peripheral blood cell values. At 12 weeks of age, 150 μL of peripheral blood was drawn by retro-orbital (RO) puncture from 14 male (black bars) and 15 female (light bars) C57BL/6J mice and analyzed with a Cell-Dyn 3500R automated hematology analyzer. Two weeks subsequent to RO bleeding, 250 μL of blood was drawn from the same group of mice by cardiac puncture (CP) with a 23G needle mounted on a 1-mL syringe, and the same analysis was performed; male (dark bars) and female (white bars). Blood drawn by cardiac puncture generated lower erythrocyte counts, hemoglobin levels, and hematocrits, presumably the result of red cell lysis. Platelet numbers were also lower, potentially the result of undetected micro-clots. For reasons not entirely clear, differentials were more variable when blood was drawn by cardiac puncture. Given that WBC numbers tended to be lower, we speculate that this variability may be due to random lysis of leukocytes during blood drawing. Previous work in this laboratory confirmed that any CBC perturbations resulting from RO bleeding are resolved within 2 weeks (data not shown), and we therefore discount the possibility that prior RO bleeding affected the CP results in the current study (* = $P < 0.0005$). B. Comparison of Cell-Dyn 3500R automated hematology analyzer leukocyte differential counts performed on 14 male (black bars) and 15 female (light bars) C57BL/6J peripheral blood samples, with manual differential counts performed on peripheral blood smears from the same mice; male (dark bars) and female (white bars).

baseline characterization by the Steering Committee of the MPP (Paigen and Eppig 2000), and these strains form the basis of the present study. In addition, CBA/J, CBA/CaJ, 129S6/SvEvTac, BALB/cJ, C3HeB/FeJ, and C57BL/10J were also included. Peripheral blood cell parameters exhibit differing levels of natural variation between mice of the same sex and strain. Although hemoglobin levels (HGB), erythrocyte numbers (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCV), and mean corpuscular hemoglobin concentration (MCHC) show little fluctuation, leukocyte (WBC) and platelet numbers (PLT) vary considerably between sex and age-matched animals.

In general, the CBCs of each inbred strain exhibited minimal sexual dimorphism, although the majority demonstrated small, but statistically significant ($P < 0.005$ after Bonferroni correction using Student's *t*-test) differences in one or more values, typically centered on the various erythrocyte indices. More tangible were the differences observed in platelet numbers, with several strains (e.g., C57BL/6; male 1311 ± 79 K/μL, female 1080 ± 79 K/μL, $P < 3.2 \times 10^{-7}$), exhibiting an apparently real disparity between the sexes. Table 1 also illustrates that leukocyte differentials for male mice are often less consistent than those of female counterparts. In addition, total white cell counts are typically higher in males. These observations can probably, at least in part, be explained by the fact that males were housed in groups of three to five for this study, with general scuffling and fighting resulting in raised neutrophil counts in some mice.

Inter-strain comparisons reveal a large number of statistically significant differences across the full range of peripheral blood cell values; while too numerous to discuss, several general themes emerge. As expected, a high degree of similarity was seen in the CBCs of related strains, particularly substrains. While MCHC exhibited very little fluctuation, the remaining red cell parameters varied markedly between strains (Fig. 2). Notably, FVB/NJ mice demonstrated the lowest hemoglobin levels and hematocrits (male and female averaged values of 14.1 g/dL HGB, and 41.8% HCT), whereas 129S1/SvImJ mice showed the highest (16.1 g/dL HGB, 48.7% HCT). A general inverse correlation between erythrocyte numbers and corpuscular volume was observed, best exemplified by DBA/2J and C3H/HeJ, which represent the highest and lowest values for both parameters (male and female averaged RBCs DBA/2J 10.8 M/μL C3H/HeJ 8.7 M/μL; MCVs DBA/2J 41.5 fL, C3H/HeJ 49.1 fL). All strains exhibited a similar red cell distribution width (RDW), with the exception of CAST/Ei, which displayed a signifi-

