

Basal and induced granulopoiesis in outbred, F₁ hybrid and inbred mice: can inbreeding depression influence the experimental practice?

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Abstract

In this study we examined differences in selected indices of granulopoiesis in outbred, F₁ hybrid and inbred mouse strains. Specifically, serum granulocyte colony-stimulating factor (G-CSF) levels, numbers of marrow granulocyte-macrophage progenitor cells and morphologically recognizable proliferative marrow granulocytic precursor cells were evaluated. These parameters were determined in untreated controls, and in mice exposed either to a non-specific stimulus (injection of saline) or to a granulopoiesis-enhancing stimulus (administration of a cyclooxygenase-2 inhibitor, meloxicam). Lower levels of G-CSF were detectable in the outbred ICR mice, which also demonstrated an enhanced response to both types of the stimuli. Considering the fact that outbred mice are closer to natural mammalian populations, including human ones, the possibility of using outbred mice, instead of the often used inbred strains, for experiments evaluating the effects of pharmacological interventions on hematopoiesis should be investigated.

Keywords: hematopoiesis, outbred mice, inbreeding depression

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Introduction

Deleterious effects of inbreeding on fitness in a wide spectrum of mammalian species, including humans, have been often reported (e.g. refs^{1–3}). Among the parameters of fitness discussed in the literature, those of blood and/or hematopoiesis seldom appear. Inbreeding depression in populations with high inbreeding levels was reported to be responsible for disorders such as immunocompetence in lemurs,⁴ abnormalities in blood leukocyte and lymphocyte counts in robins⁵ or in neutrophil dysfunction in rabbits.⁶ Only episodic information is available on the functional differences in hematopoiesis between various mouse strains differing in their breeding status; remarkable differences in serum levels of a number of cytokines including granulocyte colony-stimulating factor (G-CSF) between an outbred and an inbred mouse strain with experimentally induced sepsis were reported.⁷ Some basic peripheral blood parameters in various outbred and inbred mice were summarized in ref.⁸ However, we found no paper reporting the influence of inbreeding on cell proliferation and differentiation in the hematopoietic organs. In this

study, we compared the values of selected basic parameters of granulopoiesis among various inbred, outbred and F₁ hybrid mouse strains. Furthermore, we tested the ability of the hematopoietic system in selected inbred, outbred and F₁ strains of mouse to react to both non-specific stress (injection of saline) and specific stimulus (administration of a granulopoiesis-stimulating agent, meloxicam). Meloxicam is an inhibitor of cyclooxygenase-2 synthesis, which acts by inhibiting the production of prostaglandins that inhibit granulopoiesis.^{9,10} The positive influence of meloxicam on granulopoiesis was demonstrated previously.^{11,12}

Materials and methods

All mice used in the study, i.e. male outbred ICR and NMRI mice, inbred C57BL6, C57BL10, BALB/c and CBA mice, as well as hybrid (CBAxC57BL10)F₁ mice aged three months and weighing an average 30 g were obtained from the breeding facility of the Medical Faculty, Masaryk University, Brno, Czech Republic, and represent the whole spectrum of mouse strains produced by the facility. Mice

Table 1 Serum concentration of G-CSF in untreated controls of various mouse strains

Strain	G-CSF (pg/mL)
ICR (outbred)	72.7 ± 15.2
NMRI (outbred)	119.2 ± 24.5
(CBAx57BL10)F ₁ *	130.8 ± 49.6
C57BL6 (inbred)	253.2 ± 74.8
BALB (inbred)	478.5 ± 151.5
C57BL10 (inbred)	686.9 ± 64.9
CBA (inbred)	719.5 ± 65.0

G-CSF, granulocyte colony-stimulating factor

Five animals per group were used

*Value in (CBAx57BL10)F₁ mice was taken from ref.¹²

from a single source were used to exclude the possibility of false interstrain differences stemming from different breeder conditions. The use and treatment of the animals followed the European Community Guidelines as accepted principles for the use of experimental animals. All experiments were performed with the approval of the Institute's Ethical Committee.

Meloxicam (Sigma, St Louis, MO, USA) was dissolved in sterile saline and administered intraperitoneally at a dose of 20 mg/kg in a volume of 0.2 mL of saline. Another group of mice were treated with 0.2 mL of saline. Untreated animals were used as controls.

Concentrations of G-CSF in mouse serum were determined using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) in control animals or six hours after administration of meloxicam. This time interval was chosen on the basis of a previous study.¹² The numbers of nucleated cells of the femoral marrow were determined using a Coulter Counter (Model ZF, Coulter Electronics, Luton, UK). Granulocyte-macrophage colony-forming cells (GM-CFC) were assayed using a standard semi-solid plasma clot technique.¹² For evaluating the compartment of morphologically recognizable granulocytic precursor cells, differential counts were performed on marrow smears stained by the May Grünwald-Giemsa method. In the granulocytic lineage, myeloblasts through

myelocytes were designated as proliferative cells, and meta-myelocytes through segmented stages as non-proliferative cells.

Results are given as means ± standard error of mean. One-way analysis of variance and Tukey's *post hoc* test were used for statistical evaluations.

Results

Low values of serum G-CSF were found in the serum of outbred strains of mice (Table 1). Analysis of variance revealed statistical significance at the $P < 0.001$ level when the values were compared between the individual strains (seven groups), as well as between the group of aggregated outbred strains, that of aggregated inbred strains and that of the F₁ strain.

