ORIGINAL ARTICLE

Peromyscus leucopus mice: a potential animal model for haematological studies

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SUMMARY

Peromyscus leucopus mice share physical similarities with laboratory mice *Mus musculus* (MM) but have higher agility and longer lifespan. We compared domesticated *P. leucopus linville* (PLL) and *M. musculus* C57BL/6 (MMB6) mice for cellular composition of peripheral blood (PB), bone marrow (BM) and spleen. PLL mice had significantly fewer platelets and significantly more monocytes in the blood, and notably fewer megakaryocytes in the BM. Spleens of PLL mice were significantly smaller, with 50% fewer cells and reduced 'red pulp'. There was no obvious haematological change in PLL mice between 2–8 and 16–26 months of age, except for a significant increase in blood monocytes. Cellular reactive oxygen species (ROS) content showed no change with age but differed significantly between different cell types. Treating two to eight month-old PLL mice with antioxidant N-acetylcysteine in drinking water for three months did not affect cellular ROS content, but increased blood leucocytes especially the concentration of monocytes. The low platelets, low megakaryocytes, high monocytes and low splenic erythropoiesis in PLL mice resemble human measurements better than the values seen in MMB6.

Peromyscus leucopus linville (PLL) is a mouse strain of the species *P. leucopus* commonly referred to as 'white-footed' mice. These animals share many physical features with the popular laboratory mice *Mus musculus* (MM) and are the most populous rodent species in the north-eastern United States (Joyner *et al.* 1998). *P. leucopus* have been used for studies of social interactions and stress physiology (Southwick 1964), as well as for the investigation of photoperiodism and the action of melatonin on the regulation of hormone secretion and reproductive physiology (Glass & Lynch 1982; Glass & Knotts 1987; Glass & Dolan 1988; Weaver *et al.* 1990). These animals came to public attention as the primary reservoir host for *Borrelia burgdorferi sensu stricto*, the aetiological agent of lyme disease (Magnarelli *et al.* 1988, 1994; Hofmeister & Childs 1995; Hofmeister *et al.* 1999), and they may also be hosts for species of hantavirus (Hjelle *et al.* 1995; Lyubsky *et al.* 1996; Morzunov *et al.* 1998). More recent studies have focused on

behavioural and reproductive physiology of *P. leucopus* mice using different breeding practices under long-term captivity, with data showing that adaptation can be rapid, affecting reproductive patterns and behaviour, even under breeding protocols designed to minimize the rate of genetic change due to random drift and inadvertent selection (Malo *et al.* 2010; Lacy *et al.* 2013).

Peromyscus leucopus have long lifespans that distinguish them from MM mice and from sister species within the genus *Peromyscus* (Sacher & Hart 1978; Steger *et al.* 1980; Cohen *et al.* 1987; Burger & Gochfeld 1992). *Peromyscus* show extended reproductive lifespan (Steger *et al.* 1980; Burger & Gochfeld 1992), accelerated wound repair (Cohen *et al.* 1987), reduced cellular reactive oxygen species (ROS) (Labinskyy *et al.* 2009; Shi *et al.* 2013) and increased vascular resistance to oxidative stress and inflammatory damages (Labinskyy *et al.* 2009). Despite their broad utilization in biomedical research, *P. leucopus* mice have not been well

characterized. For example, haematological parameters are not fully available, because previous studies only provided partial measurements: one study reported haematocrit and total and differential WBC counts in a group of young *P. leucopus* mice, and another immunological and haematological values in pups born to normal dams or dams exposed to aroclor 1254 (Wu *et al.* 1999a,b). In the current work, we performed haematological measurements in PLL mice with reference to MM mice using C57BL/6 (B6) as representatives. We also attempted to test the effects of ageing and antioxidant N-acetylcysteine (NAC) treatment on haematological changes in *P. leucopus* animals. Here, we report that *P. leucopus* have lower platelet counts, fewer megakaryocytes and more monocytes than do MMB6 mice, with values closely resembling those of humans, suggesting that *P. leucopus* mice could potentially be a good model of some aspects of human haematology.

Materials and methods

Animals

Peromyscus leucopus linville mice were obtained from the Peromyscus Genetic Stock Center (PGSC) at the University of South Carolina (Columbia, SC, USA), after 28 years of domestication and restricted free mating (to avoid sister–brother mating). PLL mice at 16–26 months of age were obtained from PGSC directly, while 2- to 8-month-old PLL mice were produced at NIH from young breeders (sister–brother mating avoided). Normal C57BL/6 (MMB6) mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and were bred and maintained in NIH animal facilities with standard care and nutrition.

Ethical approval

All animal studies were approved by the Animal Care and Use Committee at the National Heart, Lung and Blood Institute.

Complete blood counts and cellular analyses

Blood was collected from the retro-orbital sinus into EDTA-added Eppendorf tubes, and complete blood counts (CBCs) were performed using a HemaVet 950 analyzer (Drew Scientific, Inc., Waterbury, CT, USA). Mice were euthanized by CO_2 inhalation from a compressed source. Bone marrow (BM) cells were extracted from bilateral tibiae and femurs. The spleen of each animal was homogenized to obtain a single cell suspension. BM and spleen cells were filtered through 95-μM nylon mesh and counted using a Vi-Cell counter (Coulter Cooperation, Miami, FL, USA).