Table. 1. Complete peripheral blood cell counts for 16 inbred mouse strains

Strain/Sex (n)	*RBC (M/μL)*	*HGB (g/dL)*	*HCT (%)*	*MCV (fL)*	*MCH (pg)*	*MCHC (%)*	*PLT (K/μL)*	*MPV (fL)*	*RDW (%CV)*	*WBC (K/μL)*	*N (%)*	*L (%)*	*M (%)*	*E (%)*
C57BL/6J														
M (14)	9.6 ± 0.3	14.8 ± 0.5	45.4 ± 1.7	47.2 ± 1.5	15.4 ± 0.3	32.6 ± 0.5	1311 ± 79	4.1 ± 0.1	18.8 ± 1.2	6.2 ± 2.7	9.5 ± 3.6	86.2 ± 4.1	1.8 ± 2.4	2.0 ± 1.1
F (15)	10.2 ± 0.3	15.4 ± 0.3	46.2 ± 1.2	45.1 ± 0.5	15.1 ± 0.2	33.4 ± 0.3	1080 ± 79	4.2 ± 0.2	17.5 ± 0.7	5.9 ± 1.1	7.7 ± 1.9	87.9 ± 2.2	1.3 ± 0.8	2.8 ± 1.0
C57BL/10J														
M (10)	9.8 ± 0.3	14.1 ± 0.4	43.3 ± 1.3	44.5 ± 0.4	14.4 ± 0.2	32.5 ± 0.3	1162 ± 68	4.2 ± 0.2	17.7 ± 0.9	7.5 ± 12	5.9 ± 1.2	88.8 ± 1.9	2.2 ± 1.4	2.9 ± 0.9
F (9)	9.9 ± 0.2	14.6 ± 0.3	44.4 ± 1.0	44.7 ± 0.5	14.7 ± 0.1	32.8 ± 0.2	1086 ± 63	4.5 ± 0.2	17.7 ± 0.7	5.5 ± 1.0	6.1 ± 1.3	90.0 ± 2.7	1.8 ± 1.7	2.0 ± 1.3
129S6/SvEv														
M (10)	9.4 ± 0.7	14.9 ± 1.1	45.7 ± 3.4	48 5 ± 0.4	15.9 ± 0.2	32.7 ± 0.3	762 ± 141	3.7 ± 0.1	19.0 ± 1.4	8.8 ± 1.5	12.5 ± 3.7	84.7 ± 4.3	1.3 ± 0.4	1.6 ± 0.6
F (10)	9.6 ± 0.3	15.5 ± 0.4	46.9 ± 1.3	48.6 ± 0.6	16.1 ± 0.3	33.1 ± 0.2	784 ± 87	3.6 ± 0.1	18.1 ± 0.5	9.4 ± 2.3	13.4 ± 2.1	82.0 ± 3.7	1.5 ± 0.8	3.0 ± 2.4
129S1/SvImJ														
M (15)	10.4 ± 0.2	16.0 ± 0.5	48.5 ± 1.2	46.6 ± 1.1	15.3 ± 0.3	32.9 ± 0.5	733 ± 47	3.7 ± 0.1	18.7 ± 1.0	8.0 ± 1.3	14.0 ± 3.0	81.5 ± 3.8	2.6 ± 1.9	1.8 ± 0.7
F (15)	10.4 ± 0.2	16.2 ± 0.4	48.8 ± 0.8	47.1 ± 0.5	15.7 ± 0.1	33.3 ± 0.3	746 ± 64	3.6 ± 0.1	18.3 ± 0.9	7.6 ± 1.4	10.5 ± 2.3	82.9 ± 5.1	1.0 ± 0.9	5.6 ± 3.2
CBA/CaJ														
M (15)	9.7 ± 0.3	15.2 ± 0.5	45.2 ± 1.4	46.5 ± 0.5	15.7 ± 0.3	33.7 ± 0.7	1090 ± 179	4.5 ± 0.3	16 5 ± 0.7	8.1 ± 1.6	13.0 ± 3.7	81.7 ± 3.5	2.1 ± 2.5	2.3 ± 0.7
F (15)	9.5 ± 0.4	15.5 ± 0.4	45.3 ± 1.7	47.7 ± 1.0	16.3 ± 0.5	34.2 ± 0.9	931 ± 110	4.6 ± 0.4	17.5 ± 1.1	6.4 ± 1.9	14.5 ± 3.4	81.2 ± 2.8	1.9 ± 1.8	2.1 ± 0.4
