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Selected peripheral blood cell parameters in twelve inbred strains of laboratory mice

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In 230 mice from 12 inbred strains (A.CA/W, AKR/W, BALB/cW, BN/aW, CBA/W, CBA-T6/W, C3H/W, C57BL/6W, C57BL/10W, DBA2/W, 129S1/SvW, HLB219/J) the following parameters were measured: the total number of white (WBC) and red (RBC) blood cells, haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT) number. Highly significant interstrain differences were shown within RBC, HGB, HCT, MCV and PLT.

KEY WORDS: blood cells / inbred strains / mice

Inbred strains of laboratory mice are useful in function analysis of different genes active in specific biological processes. They are often used for basic studies of selected physiological processes as animal models of numerous human diseases [Gao *et al*. 1997, Arcasay *et al.* 1999, Morrison *et al.* 2002, Lopez *et al*. 2002, Hall *et al*. 2004].

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The differences within selected blood parameters taking place between different inbred strains of mice may be due to mutations in genes responsible for the synthesis of haematopoiesis-associated peptides or with the genetic background. Blood parameters are quantitative traits, and therefore the differences in the level thereof between distinct inbred strains could result from changes in several genes. This assumption was confirmed by Cheung *et al*. [2004] who identified a few quantitative trait *loci* (QTLs) for platelets (PLT) number and by Peters *et al*. [2005], who found QTLs for white blood cell counts (WBC). They found a QTL which accounted for up to 31% of the total variance in the baseline of WBC count and 30% of the total variance for PLT number using F2 generations of the NZW/LacJ × SM/J and C57BLKS/J × SM/J crossbreds. Moreover, Tsai *et al*. [2002] demonstrated the environmental effect on haematologic indicators in different inbred strains of mice.

Kile *et al*. [2003] presented preliminary information about the baseline level of peripheral blood haematological parameters in 16 mostly used inbred strains of mice bred in the Jackson Laboratory.

Similar characterization of inbred mice strains bred by the Cancer Centre and Institute of Oncology in Warsaw (Oncology Centre) was subjected to analysis in this study. Our breeding of laboratory animals has the status of a Reference Centre for Biomedical Research in Immunooncology of the International Council for Laboratory Animal Science (ICLAS) and supplies Polish scientific institutions with inbred strains of mice which show very important data in a wide variety of parameters. The results concerning a genetic characterization of inbred strains with the aid of microsatellite markers have already been published on the Jackson Laboratory website [Wirth-Dzięciołowska and Gajewska 2002].

The aim of this study was to determine the parameters of peripheral blood in 12 inbred strains of mice bred at the Oncology Centre in Warsaw.

Material and methods

Animals

Considered were 230 mice of both sexes from 12 inbred strains: A.CA/W, AKR/W, BALB/cW, BN/aW, CBA/W, CBA-T6/W, C3H/W, C57BL/6W, C57BL/10W, DBA2/W, 129S1/SvW and HLB/219J. The HLB/219J strain, obtained from the Jackson Laboratory, USA, has been characterized by a very low number of platelets. This mutation occurred in C57Bl/6J after chemical induction of N-ethyl-N-nitrosourea (ENU) (www.jax.org).

All animals were kept under specific pathogen-free conditions. Health and genetics were surveyed according to the LABAAS schedule [Nickles *et al*. 2002]. Six to eight animals of the same sex and strain were kept in each cage on wood chip bedding (chip classic, RETTENMAIER, Germany). The light/dark period of 12h/12h was kept at 22±2°C and 40-60 % relative humidity. The room was air-conditioned (15 ventilation cycles per hour). Animals were fed to appetite a commercial pelleted diet (Labofeed

H) manufactured by MORAWSKI Co., Poland, and had free access to water. Numbers of females and males within each strain are given in Tables 1 and 2.

The experiment was approved by the local ethics committee.

Blood sampling

Blood samples of 150-200 μl were collected retroorbitally under anaesthesia into K_2-EDTA-coated tubes (MICROVETTE Sarstedt, Germany) from 4-month-old animals. Eight to 15 samples of blood were withdrawn on each sampling day between 10 and 11 am, and analysed within half to one hour after collection.

