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Age-dependent selection against hypoxanthine phosphoribosyl transferase-deficient cells in mouse haematopoiesis

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SUMMARY

The basis of a previously observed difference in the level of contribution of hypoxanthine phosphoribosyltransferase-deficient cells between the haematopoietic and non-haematopoietic tissues of chimaeric and heterozygous mice has been clarified by studying two populations of female mice that differ only in that one is heterozygous for a null allele at the *hprt* locus and the other is wild type at this locus. Both populations are heterozygous for an electrophoretic variant allele at the X-linked *Pgk-1* locus, so that X-chromosome inactivation generates cells expressing different isozymes of phosphoglycerate kinase which can be assayed to monitor cell selection. The results show that hypoxanthine phosphoribosyltransferase deficiency itself, rather than an effect of another X-linked gene, causes a reduced level of contribution to haematopoietic tissues. Further, the extent of the depletion increases significantly with age, and this effect is due to a progressive reduction in the level of contribution to haematopoietic tissues rather than to an increase in the level of contribution to non-haematopoietic tissues.

Key words: haematopoiesis, HPRT, Lesch-Nyhan syndrome

INTRODUCTION

In humans, genetic deficiency of the enzyme hypoxanthine phosphoribosyl transferase (HPRT; EC 2.4.2.8), which is encoded by a gene on the X chromosome, causes Lesch-Nyhan syndrome (reviewed by Stout and Caskey, 1989). Lesch-Nyhan patients show a characteristic group of behavioural abnormalities, including spastic cerebral palsy, choreoathetosis and compulsive selfmutilation. It has been proposed that at least some of these abnormalities result from a deficiency in the dopamine-mediated pathways of the basal ganglia of the brain, since 70-90% depletion of dopamine was seen in all three Lesch-Nyhan patients autopsied by Lloyd et al. (1981) and reduced dopamine metabolite levels have been reported in the cerebrospinal fluid of Lesch-Nyhan patients throughout life (Silverstein et al., 1983). As well as these behavioural abnormalities, effects on haematopoiesis are also present. In different studies, megaloblastic anaemia, macrocytic and megaloblastic changes, haemolytic anaemia and subnormal populations of B lymphocytes have been reported (reviewed by Stout and Caskey, 1989). In heterozygous female carriers, where X-chromosome inactivation produces a mixture of functionally $HPRT^+$ and $HPRT^-$ cells, there is strong selection against the $HPRT^-$ population in haematopoietic tissues, to the extent that this population is undetectable in adult lymphocytes and erythrocytes (Dancis et al., 1968; McDonald and Kelley, 1972). However, in three young female heterozygotes (7 to 17 years old), between 5 and 10% of lymphocytes were HPRT deficient, suggesting that the extent of selection is age dependent (Albertini and De Mars, 1974).

We and others have produced mice deficient in HPRT by breeding from germ-line chimaeras generated by blastocyst injection of HPRT-deficient embryonal stem cells (Hooper et al., 1987; Kuehn et al., 1987). These mice do not show the behavioural abnormalities of the human condition (Finger et al., 1988; Hooper, 1988). Trauma to the ears and flanks caused by overgrooming has been seen in some old HPRT-deficient mice, but it remains to be established whether this is due to the mutation (Williamson et al., 1992). There is a significant reduction in dopamine levels in the basal ganglia, but the extent of the reduction is much smaller than in human patients (Finger et al., 1988; Dunnett et al., 1989; Williamson et al., 1991). Recent work suggests that HPRT deficiency has less severe neurological and behavioural consequences in mice than in humans because mice are more reliant on adenine phosphoribosyl transferase (APRT) than HPRT for purine salvage (Wu and Melton, 1993).

We have previously detected a difference in the level of contribution of HPRT-deficient cells between haematopoietic and non-haematopoietic tissues of both chimaeric and heterozygous mice (Ansell et al., 1991). However, in neither case could we rigorously conclude that this was a consequence of the HPRT deficiency per se, since the $HPRT^-$ and $HPRT^+$ cells in the chimaeras were from different mouse strains and the heterozygotes were derived from a stock that resulted from crossing to outbred mice. The extent of this difference showed an increase with age, but this was of only borderline statistical significance and could have resulted either from a reduction in the level of contribution to haematopoietic tissues or from an increase in the level of contribution to non-haematopoietic tissues. To clarify these points, we have established the *hprt$^{b\text{-}m3}$* null mutation on an inbred background and we present here a comparison of heterozygotes bred from mutant animals with control animals bred from the corresponding wild-type strain. The results demonstrate that the depletion of the $HPRT^-$ cell population is indeed a consequence of HPRT deficiency, that it increases in extent significantly with age, and that the latter effect is mediated in haematopoietic rather than non-haematopoietic tissues.