CBA/J														
M (10)	8.9 ± 0.2	14.2 ± 0.4	42.0 ± 0.8	47.3 ± 0.6	15.9 ± 0.2	33.7 ± 0.4	1036 ± 54	4.6 ± 0.3	16.2 ± 1.0	4.8 ± 1.0	13.4 ± 5.1	81.5 ± 3.9	3.3 ± 2.4	1.3 ± 0.4
F (10)	9.0 ± 0.4	14.3 ± 0.4	42.1 ± 1.4	47.0 ± 0.5	15.9 ± 0.3	33.9 ± 0.5	912 ± 129	4.5 ± 0.2	16.3 ± 0.8	4.6 ± 1.2	14.9 ± 2.9	81.8 ± 3.4	1.4 ± 0.6	1.5 ± 0.7
C3H/HeJ														
M (10)	8.6 ± 0.4	14.6 ± 0.6	42.6 ± 1.3	49.5 ± 1.0	16.9 ± 0.3	34.3 ± 0.4	1052 ± 68	4.8 ± 0.4	17.3 ± 0.8	6.4 ± 13	21.7 ± 4.0	72.3 ± 4.0	3.9 ± 1.2	1.8 ± 0.5
F (10)	8.8 ± 0.3	14.7 ± 0.4	42.8 ± 1.3	48.9 ± 0.3	16.9 ± 0.2	34.4 ± 0.3	986 ± 77	4.9 ± 0.3	16.6 ± 0.9	5.2 ± 0.8	19.6 ± 3.9	75.7 ± 4.3	2.3 ± 0.7	2.0 ± 0.6
C3HeB/FeJ														
M (15)	9.1 ± 0.3	14.7 ± 0.4	43.0 ± 1.1	47.5 ± 0.6	16.2 ± 0.2	34.2 ± 0.4	976 ± 164	4.8 ± 0.6	17.0 ± 0.6	6.2 ± 1.5	15.2 ± 3.6	80.0 ± 3.6	2.1 ± 1.1	2.4 ± 0.9
F (15)	8.9 ± 0.3	14.7 ± 0.5	43.0 ± 1.3	48.4 ± 0.4	16.6 ± 0.2	34.3 ± 0.4	977 ± 164	4.7 ± 0.2	17.2 ± 1.1	5.8 ± 1.2	14.9 ± 2.4	81.8 ± 2.6	1.3 ± 0.9	1.8 ± 0.6
DBA/2J														
M (14)	11.1 ± 0.7	15.0 ± 0.6	45.5 ± 2.3	41.1 ± 0.6	13.5 ± 0.3	32.9 ± 0.4	1121 ± 109	4.7 ± 0.7	19.5 ± 1.2	7.7 ± 1.3	13.5 ± 5.2	78.9 ± 6.6	4.5 ± 3.5	2.1 ± 0.9
F (15)	10.5 ± 0.3	14.7 ± 0.3	44.0 ± 1.1	41.9 ± 0.6	14.0 ± 0.1	33.3 ± 0.4	1182 ± 150	4.5 ± 0.3	18.3 ± 0.8	6.6 ± 1.0	11.1 ± 5.2	80.0 ± 4.4	5.0 ± 5.4	2.9 ± 1.3
FVB/NJ														
M (15)	9.3 ± 0.3	14.1 ± 0.3	41.8 ± 1.0	45.0 ± 0.3	15.2 ± 0.1	33.7 ± 0.2	1453 ± 145	3.8 ± 0.2	16.7 ± 0.6	5.6 ± 1.4	11.0 ± 2.2	84.5 ± 3.5	1.9 ± 1.2	2.4 ± 1.0
F (15)	9.0 ± 0.4	14.2 ± 0.4	41.7 ± 1.4	46.3 ± 0.6	15.8 ± 0.4	34.1 ± 0.8	1275 ± 105	4.0 ± 0.1	16.7 ± 0.6	5.7 ± 0.8	10.2 ± 2.2	86.3 ± 2.0	1.3 ± 0.4	2.2 ± 0.9
A/J														
M (8)	9.6 ± 0.4	14.5 ± 0.5	42.9 ± 1.6	44.8 ± 0.5	15.1 ± 0.2	33.7 ± 0.3	1103 ± 120	4.3 ± 0.1	17.4 ± 1.0	6.2 ± 1.8	11.4 ± 6.0	86.2 ± 6.0	0.9 ± 0.7	1.4 ± 0.6