Flow cytometry

Blood, BM and spleen cells were first incubated with ACK solution twice for 10 minutes to lyse red blood cells (RBCs). Nucleated cells were stained with antibody mixtures on ice

for 30 min. Anti-human CD3, CD4, CD8, CD14 and CD19 antibodies were all from BD Biosciences (San Diego, CA, USA). Anti-mouse CD3 (clone 145-2C11), CD4 (clone H129.19), CD8 (clone 53-6.7), CD41 (clone MW Reg30), CD44 (clone 1M7), CD45R (clone RA3-6B2), CD61 (clone 2C9.G2), CD62L (clone MEL-14), CD95 (clone Jo2), Gr1 (clone RB6-8C5) and Sca-1 (clone D7) antibodies were also from BD Biosciences. Anti-mouse CD11b (clone M1/70), CD34 (cloneMEC14.7) and CD117 (clone 2B8) antibodies were from Biolegend (San Diego, CA, USA). Anti-mouse CD25 (clone PC61.5) and CD150 (clone 9D1) antibodies were from eBiosciences (San Diego, CA, USA). Each antibody was conjugated to either fluorescein isothiocyanate, phycoerythrin (PE), PE-cyanin 5 (PE-Cy5), PE-cyanin 7 (PE-Cy7), allophycocyanin (APC) or allophycocyanin 7 (APC-Cy7). For the detection of cellular ROS, blood and BM cells were first stained with specific antibodies on ice for 30 min and then incubated with 20 μM fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCF-DA; Invitrogen Corporation, Camarillo, CA, USA) at 37 °C for 30 min.

Stained cells were acquired and analysed on a LSR II or Canto II flow cytometer using the FACSDiva software (Becton Dickson, San Diego, CA, USA). We gated cells to exclude residual RBCs and debris based on FSC-A/SSC-A display.

Cytology and histology

Blood smears were prepared from venous blood and were stained with Wright staining solution (Electron Microscopy Sciences, Hatfield, PA, USA) according to the manufacturer's instructions. Spleen and sternums were preserved in 10% formalin, sectioned and Hematoxylin & eosin stained (Vitrovivo Biotechnology, Rockville, MD, USA). Slides from blood smears and spleen/sternum sections were examined under a Zeiss Axioskop2 plus microscope with images captured at 20 × by a Zeiss AxioCam HRC camera using the AxioVision 4.5 software (Carl Zeiss MicroImaging GmbH, Jena, Germany). For sternum samples, we counted the number of megakaryocytes–megakaryoblasts per field of view under 20× microscopic magnification based on their 'mega cell' characteristic. Average megakaryocytes–megakaryoblasts was calculated for each sample based on examination of three separate view fields.

N-acetylcysteine treatment

Chemical grade NAC (Sigma-Aldrich, St. Louis, MO, USA) of 16.3 g was dissolved in 2.5 l of de-ionized water to obtain 40 mM NAC water solution, adjusted to pH 3.0–4.0, filtered through 0.22 μM and then given to PLL mice as drinking water for 3 months. Control PLL mice were provided with normal water without NAC. Animals were bled at 2 and 3 months for CBC and flow cytometry analyses. BM cells were extracted after animal euthanasia at the third month and were analysed by flow cytometry to measure cellular ROS contents as described above.

Data analysis

Data were analysed by JMP statistical discovery software (SAS Institute Inc. 1998) through variance analysis and were shown as means with standard errors. Respective p values were given to indicate statistical significance ($P < 0.05$ being regarded as statistically significant).

Results

We first measured blood counts of two to eight month-old MMB6 and PLL mice. Haematologic parameters were relatively stable within the age range of mature young adult mice, and the majority of measurements were similar between the two rodent species, with modest levels of individual variation (Table 1). The most notable difference was a significantly lower ($P < 0.0001$) platelet count in PLL mice, <50% of that in MMB6 mice (Figure 1a). Platelets were also significantly smaller in PLL mice, with a 19% reduction ($P < 0.0001$) in mean platelet volume (MPV) relative to the size of MMB6 platelets (Figure 1a). Another notable difference was a 15% higher concentration ($P < 0.0001$) of circulating RBCs in PLL mice (Figure 1b). This RBC increase, however, was associated with a 13% reduction ($P < 0.0001$) in mean corpuscular volume (MCV)