Analytical

The following blood parameters were determined: total number of white blood cells (WBC), total number of red blood cells (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume of single red cell (MCV), mean corpuscular haemoglobin weight (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT) number. All blood samples were analysed with an automatic haematology analyser using the volume impedance principle (Sysmex K4500, SYSMEX Corporation, Kobe, Japan).

Statistical

All data were expressed as mean values and their standard deviations (SD). For statistical evaluation the analysis of variance (ANOVA) and *post-hoc* comparisons were made with a Duncan test. The differences between sexes were tested with a T test and for comparison, with a nonparametric U test (Mann-Whitney).

Results and discussion

Means and their SD of all analysed parameters of peripheral blood cells are presented in Tables 1-3.

Intrastrain comparisons showed some differences in blood parameters between the sexes in a number of strains (Tab. 4 and 5) The parameters in question occurred higher in females than in males, especially within BALB/cW and C3H/W strains.

HGB level, HCT and MCV were shown to be lower in males than in females in the majority of strains, whereas the number of WBC and PLT revealed the opposite trend. Males had a higher number of WBC in some of the inbred strains and a significantly higher number of PLT than females in almost all strains considered (Tab.1 and 2). The differences between sexes for WBC counts and for MCHC and HCT were not significant, however, significance was identified for PLT and other blood traits measured (Tab. 4). Marked differences occurred between males and females in PLT numbers within C57Bl/10W strain. Quite opposite results were reported by Kile *et al*. [2003], who observed more tangible variances between sexes in the platelet number in C57BL/6J, but not in C57BL/10J strain.

Table 1. Means and their standard deviations (SD) for peripheral blood cell indicators in female mice from 12 inbred strains

Strain	n		Indicator							
			WBC (k/nl)	RBC (M/nl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	PLT (k/μl)
129S1/SrW	11	mean	4.7	9.5	15.9	53.8	56.4	16.7	29.6	748.2
		SD	0.8	0.2	0.4	1.0	0.5	0.4	0.6	154.8
A.CA/W	9	mean	4.5	9.5	15.3	51.2	53.8	16.1	29.9	989.6
		SD	0.8	0.3	0.6	1.2	1.2	0.5	0.4	105.9
AKR/W	14	mean	7.8	10.1	15.4	52.4	51.9	15.3	29.5	788.9
		SD	1.7	0.3	0.4	1.3	0.8	0.3	0.4	124.7
BALB/cW	12	mean	9.1	10.4	16.7	54.0	52.0	16.1	30.9	826.7
		SD	1.4	0.2	0.3	1.1	0.8	0.2	0.3	72.1
BN/aW	8	mean	5.0	10.3	15.6	53.6	51.7	15.2	29.2	953.9
		SD	0.8	0.3	0.4	1.6	0.8	0.2	0.6	71.7
B6/W	11	mean	6.9	9.8	15.3	53.2	54.6	15.7	28.7	939.4
		SD	0.9	0.5	0.5	2.1	0.8	0.3	0.2	122.3
B10/W	10	mean	7.8	10.4	15.3	54.0	52.0	14.8	28.4	946.4
		SD	2.0	0.3	0.5	1.8	0.5	0.2	0.4	58.4
C3H/W	12	mean	7.5	8.5	14.4	47.0	55.2	16.9	30.6	615.1
		SD	2.1	0.3	0.4	1.7	0.6	0.4	0.4	130.4
CBA/W	4	mean	7.9	9.2	14.7	48.2	52.6	16.1	30.6	997.7
		SD	2.0	0.6	0.4	2.3	1.1	0.9	1.1	125.2
CBA-T6W	8	mean	8.9	9.6	15.9	50.6	52.9	16.6	31.4	859.5
		SD	1.0	0.3	0.4	0.9	0.6	0.2	0.2	33.0
DBA/2W	10	mean	9.4	11.0	15.3	53.4	48.3	13.8	28.6	977.2
		SD	1.9	0.2	0.3	1.2	0.4	0.2	0.4	85.4
HLB219/J	2	mean	6.9	10.1	15.8	53.8	53.3	15.7	29.4	114.0*
		SD	-	-	-	-	-	-	-	-
Strains pooled	111	mean	7.3	9.9	15.5	52.2	52.9	15.7	29.7	853.0
		SD	2.3	0.7	0.7	2.7	2.2	0.9	1.0	157.0

*While computing grand mean for platelets, the HLB219/J PLT values were not considered.