F (10)	9.8 ± 0.4	15.0 ± 0.3	44.2 ± 1.2	45.1 ± 0.8	15.4 ± 0.3	34.0 ± 0.3	1177 ± 87	4.3 ± 0.2	17.9 ± 0.8	4.7 ± 0.8	11.4 ± 3.0	84.4 ± 2.5	2.7 ± 2.3	1.4 ± 0.6
CAST/Ei														
M (9)	10.0 ± 0.6	15.5 ± 0.4	45.8 ± 1.5	45.9 ± 1.3	15.6 ± 0.5	33.9 ± 0.3	1201 ± 167	4.1 ± 0.8	23.0 ± 2.4	5.5 ± 1.8	8.7 ± 3.2	87.7 ± 3.5	1.2 ± 0.7	2.3 ± 2.0
F (10)	10.6 ± 0.6	15.4 ± 0.3	45.3 ± 1.3	42.9 ± 1.4	14.5 ± 0.6	33.9 ± 0.4	817 ± 61	3.5 ± 0.4	20.2 ± 1.3	5.1 ± 1.8	6.7 ± 0.7	90.2 ± 2.7	0.7 ± 0.5	1.6 ± 0.5
BTBR/NJ														
M (10)	9.0 ± 0.2	14.4 ± 0.4	42.5 ± 0.9	47.2 ± 0.5	16.0 ± 0.2	34.0 ± 0.3	1137 ± 65	3.6 ± 0.1	16.5 ± 0.6	8.9 ± 1.3	14.5 ± 2.4	81.5 ± 2.8	2.3 ± 1.5	1.3 ± 0.3
F (10)	8.9 ± 0.2	14.7 ± 0.2	43.2 ± 0.8	48.4 ± 0.5	16.5 ± 0.2	34.1 ± 0.2	1044 ± 58	3.7 ± 0.1	17.0 ± 0.8	9.4 ± 1.2	13.5 ± 1.9	82.0 ± 2.5	2.5 ± 0.8	1.4 ± 0.5
BALB/cByJ														
M (15)	9.6 ± 0.4	15.2 ± 0.4	44.4 ± 1.5	46.2 ± 1.0	15.8 ± 0.3	34.2 ± 0.3	1315 ± 141	4.4 ± 0.1	17.5 ± 0.6	5.7 ± 1.6	13.6 ± 2.9	81.1 ± 3.6	1.9 ± 1.3	3.2 ± 1.2
F (15)	10.0 ± 0.3	16.0 ± 0.5	46.0 ± 1.3	45.9 ± 0.4	16.0 ± 0.2	34.8 ± 0.2	1087 ± 124	4.5 ± 0.2	17.2 ± 0.8	6.2 ± 1.2	11.1 ± 3.3	84.1 ± 3.1	1.2 ± 0.6	3.3 ± 1.0
BALB/cJ														
M (9)	9.2 ± 0.3	14.8 ± 0.6	43.0 ± 1.4	46.7 ± 0.5	16.0 ± 0.2	34.3 ± 0.3	1132 ± 147	4.6 ± 0.3	16.8 ± 0.7	3.6 ± 1.7	20.2 ± 3.4	75.8 ± 4.0	1.4 ± 0.9	2.5 ± 0.9
F (10)	9.4 ± 0.3	15.5 ± 0.3	44.1 ± 0.8	47.2 ± 0.8	16.6 ± 0.3	35.1 ± 0.2	1181 ± 67	4.5 ± 0.2	16.0 ± 0.8	4.8 ± 1.2	14.8 ± 3.1	80.0 ± 3.6	2.0 ± 0.8	2.9 ± 1.0
SJL/J														
M (14)	9.0 ± 0.4	13.7 ± 0.5	42.6 ± 1.5	47.2 ± 0.6	15.1 ± 0.3	32.1 ± 0.5	1012 ± 207	5.3 ± 0.6	19.6 ± 2.0	10.5 ± 2.7	5.8 ± 1.5	90.6 ± 2.1	1.4 ± 0.4	2.0 ± 0.7
F (15)	9.5 ± 0.4	14.6 ± 0.5	44.5 ± 1.6	46.8 ± 1.0	15.3 ± 0.3	32.8 ± 0.3	1054 ± 152	5.2 ± 0.3	18.4 ± 1.2	7.9 ± 1.7	7.4 ± 1.6	88.2 ± 2.4	2.1 ± 0.6	2.0 ± 0.9