MATERIALS AND METHODS

Mice

To establish the *hprt$^{b\text{-}m3}$* mutation on an inbred strain 129/Ola background, male chimaeras produced by blastocyst injection of the 129/Ola-derived diploid male embryonal stem cell line E14TG2a as previously described (Hooper et al., 1987) were mated to strain 129/Ola females and ES cell-derived progeny identified by coat colour and bred to homozygosity as monitored by tail blotting (Thompson et al., 1989). Doubly heterozygous *Pgk-1^a Hprt^b / Pgk-1^b hprt$^{b\text{-}m3}$* females were obtained by mating males from the above stock to females of strain CBA/Ca-*Pgk-1^a*, which are homozygous for the *Pgk-1^a* allele (Forrester and Ansell, 1985). Control *Pgk-1^a Hprt^b / Pgk-1^b Hprt^b* females were obtained by mating wild-type strain 129/Ola males to CBA/Ca-*Pgk-1^a* females.

Isozyme assays

Phosphoglycerate kinase isozyme assays were carried out as described by Ansell and Micklem (1986). Estimates of the percentage of PGK-1B in each tissue, standardised for variation in overall extent of mosaicism between mice, were determined by logistic regression analysis (McCullagh and Nelder, 1983). Data were fitted using the GLIM statistical package (Numerical Algorithms Group, Oxford) to a model $\ln\{x/(100\text{-}x)\} = A+B_M+C_T$, where x is the percentage of PGK-1B, A is a constant, B_M takes a different value for each mouse and C_T takes a different value for each tissue. The estimates presented in Fig. 1 are calculated using the mean value of B_M for each group of mice. Estimates of mean percentages for haematopoietic and non-haematopoietic tissue groups were obtained similarly, and the statistical significance of differences was assessed by computing an *F* statistic from the scaled deviances of a complete regression model and a reduced model in which the estimates were constrained to be identical. The quantities plotted in Fig. 2 were computed as follows for each mouse: HEM is the mean of the logit values of PGK-1B proportion in haematopoietic tissues; NON is the corresponding mean for non-haematopoietic tissues; DIF=HEM−NON. The statistical significance of the age dependence of these parameters was assessed by fitting a linear regression using the GLIM package and testing whether the slopes differed significantly from zero as described in Table 1.

Table 1. Statistical analysis of age dependence

	10^4×slope of regression line (month^{-1})		
	control	double heterozygote	10^4×difference in slopes (month^{-1})
HEM	−43±101	−371±105***	−328±146*
NON	−6±99	35±103	40±143
DIF	−37±94	−405±98***	−368±136**

Linear regression analysis was carried out on the points plotted in Fig. 2. Each value tabulated in the first two columns is estimate ± standard error of slope of regression line; in the third column, the difference between the first two columns ± its standard error. The statistical significance of the difference between each estimate and zero was determined by Student's *t*-test: *, $0.01<P<0.05$; **, $0.001<P<0.01$; ***, $P<0.001$; otherwise, not significant.

RESULTS

By establishing the *hprt$^{b\text{-}m3}$* null allele on an inbred strain 129/Ola background and then performing appropriate crosses, as described in Materials and Methods, we have been able to derive two populations of female mice, both heterozygous for an electrophoretic variant at the X-linked *Pgk-1* locus, which encodes the somatic cell isoform of phosphoglycerate kinase (EC 2.7.2.3). Members of each population are genetically identical and the populations differ only in that one is heterozygous for the *hprt$^{b\text{-}m3}$* allele and the other is wild type at this locus. In the double heterozygotes, cells in which the wild-type *Hprt^b* allele is inactivated also suffer inactivation of the linked *Pgk-1^a* allele, so that HPRT-deficient cells express PGK-1B and $HPRT^+$ cells express PGK-1A. Thus, as in our previous study (Ansell et al., 1991), selection against HPRT-deficient cells can be monitored by determining the proportions of the two PGK alleles. In the present study, however, it is possible to control for effects of other X-linked genes, by carrying out parallel assays on the control population which does not carry the *hprt* mutation.