Interstrain comparisons revealed many significant differences within the parameters analysed (Tab. 1 and 2). Extreme differences were observed in the number of WBC, what is not surprising, and also in the number of PLT. The highest PLT numbers (near $1000\times10^3/\mu l$) were found in C57BL/10W, C57BL/6W, A.Ca/W and DBA/2W strains. Much lower values were obtained in C3H/W strain ($660\times10^3/\mu l$), the differences, however, being still significant. Highly significant differences between strains were also found in the mean volume of a single platelet. In particular, big platelets were observed in C3H/W and AKR/W strains (6.9fL and 6.5fL, respectively). The participation of big platelets (P-LCR) was extremely high in these strains (9.42% and 4.62%, respectively). Strain129S1/SvW exhibited the smallest volume of platelets (5.3fL) and the lowest P-LCR value (2.18%). HLB/219 strain was found to exhibit thrombocytopenia. The lowest PLT number (female and male means amounting to $114\times10^3/\mu l$ and $212\times10^3/\mu l$, respectively) distinguished the latter from the related C57Bl/6J strain ($939.36\times10^3/\mu l\pm122.34$ and $1065.92\times10^3/\mu l\pm80.86$ for females and males). Other traits analysed reached almost the same level in both related strains.

Table 2. Means and their standard deviations (SD) for peripheral blood cell indicators in male mice from 12 inbred strains

Strain	n		Indicator							
			WBC (k/nl)	RBC (M/nl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	PLT (k/µl)
129S1/SrW	9	mean	5.7	9.7	15.5	53.2	56.9	16.6	29.1	886.6
		SD	2.7	0.6	0.5	2.0	2.3	0.7	0.4	92.5
A.CA/W	11	mean	4.6	9.5	14.8	50.0	52.5	15.5	29.6	1030.4
		SD	0.8	0.3	0.5	1.1	0.9	0.4	0.6	79.2
AKR/W	8	mean	7.6	9.9	15.1	51.0	51.3	15.2	29.5	808.2
		SD	1.4	0.3	0.6	1.8	0.8	0.3	0.4	94.8
BALB/cW	15	mean	7.4	10.3	16.0	53.4	51.8	15.6	30.0	994.3
		SD	2.8	0.4	0.5	1.8	0.4	0.3	0.4	122.7
BN/aW	11	mean	6.2	10.1	15.3	52.8	52.2	15.1	29.0	929.8
		SD	2.2	0.5	0.6	2.6	0.4	0.3	0.6	82.5
B6/W	12	mean	5.8	10.0	15.5	54.8	53.8	15.2	28.2	1065.9
		SD	2.5	0.3	0.5	1.9	0.4	0.1	0.2	80.9
B10/W	12	mean	8.8	10.3	15.3	54.2	52.5	14.8	28.2	1108.1
		SD	2.1	0.3	0.4	1.4	0.3	0.2	0.3	183.0
C3H/W	8	mean	6.6	8.2	14.1	45.0	54.6	17.1	31.3	745.1
		SD	1.6	0.2	0.3	1.0	0.3	0.2	0.2	49.2
CBA/W	10	mean	8.7	9.7	15.6	50.8	52.2	16.1	30.8	889.4
		SD	1.1	0.3	0.3	1.1	0.9	0.4	0.3	138.1
CBA-T6W	12	mean	8.8	9.5	15.5	50.2	52.9	16.3	30.9	913.3
		SD	1.1	0.3	0.3	1.1	0.6	0.3	0.4	174.4
DBA/2W	8	mean	8.5	10.6	14.9	50.8	47.9	14.1	29.4	1007.5
		SD	3.3	0.4	0.3	1.9	0.6	0.3	0.6	41.0
HLB219/J	3	mean	10.4	10.0	15.1	52.4	52.3	15.1	28.9	212.5*
		SD	-	-	-	-	-	-	-	-
Strains pooled	119	mean	6.9	9.9	15.2	51.7	52.5	15.5	29.5	960.3
		SD	2.3	0.7	0.6	3.0	2.1	0.8	1.0	144.7

*While computing grand mean for platelets, the HLB219/J PLT values were not considered.