n, Number of mice from which blood was analyzed. Values given are mean ± standard deviation.

RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; RDW, red cell distribution width; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; MPV, mean platelet volume; WBC, white blood cells; N, neutrophils; L, lymphocytes; M, monocytes; E, eosinophils; basophils (not shown) <1% in all strains tested.

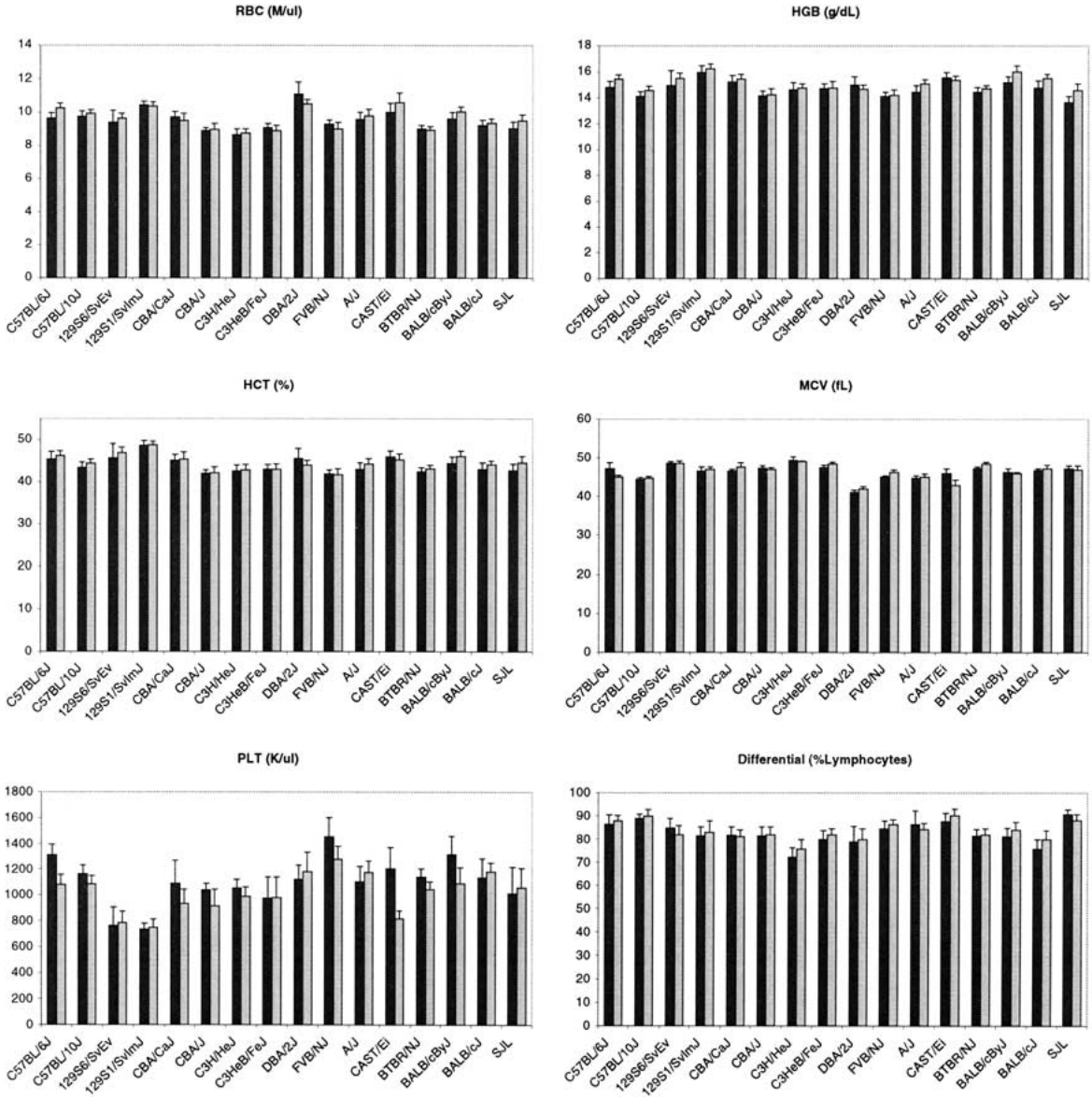

Fig. 2. Selected erythrocyte, platelet, and leukocyte values in the peripheral blood of commonly used laboratory inbred mouse strains; male (black bars) and female (light bars).

cantly higher degree of anisocytosis than all other strains.

Platelet number and mean platelet volume (MPV) displayed highly significant variation. Interestingly, platelet levels in the two 129 strains 129S6/SvEvTac (male 762 ± 141 K/μL, female 784 ± 87 K/μL) and 129S1/SvImJ (male 733 ± 47 K/μL, female 746 ± 64 K/μL) were lower than in all other strains examined, of which FVB/NJ mice exhibited the highest (male 1453 ± 145 K/μL, female 1275 ± 105 K/μL). 