At all ages studied between 3 weeks and 2 years, there was a highly significant deficiency in PGK-1B proportion in the haematopoietic tissues of doubly heterozygous mice compared with non-haematopoietic tissues (Fig. 1). The deficiency was seen in all tissues, but was least marked in tissues such as whole spleen and lymph nodes that have a relatively high stromal component. In the control mice, the difference between PGK-1B proportion in haematopoietic and non-haematopoietic tissues in some cases reached significance at the 5% level but was in all cases clearly much less than in the double heterozygotes. To examine the effect of the *hprt* mutation more rigorously, PGK-1B proportions were normalised by logistic transformation (Armitage and Berry, 1987) and mean values for haematopoietic and non-haematopoietic tissues were calculated for each mouse (parameters HEM and NON respectively). These parameters and their difference, DIF, are plotted in Fig. 2. Effects of the *hprt* mutation and of age were tested by carrying out linear regression analysis of the age dependence of these

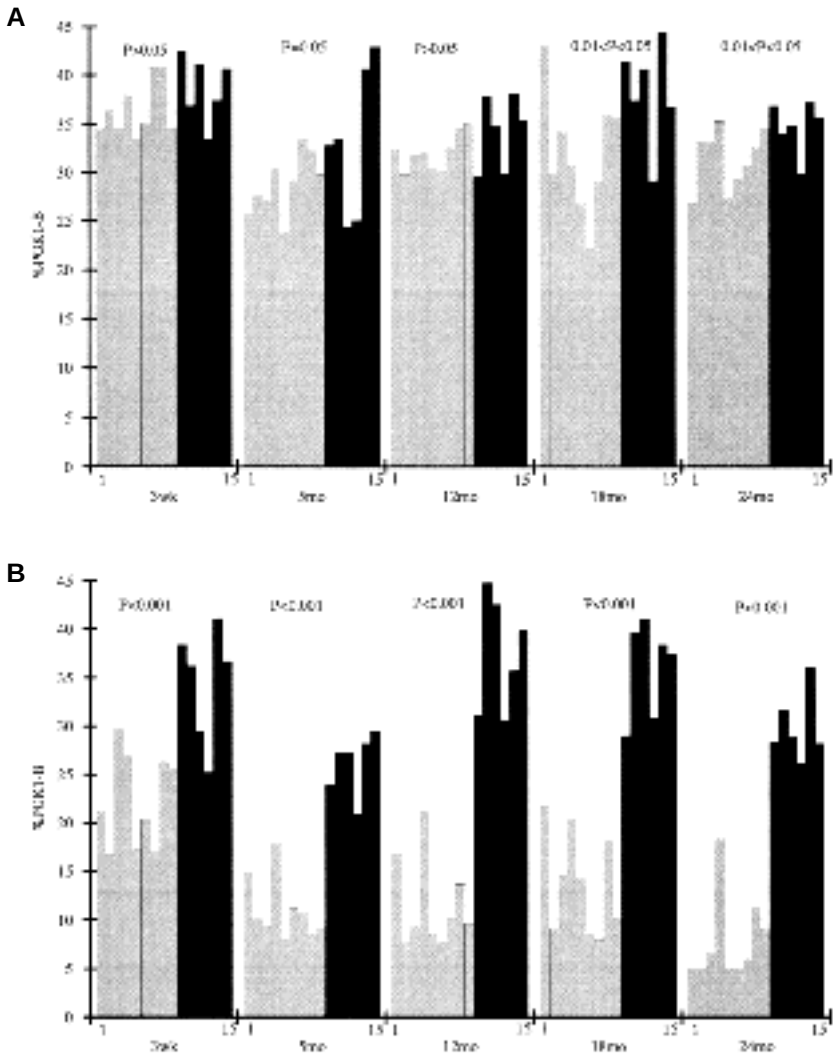

Fig. 1. Phosphoglycerate kinase isozyme analysis in female mice of genotype (A) *Pgk-1*^{a} *Hprt*^{b} / *Pgk-1*^{b} *Hprt*^{b} and (B) *Pgk-1*^{a} *Hprt*^{b} / *Pgk-1*^{b} *hprt*$^{b\text{-}m3}$. Each group of five panels represents data from mice of age (from left) 0.75, 5, 12, 18 and 24 months. Within each panel, estimates of the percentage of PGK-1B, determined from analysis of 5 mice as described in Materials and Methods, are plotted for tissues in the following order: 1, peritoneal exudate cells; 2, thymus; 3, spleen cell suspension; 4, whole spleen; 5, peripheral blood lymphocytes; 6, peripheral lymph nodes; 7, mesenteric lymph notes; 8, bone marrow; 9, blood; 10, skeletal muscle; 11, kidney; 12, liver; 13, lung; 14, brain; 15, heart. Bars 1 to 9, data for the haematopoietic tissues, are shown in grey. The *P* values given within each panel relate to a test of the null hypothesis that the mean proportion of PGK-1B in haematopoietic and non-haematopoietic tissues are equal. This test was carried out as described in Materials and Methods.