Table 1 CBC in MMB6 and PLL mice

Measurements	*M. musculus B6*		*P. leucopus linville*	
	Females	Males	Females	Males
n	32	27	45	29
WBCs ($\times 10^9$/l)	7.6 ± 0.5	9.9 ± 0.5	8.8 ± 0.4	8.2 ± 0.5
Neutrophils ($\times 10^9$/l)	1.4 ± 0.1	1.8 ± 0.2	1.3 ± 0.1	1.2 ± 0.1
Lymphocytes ($\times 10^9$/l)	5.9 ± 0.4	7.9 ± 0.4	7.0 ± 0.3	6.5 ± 0.4
Monocytes ($\times 10^9$/l)	0.24 ± 0.03	0.19 ± 0.03	0.38 ± 0.02**	0.38 ± 0.03**
RBCs ($\times 10^{12}$/l)	9.9 ± 0.3	9.7 ± 0.3	11.2 ± 0.3**	11.4 ± 0.3**
Haemoglobin (g/dl)	13.8 ± 0.4	13.1 ± 0.4	13.0 ± 0.3	13.2 ± 0.4
Haematocrit (%)	43.4 ± 1.3	42.3 ± 1.4	41.1 ± 1.1	43.0 ± 1.4
MCV (fL)	44.0 ± 0.5	43.4 ± 0.6	36.7 ± 0.4**	37.8 ± 0.5**
MCH (pg)	14.0 ± 0.1	13.5 ± 0.2	11.6 ± 0.1**	11.6 ± 0.2**
RDW (%)	18.4 ± 0.2	18.4 ± 0.2	18.4 ± 0.2	17.6 ± 0.2
Platelets ($\times 10^9$/l)	905 ± 40	827 ± 43	400 ± 33**	421 ± 42**
MPV (fl)	4.44 ± 0.04	4.36 ± 0.05	3.56 ± 0.04**	3.54 ± 0.05**

CBC, Complete blood count; MCH, Mean corpuscular haemoglobin; MPV, mean platelet volume; PLL, *Peromyscus leucopus linville*; RDW, RBC distribution width.
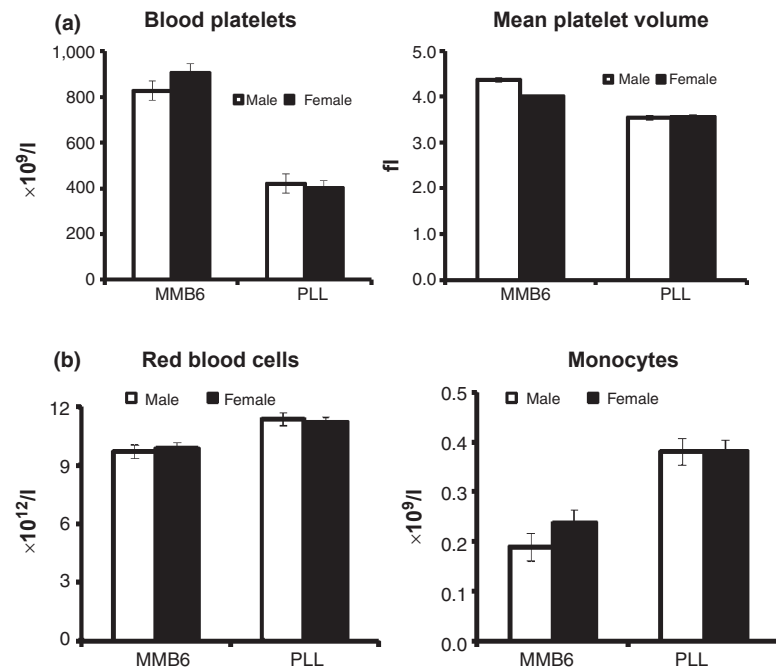**Strain difference $P < 0.01$.

(a) Blood platelets
Mean platelet volume
(b) Red blood cells
Monocytes

Figure 1 Cytological measurements in MMB6 and PLL mice. Orbital sinus blood from MMB6 female ($n = 32$), MMB6 male ($n = 27$), PLL female ($n = 42$) and PLL male ($n = 29$) mice was analysed at 2–8 months of age. Most blood measurements were relatively similar between MMB6 and PLL mice, as listed in Table 1. A major difference was a significantly lower ($P < 0.0001$) platelet concentration in PLL mice which is less than half of that in MMB6 mice (a). PLL mean platelet volume was also 19% smaller ($P < 0.0001$) than that of MMB6 platelets (a). There were significantly higher concentrations of red blood cells ($P < 0.0001$) and monocytes ($P < 0.0007$) in PLL mice relative to MMB6 animals (b). PLL, *Peromyscus leucopus linville*.

and a 16% reduction ($P < 0.0001$) in mean corpuscular haemoglobin (MCH) concentration (Table 1). A third difference was a significantly higher concentration of circulating monocytes ($P < 0.0001$) in PLL mice, a 79% increase compared to monocyte concentration in MMB6 animals (Figure 1b).