Table 3. Means and their standard deviations (SD) for peripheral blood cell indicators in mice from inbred strains (sexes and strains pooled)

Statistic	n	Indicator							
		WBC (k/nl)	RBC (M/nl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	PLT (k/µl)
Mean	230	7.1	9.9	15.4	51.9	52.7	15.6	29.6	907.1
SD		2.3	0.7	0.7	2.8	2.2	7.9	1.0	159.6

The number of RBC differed significantly among strains. C3H/W exhibited the lowest erythrocyte counts (8.4 M/µl) while DBA/2W demonstrated the largest (10.86 M/µl). Within this parameter significant differences were identified between C57BL/6W and C57BL/10W ($P<0.05$), in spite of a close relationship between the strains.

MCHC was another trait that exhibited significant differences between the strains. C3H/W and CBA-T6 mice demonstrated the highest values of MCHC, while C57BL/6W and C57BL/10W the lowest. MCH and MCV represented high interstrain

Table 4. Significant intersex differences in peripheral blood cell indicators within inbred mice strains (T test, P<0.01, M>F)*

Strain	Indicator						
	WBC	RBC	HGB	HCT	MCH	MCHC	PLT
C57BL/6W	M>F	M>F			M>F		M>F
AKR/W		M>F	M>F	M>F			
BALB/cW			M>F		M<F	M<F	M>F**
A.CA/W					M>F		M>F
C57BL/$_{10}$W							M>F

*Significant differences according to non-parametric U test are identical with those identified with T test.
**P>0.001.
M – males; F – females.

Table 5. Final specification of peripheral blood cell indicators across mice sexes

Trait	Females			Males		
	median value	maximum	minimum	median value	maximum	minimum
WBC (10^3/μl)	7.30	13.30	3.40	6.65	12.60	2.50
RBC (10^6/μl)	9.98	11.33	7.60	9.93	11.62	7.93
HGB (g/dl)	15.50	17.20	13.60	15.20	16.90	13.60
HCT (%)	52.85	56.60	43.00	51.75	59.40	43.80
MCV (fl)	52.60	57.10	41.60	52.25	62.80	47.20
MCH (pg)	15.80	17.90	13.50	15.40	18.30	13.80
MCHC (%)	29.65	32.10	27.70	29.60	31.50	27.80
PLT* (10^3/μl)	867.00	1193.00	308.00	957.50	1364.00	507.00

*The strain HLB/219 was not taken into consideration.

similarity, except for DBA/2W strain which showed the lowest values of these traits (Tab. 1 and 2). A general negative correlation between the erythrocyte number and the mean MCV was observed (r = -0.70), best exemplified by DBA/2W and 129S1/SvW, where the highest and lowest values for both parameters were estimated (Tab. 1 and 2). A similar correlation was observed between the MCH, MCHC and the erythrocyte numbers (r = -0.86 and r = -0.60, respectively, for MCH and MCHC trait). These results were identified as significant**.**

The results concerning peripheral blood parameters are among characteristic traits of the inbred mice strains from the Oncology Centre in Warsaw. The inbred strains considered in this study are bred in the Oncology Centre for many generations and are of different origin. They originate from the Netherlands Cancer Institute (AKR/W, C57Bl/10W), the Karolinska Institute (A.Ca/W), the Institute of Zoology of Warsaw University (CBA/W, CBAT6/W), the Jackson Laboratory (129S1/Sv/W, DBA/2W, C57BL/6W), the Institute of Oncology in Budapest (C3H/W) and the Polish Institute

Table 6. Genetic distances between analysed inbred strains of mice based on microsatellite sequences