parameters for each genotype. As documented in Table 1, none of the slopes of the regression lines for control mice differed significantly from zero. However, the slopes of the regression lines for HEM and DIF for doubly heterozygous mice were significantly different both from zero and from the corresponding control slopes. In contrast, the slope of the regression line for NON in heterozygous mice did not differ significantly either from zero or from the control slope. It should be emphasized that the choice of a linear function for each regression analysis was made solely to enable a test to be made of the null hypothesis that the parameter was not age dependent. In the case of the data for HEM and DIF in the heterozygote, not only is this null hypothesis rejected, but visual inspection suggests that the age dependence may be non-linear. No physical significance should therefore be attached to the regression lines, or in particular to their extrapolation back to embryonic stages of development. We conclude that the depletion of the HPRT-deficient cell population occurring in the doubly heterozygous animals is a consequence of HPRT deficiency, that it increases in extent significantly with age, and that the latter effect is mediated in haematopoietic rather than non-haematopoietic tissues.

DISCUSSION

We demonstrate here that, in mice that are heterozygous for a null allele of *hprt* and therefore contain both HPRT$^+$ and HPRT$^-$ cell populations arising as a consequence of X-chromosome inactivation, there is depletion of the HPRT-deficient population in haematopoietic tissues as a direct consequence of their HPRT deficiency, and this depletion increases in extent significantly with age. No age-dependent selection is seen in non-haematopoietic tissues; nor, in a separate study, were we able to detect any significant age dependence of the ratio of forebrain dopamine levels in HPRT-deficient and wild-type strain 129/Ola mice aged between 2 and 20 months (D. J. Williamson, L. Dobbie, S. Butcher and M. L. Hooper, unpublished data).

If allowance is made for the presence of stromal cells in the tissues analysed, the extent of depletion of the HPRT-deficient cell population is similar in different haematopoietic tissues. This, together with the age dependence demonstrated here and the absence of any detectable depletion in 20-day embryos (Ansell et al., 1991), suggests that the depletion occurs because HPRT deficiency leads to a selective disadvantage in the division rate of pluripotent