Microscopic examination of blood smears from two to eight month-old MMB6 and PLL mice revealed smaller RBCs in PLL than in MMB6 mice (Figure 2a), consistent with a 13% decline in MCV in PLL animals (Table 1). Spleens were significantly smaller in PLL mice, with an aver-

age $94 \pm 13 \times 10^6$ cells/spleen, less than half ($P < 0.0001$) of the $195 \pm 12 \times 10^6$ cells/spleen in MMB6 mice. Histologically, spleen sections from PLL mice had reduced red pulp areas in comparison with those of MMB6 mice (Figure 2b). Overall BM cellularity was similar between MMB6 and PLL mice; however, more megakaryocytes–megakaryoblasts were found in sections from MMB6 than from PLL mice (Figure 2c). The number of megakaryocytes–megakaryoblasts per view field was significantly higher ($P < 0.0027$) in MMB6 (16 ± 1) than in PLL (11 ± 1) BM (Figure 2d).
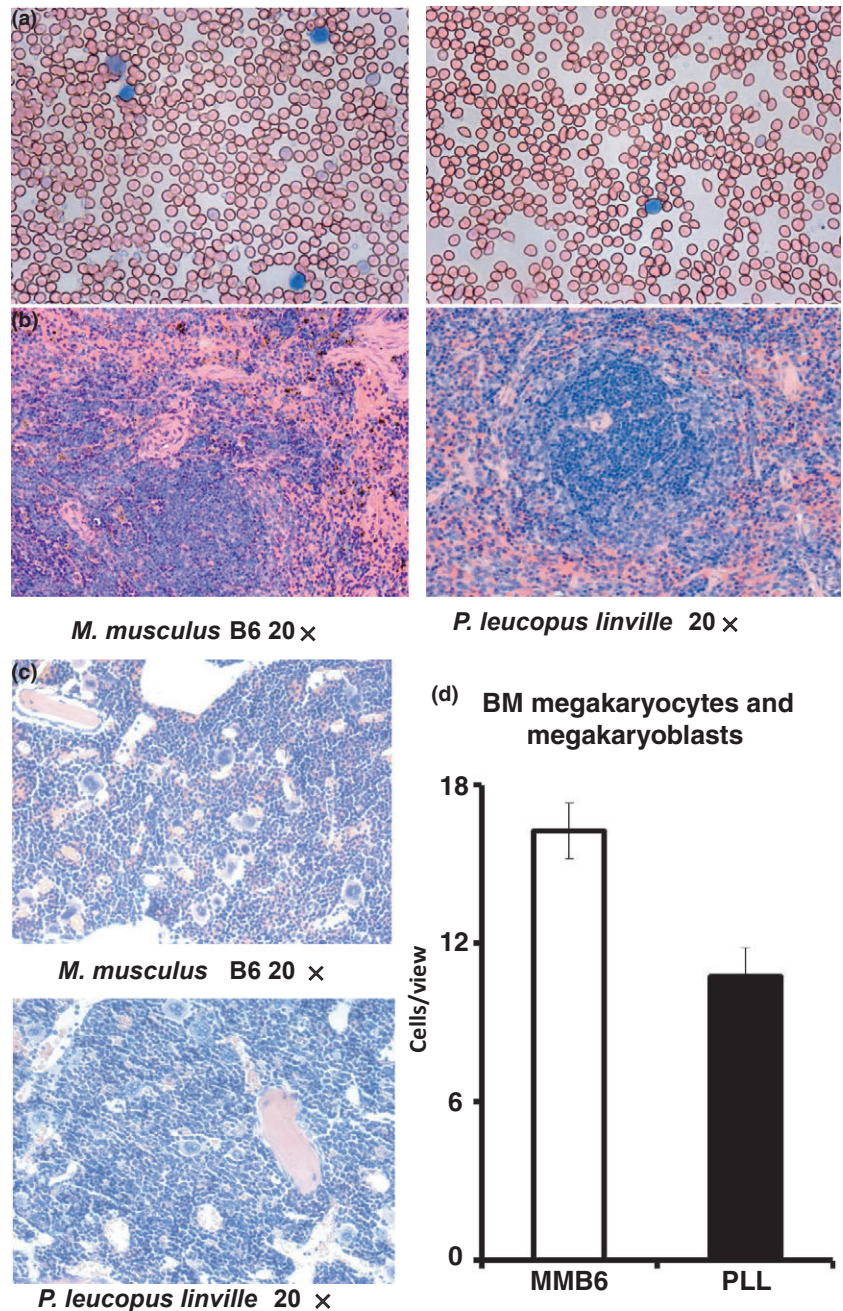

Figure 2 Histological comparisons between MMB6 and PLL mice. Blood smears prepared from young MMB6 ($n = 8$) and PLL ($n = 8$) mice were stained with Wright solution and examined under a Zeiss Axioskop2 plus microscope. Red blood cells from PLL mice were smaller than RBCs from MMB6 mice (a). Sections of spleens from PLL mice had much smaller red pulp areas of active erythropoiesis than those from MMB6 mice (b). sternum sections from PLL mice contained fewer megakaryocytes–megakaryoblasts relative to those from MMB6 mice (c). The number of megakaryocytes–megakaryoblasts per view field under $20\times$ magnification was significantly lower in PLL ($P < 0.0027$) than in MMB6 mice when eight animals from each strain were examined (d). PLL, *Peromyscus leucopus linville*.