Strain	DBA/2W	A.CA/W	BN/aW	C3H/W	CBA/W	BALB/cW	C57BL/10W	C57BL/6W	AKR/W	129S1/Sv
129S1/SvW	0.63	0.64	0.56	0.60	0.58	0.64	0.41	0.42	0.68	0.00
AKR/W	0.68	0.55	0.61	0.57	0.50	0.59	0.71	0.70	0.00	
C57BL/6W	0.67	0.70	0.64	0.72	0.64	0.69	0.05	0.00		
C57BL/10W	0.67	0.69	0.64	0.71	0.63	0.68	0.00			
BALB/cW	0.57	0.44	0.60	0.58	0.54	0.00				
CBA/W	0.52	0.46	0.58	0.24	0.00					
C3H/W	0.51	0.45	0.57	0.00						
BN/aW	0.62	0.56	0.00							
A.CA/W	0.55	0.00								
DBA/2W	0.00									

of Hygiene (BALB/cW). BN/aW strain was obtained in 1961 in Poland. These strains are maintained for many generations – from 10 (DBA/2W and C57BL/6W) to 166 (BN/aW). The differences between strains may result from differences in their genetic background and mutations that could occur in genes responsible for haematopoiesis. Another source of variation, especially between closely related strains such as C57BL/6W and C57BL/10W, could be attributed to the history of their origin.

Earlier Wirth-Dzięciołowska and Gajewska [2002], evaluated the relationship between the strains of mice in question using microsatellite markers (Tab. 6). DBA/2W and C3H/W strains were found closely related. They, however, differed significantly in almost all analysed traits.

The C57BL/6W and C57BL/10W strains are often treated as one strain. Our earlier study based on microsatellite markers confirmed the existence of differences between these strains on chromosomes 2 and 13 reported by Slingsby *et al*. [1995], and revealed some new polymorphisms on chromosomes 4, 17 and 18 [Gajewska *et al.* 2002]. No significant differences in the analysed traits between mice of these two strains were identified, but C57Bl/10W strain tended to have higher RBC and PLT counts and a lower MCH value than the other one. Similar differences between C57Bl/6J and C57BL/10J were observed by Peters *et al.* [2002]. The environmental effect and genetic plasticity of the traits analysed were observed in DBA/2 strain, best illustrated by the differences between DBA/2W and DBA/2J strains, which diverged ten generations ago. The Oncology Centre mice have 2M/µl more RBC, 10% higher HCT, a greater value of MCV and 100K/µl less PLT than the mice from the Jackson Laboratory [Peters *et al*. 2002].

The BALB/c strains from the Oncology Centre and the Jackson Laboratory differ in the majority of traits analysed. The biggest differences were found in PLT count

(800 K/µl *vs*. 1250 K/µl) and HCT (53.7% *vs*. 42%). Some differences between the Oncology Centre and the Jackson Laboratory mice in HCT and MCV may result from the difference in the type of automated haematological analysers applied. Obviously, this can be indicated by the higher values of HCT and MCV in our mice as compared to the results reported by Peters *et al.* [2002].

To sum up, the differences in the analysed blood parameters shown among the inbred strains of mice bred in the specified, constant conditions allow and help the use of these animals in various research, especially in studies on genetic background of peripheral blood morphology indicators. The differences identified among strains support the argument for further studies, particularly aimed at identifying genes that determine haematopoiesis and analysing quantitative factors involved in these processes.

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Parametry krwinkowe krwi obwodowej myszy dwunastu szczepów wsobnych

S t r e s z c z e n i e

Badania morfologii krwi obwodowej przeprowadzono na myszach z 12 szczepów wsobnych (A.CA/W, AKR.W, BALB>cW, BN/aW, CBA/W, CBA-T6/W, C57BL/6W, C57BL/10W, DBA2/W, 129S1/SvW, i HLB219/J). Oznaczono następujące parametry krwi 239 czteromiesięcznych myszy obu płci: ogólną liczbę leukocytów (WBC), liczbę erytrocytów (RBC), koncentrację hemoglobiny (HGB), hematokryt (HCT), średnią objętość erytrocytu (MCV), średnie stężenie hemoglobiny w krwince (MCHC), średnią masę hemoglobiny w krwince (MCH) oraz liczbę płytek krwi (PLT). Między myszami szczepów wsobnych stwierdzono wysokoistotne różnice w większości badanych parametrów krwi. W niektórych szczepach wykazano istotne różnice między płciami w liczbie płytek krwi i w jednostkowych przypadkach w obrębie innych analizowanych cech.