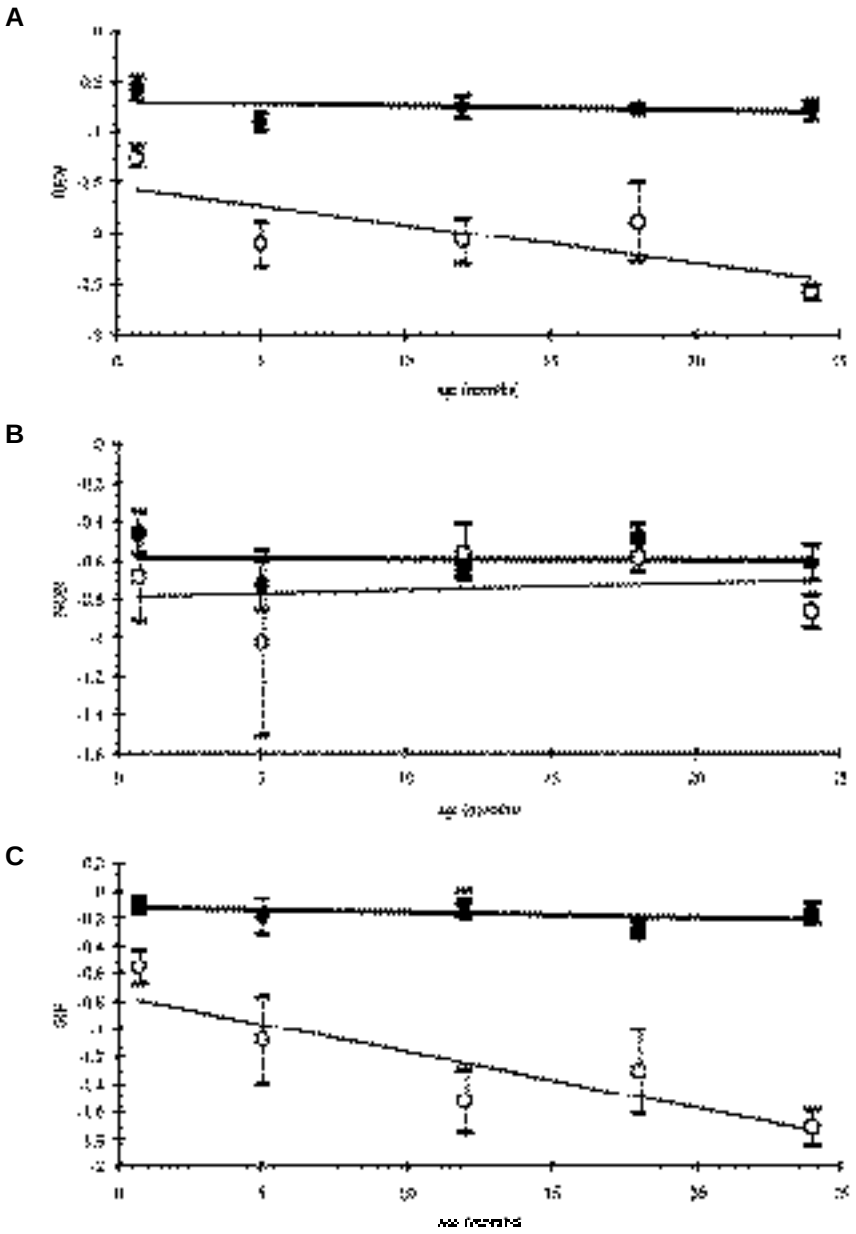

Fig. 2. Age dependence of the parameters (A) HEM, (B) NON and (C) DIF. Parameters for each mouse were calculated as described in Materials and Methods, and mean and s.e.m. values for each group of 5 mice are plotted. Lines plotted through the points are linear regression fits used to test the null hypothesis that the parameters are independent of age (see Table 1). Closed symbols and heavy lines, control *Pgk-1*^{a} *Hprt*^{b} / *Pgk-1*^{b} *Hprt*^{b} mice; open symbols and light lines, doubly heterozygous *Pgk-1*^{a} *Hprt*^{b} / *Pgk-1*^{b} *hprt*$^{b\text{-}m3}$ mice.

haematopoietic stem cells. McDonald and Kelley (1972) proposed such a mechanism to account for the absence of a detectable population of HPRT-deficient erythrocytes in adult human heterozygotes. Arguing from the demonstration by Lajtha and Vane (1958) that bone marrow preferentially uses salvage of purines synthesised in the liver, rather than de novo synthesis of purines for nucleic acid biosynthesis, they suggested that HPRT deficiency in haematopoietic stem cells reduces their level of guanine nucleotides and thereby causes a reduction in the rates of stem cell DNA synthesis and proliferation.

While the extent of depletion of HPRT-deficient cells at comparable stages of maturity is less in mice than in humans (cf. Dancis et al., 1968; McDonald and Kelley, 1972; Albertini and De Mars, 1974), the rate of depletion per unit time is comparable, if not greater, in the mouse. In the light of the mechanism proposed above, it would be of interest to compare the rates of depletion per haematopoietic stem cell generation in the two species. Unfortunately, estimation of the latter parameter is critically dependent upon the assumptions that are made about stem cell compartmentation (see, for instance, Micklem, 1986) so that estimates of sufficient accuracy to permit such a comparison are at present unavailable.

The non-haematopoietic tissues analysed here do not include any that undergo rapid turnover as does the haematopoietic system. Rapid turnover involving differentiation from a stem cell population occurs throughout life in tissues such as intestinal epithelium and epidermis (Potten and Loeffler, 1990). However, in the adult, unless there is substantial tissue loss as a result of injury, the progeny of individual stem cells colonise a spatially restricted region in these organised tissues. For example, each intestinal crypt is populated by descendants of stem cells clonally derived from a single precursor cell that has already undergone X-chromosome inactivation (Ponder et al., 1985). Populations

with different X chromosomes inactivated are therefore not in direct competition as they are in haematopoiesis. On the basis of the mechanism discussed above, therefore, we would not expect to find age-dependent selection against HPRT-deficient cells outside the haematopoietic system, at least in the absence of injury.

There have been two case reports describing treatment of Lesch-Nyhan patients by bone marrow allografts. Nyhan et al. (1986) treated a 22-year-old patient, resulting in normal HPRT activity in peripheral blood cells, but no change in neurological status or behaviour, possibly due to the advanced state of the disease. Endres et al. (1991) treated a 16-month-old patient already showing some signs of psychomotor retardation but, for reasons that were not clear, the child died 10 days after the operation. On the basis of the results reported here, we would predict that in such cases there should be a tendency towards selection, with time, for allograft-derived cells at the expense of host bone marrow-derived cells, although this effect may be masked in individual cases by effects of other differences between graft and host.

If the depletion of HPRT-deficient cells in the haematopoietic tissues of heterozygous mice is indeed due to selection operating during haematopoietic stem cell proliferation, it should be demonstrable in bone marrow cultures in vitro. If so, an analysis of the effects of concentrations of purines and purine antimetabolites in the growth medium would enable the model to be tested further.

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