We attempted to define cellular differences between PLL and MMB6 mice in blood, BM and spleen by flow cytometry. No antibody specific for a *Peromyscus* antigen is commercially available, and we therefore tested an array of antibodies specific for human and MM antigens, with largely negative results. All five antibodies specific for human antigens we have tested showed no staining when incubated with PLL blood cells (Figure S1A). Ten antibodies specific for MM antigens showed very inconsistent staining patterns when incubated with peripheral blood leucocytes (Figure S1B, 1C). Thus, utilization of commercially available anti-mouse antibodies for the detection of specific cell populations in *P. leucopus* animals was not feasible.

Of the many anti-mouse antibodies we tested, we found that anti-mouse CD44 and anti-mouse CD45R antibodies produced consistent staining of distinctive cell populations in PLL mice. We used both antibodies to stain blood cells from two to eight month-old MMB6 and PLL mice in conjunction with the staining of DCF-DA to detect cellular ROS contents. Proportions of CD44 and CD45R cells were very similar between MMB6 and PLL mice, showing no significant difference (Figure 3a, Table 2). There were two populations of CD44-expressing cells in MMB6 mice:
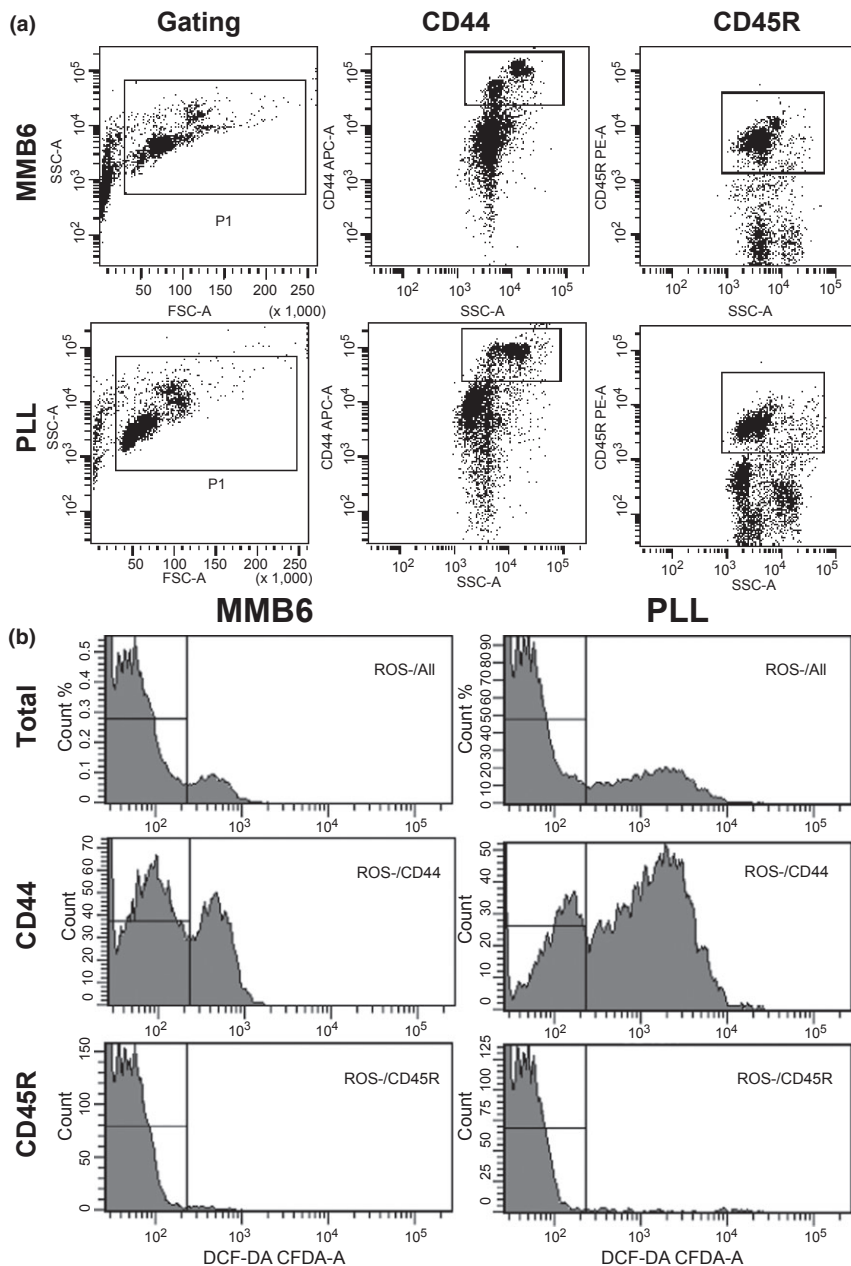

Figure 3 Cellular ROS in MMB6 and PLL mice. Blood cells from 2- to 8-month-old MMB6 ($n = 12$) and PLL ($n = 8$) mice were stained with anti-mouse CD44 and anti-mouse CD45R antibodies followed by incubation of samples with 20 μM DCF-DA at 37 °C for 30 min. PLL samples showed consistent staining of both antibodies, and no significant difference was detected between MMB6 and PLL mice in their blood CD44 and CD45R cell percentages (a). The proportion of DCF-DA$^-$ cells, reflecting the level of ROS$^-$ cells, was significantly lower ($P < 0.0044$) in PLL than in MMB6 mice, with the difference being greatest for CD44$^+$ ($P < 0.0001$) cells and less obvious for CD45R$^+$ ($P < 0.0327$) cells (b). In both MMB6 and PLL mice, the overall ROS$^-$ cell proportion was much higher in CD45R$^+$ than in CD44$^+$ cells (b). PLL, *Peromyscus leucopus linville*; ROS, reactive oxygen species.