129S6/SvEvTac and 129S1/SvImJ also displayed MPVs among the lowest of any strain (male 3.7 ± 0.1 fL, female 3.6 ± 0.1 fL for both strains). Leukocyte numbers, while subject to considerable individual fluctuation, did vary in a statistically significant fashion, both in total number and differential counts between strains. Extremes for total white blood cell numbers are represented by BALB/cJ (male 3.6 ± 1.7 K/μL, female 3.9 ± 1.2 K/μL) and 129S6/SvEvTac (male 8.8 ± 1.5 K/μL, female 9.4 ± 2.3 K/μL, $P < 6.1 \times 10^{-5}$), while the differential counts reveal that peripheral blood from C3H/HeJ mice contained the lowest proportion of lymphocytes (male 72.3 ± 14.0%, female 75.7 ± 4.3%), and C57BL/10 the highest (male 88.8 ± 1.9%, female 90.0 ± 2.7%, $P < 2.0 \times 10^{-7}$).

In conclusion, this study comprises the first comprehensive examination of CBCs in commonly used laboratory mouse strains. These data demonstrate that a high degree of strain and sex-related variation exists in the peripheral blood cell values of the 16 inbred mouse strains examined. In addition to illustrating these differences, the data presented here provide useful baseline parameters for further studies such as mutagenesis screens and analyses of quantitative factors that influence hemopoiesis.

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