In one inbred, one outbred and one F₁ hybrid strain of mice, the response of granulopoiesis to a non-specific (stress) stimulus (intraperitoneal sterile saline) and a specific granulopoiesis-stimulating stimulus (intraperitoneal meloxicam) was studied (Table 2). The outbred ICR mice showed the best ability to respond to both types of stimuli. A single injection of saline increased serum levels of G-CSF seven-fold and a single injection of meloxicam increased the level 17-fold in comparison with the values found in untreated controls. In the inbred CBA mice, the magnitude of response was much lower, achieving an increase of only 1.45-fold in the meloxicam-treated animals, compared with untreated controls. In the F₁ hybrid mice, injection of saline increased serum levels of G-CSF 4.7-fold and injection of meloxicam 9.7-fold. Approximately similar differences between the compared strains of mice, even if of a lower magnitude, were found in the numbers of progenitor and precursor granulocytic cells in the femoral bone marrow. The GM-CFC counts in the outbred mice injected with saline or meloxicam increased 1.57- and 2.12-fold, respectively, when compared with values found in untreated controls. Inbred mice and F₁ hybrid mice exhibited only a 1.33- and 1.06-fold increase, respectively, after meloxicam

Table 2 Selected hematopoietic parameters in outbred, F₁ hybrid and inbred mouse strains after administration of saline or saline + meloxicam

Strain	Untreated controls	Saline	Saline + meloxicam
(a) Serum concentration of G-CSF (pg/mL) in untreated controls and six hours after a single injection of saline or saline + meloxicam			
ICR (outbred)	72.7 ± 15.2 [†]	511.9 ± 103.8**	1224.5 ± 63.0***,†‡
(CBAx57BL10)F ₁ †	130.8 ± 49.6	620.6 ± 53.1*	1266.9 ± 63.5***,‡
CBA (inbred)	719.5 ± 65.0	649.0 ± 321.7	1045.4 ± 118.0
(b) Numbers of GM-CFC per femur (×10 ³) in untreated controls and 48 h after a single injection of saline or saline + meloxicam			
ICR (outbred)	12.32 ± 1.71	19.36 ± 1.18*,††	26.15 ± 2.98***,††
(CBAx57BL10)F ₁	22.40 ± 1.28*††	21.47 ± 2.68*††	23.75 ± 1.66 [†]
CBA (inbred)	10.49 ± 0.97	10.79 ± 0.45	13.99 ± 0.49***,‡
(c) Numbers of proliferative granuloid cells per femur (×10 ³) in untreated controls and 48 h after a single injection of saline or saline + meloxicam			
ICR (outbred)	1.482 ± 202	2.500 ± 615	6.219 ± 727***,††,††
(CBAx57BL10)F ₁	1.073 ± 423	1.446 ± 224	4.116 ± 636***,‡,††
CBA (inbred)	1.397 ± 162	2.457 ± 378*	2.204 ± 466

G-CSF, granulocyte colony-stimulating factor; GM-CFC, granulocyte-macrophage colony-forming cells

Five animals per group were used

†Values of serum concentration of G-CSF in (CBAx57BL10)F₁ mice were taken from ref.¹²* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, respectively, versus untreated controls; † $P < 0.05$ and †† $P < 0.01$, respectively, versus saline-treated mice; ‡ $P < 0.001$ and†† $P < 0.05$, respectively, versus CBA mice

administration. Similarly, meloxicam treatment increased the proliferative granulocytic cells counts in the outbred mice 4.19-fold, in the hybrid mice 3.84-fold and in the inbred mice 1.57-fold. It is evident that G-CSF and cellular responses to meloxicam are more expressive in the outbred strain of mice and least expressed in the inbred mice. With the exception of the femoral values of GM-CFC, the F₁ hybrid mice exhibit an intermediate response to meloxicam, namely in values of serum G-CSF and femoral proliferative granulocytic precursor cells, when compared with those found in outbred and inbred mouse strains. This phenomenon seems to be comprehensible because F₁ mice can be considered the first step to obtaining of the outbreeding status.

In addition, it should be noted that these data do not show any relationship between the basal control values of G-CSF and GM-CFC numbers or proliferating cell counts. Assuming the causality between G-CSF levels and the cellular responses, the decisive regulatory inputs inducing cellular response seem to be the relative increases in G-CSF concentration, regardless of the absolute values reached after the stimulatory treatment.

Discussion

Interstrain differences in hematological parameters and functions in mice were frequently investigated. These studies used different inbred strains of mice and were aimed at solving questions of the genetical conditionality of hematopoietic functions.^{13–18} As shown in the Introduction, studies dealing with the functional hematopoietic effects of inbreeding and outbreeding are rare.⁷ From this point of view our data present novel findings and provide the first evidence for the effects of inbreeding on processes of cell proliferation in the compartments of granulopoietic progenitor and precursor cells in mice. They suggest that outbred strains of mice may have a better ability to generate granulocytic cells, representing an important step towards realization of a non-specific immune response. On the contrary, the data obtained from the inbred strains investigated imply a lower magnitude of response to stimulation in their granulopoietic systems. It can be hypothesized from these findings that the phenomenon of inbreeding depression, including a decreased fitness, is expressed in the granulopoietic system of these mice.

Many animal investigations, e.g. transplantation studies or studies on the immune response to specific antigens, must be performed on inbred strains. Nevertheless, our results suggest that, contrary to the present custom of using inbred strains of mice, many studies aiming to evaluate the outcomes of pharmacological interventions on hematopoietic tissues, which are often carried with inbred strains of mice,¹⁹ should consider the use of outbred mice, as they might represent a better model for such studies. Besides the more pronounced response of outbred mice to pharmacological stimuli, these animals are closer to the natural conditions, including those in human populations, in comparison with inbred strains. Studies using both inbred and

outbred strains in parallel might be helpful in making a decision as to whether this suggestion should be applied in experimental practice.

Author contributions: MH and MP designed the research and wrote the paper; LD carried out the statistical evaluations; JH, ZH and LW carried out the experiments.

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