Table 2 Cellular ROS in blood leucocytes of MMB6 and PLL mice

Animal type	MMB6	PLL	Statistics
n	12	8	
CD44 (%)	22.8 ± 2.1	21.9 ± 2.5	*P* < 0.8025
ROS⁻%CD44	66.3 ± 2.4	33.9 ± 2.9	*P* < 0.0001
CD45R (%)	52.2 ± 2.2	48.3 ± 2.7	*P* < 0.2816
ROS⁻%CD45R	98.9 ± 0.3	98.0 ± 0.3	*P* < 0.0327
ROS⁻%	91.5 ± 1.3	84.6 ± 1.6	*P* < 0.0044

ROS, Reactive oxygen species; PLL, *Peromyscus leucopus linville*.

CD44^{high} population comprising CD11b$^+$ cells, and CD44 ^{median} population made of CD4 and CD8 T cells and some CD11b+ cells. The proportion of ROS⁻ cells in blood leucocytes was 85 ± 2% for PLL mice, significantly lower (*P* < 0.0044) than that for MMB6 mice at 92 ± 1% (Figure 3b, Table 2). This strain difference was most obvious in CD44 cells: only 34 ± 3% CD44 cells from PLL mice were ROS⁻, while 66 ± 2% CD44 cells from MMB6 mice were ROS⁻ (Figure 3b, Table 2). The ROS⁻ cell proportion was extremely high, at 97–99%, in blood CD45R+ cells from both MMB6 and PLL mice (Figure 3b, Table 2).

Next, we stained peripheral blood leucocytes from two to eight and 16- to 26-month-old PLL mice using anti-mouse CD44, anti-mouse CD45R antibodies and DCF-DA dye. The proportions of CD44$^+$ (48%±3.6% and 47%±3.6%) and CD45R$^+$ (47%±3.2% and 44%±3.2%) cells did not differ between the two age groups. No age difference was detected on ROS⁻ proportion, but the proportion of ROS⁻ cells differed significantly between CD44$^+$ and CD45R$^+$ cells: 98–99% of CD45R$^+$ cells were ROS⁻, and only 53–58% of CD44$^+$ cells were ROS⁻ (Figure 4a). There was a 5% decline in MCV (*P* < 0.0446), a 28% increase in platelets (*P* < 0.0246) and a 45% increase in monocytes in 16- to 26-month-old PLL animals relative to the younger two to eight month-old counterparts (Table 3).

As the ROS⁻ varied in different cell types but showed no change with age in PLL mice, we wondered whether antiox-

Table 3 Age effects on CBC measurements in PLL mice

Age (months)	2–8	16–26	Statistics
n	74	12	
WBCs (×10⁹/l)	8.6 ± 0.4	7.9 ± 0.9	*P* < 0.4901
Neutrophils (×10⁹/l)	1.3 ± 0.1	1.5 ± 0.2	*P* < 0.3273
Lymphocytes (×10⁹/l)	6.8 ± 0.3	5.8 ± 0.7	*P* < 0.1973
Monocytes (×10⁹/l)	0.38 ± 0.02	0.55 ± 0.04	*P* < 0.0006
RBCs (×10¹²/l)	11.3 ± 0.2	10.9 ± 0.6	*P* < 0.5587
Haemoglobin (g/dl)	13.1 ± 0.3	12.0 ± 0.7	*P* < 0.1686
Haematocrit (%)	41.8 ± 1.0	38.6 ± 2.4	*P* < 0.2119
MCV (fL)	37.1 ± 0.3	35.2 ± 0.9	*P* < 0.0446
RDW (%)	18.1 ± 0.1	17.7 ± 0.4	*P* < 0.3559
Platelets (×10⁹/l)	409 ± 19	524 ± 47	*P* < 0.0246
MPV (fl)	3.56 ± 0.03	3.60 ± 0.07	*P* < 0.0122

CBC, Complete blood count; PLL, *Peromyscus leucopus linville*; MCV, mean corpuscular volume; MPV, mean platelet volume.

Table 4 NAC treatment effect on CBC measurements in PLL mice

Treatments	NAC	Control	Statistics
n	17	17	
WBCs (×10⁹/l)	6.7 ± 0.6	5.6 ± 0.6	*P* < 0.1645
Neutrophils (×10⁹/l)	0.8 ± 0.1	0.9 ± 0.1	*P* < 0.5456
Lymphocytes (×10⁹/l)	5.4 ± 0.5	4.3 ± 0.5	*P* < 0.1350
Monocytes (×10⁹/l)	0.53 ± 0.04	0.31 ± 0.04	*P* < 0.0007
RBCs (×10¹²/l)	11.3 ± 0.3	11.1 ± 0.3	*P* < 0.6701
Haemoglobin (g/dl)	13.2 ± 0.3	13.0 ± 0.3	*P* < 0.7358
Haematocrit (%)	43.2 ± 1.1	42.5 ± 1.1	*P* < 0.6125
MCV (fL)	38.4 ± 0.6	38.2 ± 0.6	*P* < 0.8574
RDW (%)	19.4 ± 0.3	18.7 ± 0.3	*P* < 0.1236
Platelets (×10⁹/l)	441 ± 35	381 ± 35	*P* < 0.2468
MPV (fL)	3.49 ± 0.04	3.49 ± 0.04	*P* < 0.9264

NAC, N-acetylcysteine; CBC, complete blood count; PLL, *Peromyscus leucopus linville*; MCV, Mean corpuscular volume; MPV, mean platelet volume; RDW, RBC distribution width.

idant treatment would atter cellular ROS contents. We thus provided two to eight month-old PLL mice with 40 mM NAC in drinking water for three months. NAC treatment did not alter ROS⁻ cell proportion in either peripheral blood (79 ± 1.6% *vs.* 78 ± 1.6%) or BM (52 ± 3.5% *vs.* 55 ± 3.5%) cells (Figure 4b). In CD45R$^+$ cells, ROS⁻ proportion differed significantly between blood (98 ± 0.3% and 98 ± 0.3%) and BM (48 ± 3.0% and 40 ± 3.0%) without significant NAC effect (Figure 4c). Analyses of CBC also found no NAC effect except that NAC-treated mice had a 71% increase (*P* < 0.0007) in blood monocytes (Table 4).

Discussion

The main finding from this study is a consistently lower concentration of circulating platelets in PLL mice in comparison with the more commonly used MMB6 mice. At 373–470 × 10⁹/l, platelet counts for PLL mice are closer to those of humans at 161–385 × 10⁹/l (Litchtman *et al.* 2010), much lower than the 780– 901 × 10⁹/l platelets for MMB6 mice based on our current study, or the 1006–1050 × 10⁹/l average platelet counts for 72 strains of MM animals reported (The Jackson Laboratory 2013). That average platelet size for PLL is significantly smaller than platelet size for MMB6 mice suggests that the lower platelet count in PLL mice is not compensated by larger platelets. Together with fewer megakaryocytes–megakaryoblasts in PLL BM, our observation suggests that PLL mice have a much more sensitive system than MMB6 mice in the regulation of platelet production as well as in the maintenance of haemostasis. Likely, genetic elements regulate the normal platelet range, as they do to CD8 and CD4 T-cell percentages (Chen & Harrison 2002) and the BM hematopoietic stem cell pool size (de Haan *et al.* 1997).

The much smaller spleens with significantly reduced erythropoietic centres are another feature of PLL mice
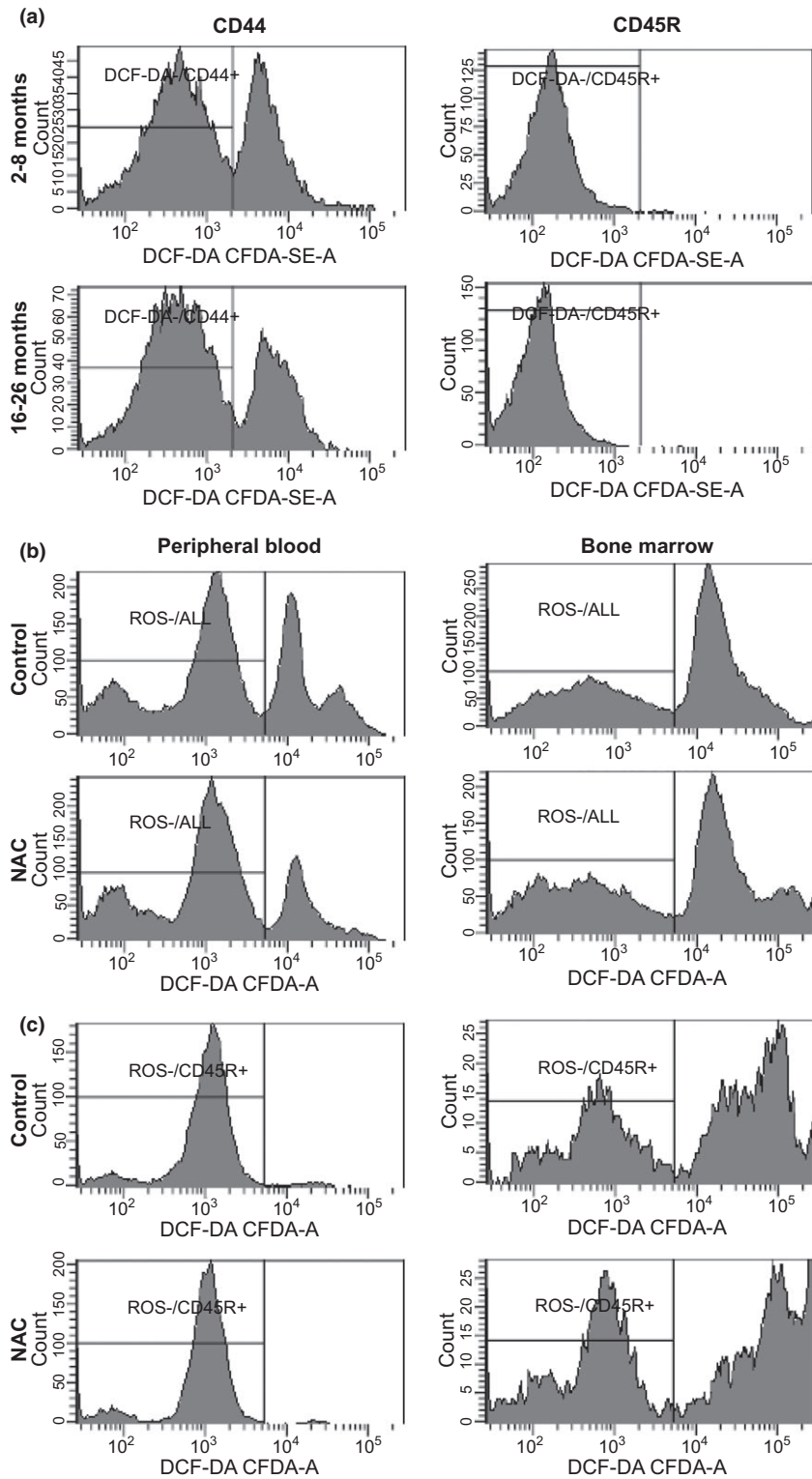
Figure 4 PLL cellular ROS unaffected by age or NAC treatment. We analysed the ROS⁻ cell proportion in blood CD44⁺ and CD45R⁺ cells in PLL mice at 2–8 ($n = 6$) and 16–26 ($n = 6$) months respectively. There was no age difference in the proportion of ROS⁻ cells (a). We treated PLL mice with 40 mm NAC water for 3 months and measured cellular ROS in blood and BM cells. ROS⁻ cell proportions in blood and BM cells showed no change in response to NAC treatment (b). The proportion of ROS⁻ cells was notably higher in blood CD45R⁺ cells than in BM CD45R⁺ cells without a significant effect of NAC treatment (c). PLL, *Peromyscus leucopus linville*; ROS, reactive oxygen species; NAC, n-acetylcysteine.

indicating that the spleen may play less of a role in erythropoiesis and hematopoiesis in PLL than in MMB6 mice. On the other hand, the 15% increase in RBC counts in PLL animals may be regarded as a compensatory response to the smaller RBCs with reduced haemoglobin content per cell.

The long lifespan of *P. leucopus* mice offers an opportunity to examine ageing over an extended time period (Sacher & Duffy 1978; Sacher & Hart 1978; Peluso *et al.* 1980; Cohen *et al.* 1987; Burger & Gochfeld 1992; Labinskyy *et al.* 2009) . In our study of PLL mice at two to

eight months and 16–26 months of age, no significant difference was found in blood cell counts, different from observations in MMB6 mice, in which increasing age was associated with declines in RBCs, haemoglobin and the relative proportion of lymphocytes (Leuenberger & Kunstyr 1976). Notably, the age group 16–26 months, which can be regarded as old for MMB6 mice, may not represent old age in PLL mice because of their long lifespan. Even at very old ages, *P. leucopus* mice had the ability to maintain normal function of their hypothalamic–pituitary–ovarian axis to maintain fertility (Steger *et al.* 1980). Old *P. leucopus* mice also had rapid wound healing ability, perhaps related to their stress-related hormone levels (Cohen *et al.* 1987). The increased lifespan in *P. leucopus* mice could also be associated with decreased cellular ROS generation and increased resistance to pro-oxidant and pro-inflammatory effects of metabolic stress (Labinskyy *et al.* 2009). We found lower proportion of ROS$^-$ blood leucocytes in young PLL than in young MMB6 mice. Our observation of no age effect on cellular ROS in blood and BM cells is consistent with early reports suggesting that *P. leucopus* mice are apparently resistant to oxidative damage. Differences in ROS levels obtained from different cell types within the same PLL animals in the current study may represent variations in metabolic activities of each cell type. For example, BM cells are metabolically more active with lower proportions of ROS$^-$ cells than are peripheral blood leucocytes, as was the case for both MMB6 and PLL mice.

The highest blood monocyte count in PLL mice resembles the monocyte concentration in humans (Litchtman *et al.* 2010). Also, monocytes further increased in 16- to 26-month-old PLL mice, as in humans (Seidler *et al.* 2010).

In conclusion, *P. leucopus* mice have circulating platelets, BM megakaryocytes and circulating monocytes that resemble measurements in humans. The reduced splenic hematopoiesis and the extended lifespan add to these characteristic, making *P. leucopus* mice a possible model for the study of human haematology.

Funding source

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Ineffective staining of PLL leucocytes with antibodies specific for human and *M. musculus* antigens.