GENETIC CONTROL OF THE HEPATOTOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN MICE

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ABSTRACT

Evidence is presented that, in strains of mice which are classified as *Ah* responsive, there are genetic loci, other than the *Ah* locus, which exert an overriding control on the induction of hepatic porphyria by 2378-TCDD in mice. These loci are not linked to the *Ah* locus.

INTRODUCTION

A major genetic element controlling the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) in various strains of mice is known to reside in the autosomal *Ah* locus. Mice can be classified as *Ah* responsive or non-responsive on the basis of the ability of 3-methylcholanthrene and analogues to induce aryl hydrocarbon hydroxylase, a cytochrome P-450IA1 mediated activity, in liver and other tissues[1]. The prototype inbred strains displaying variation at the *Ah* locus are: C57BL/6 – *Ah* responsive (Ah^b allele) and DBA/2 – *Ah* non-responsive (Ah^d allele)[1]. In crosses of these two strains the inheritance of the dominant Ah^b allele has been shown to determine whether a mouse is sensitive to various toxic effects of 2378-TCDD, *i.e.* thymic atrophy[2] or porphyria[3]. Similarly, the skin lesions induced by 3,3',4,4',5,5'-hexabromobiphenyl, a structural analogue of 2378-TCDD, are produced more easily in animals which carry the Ah^b allele as well as being homozygous at the hairless (*hr*) locus[4].

The inheritance of *Ah* responsive and *Ah* non-responsive traits is simple in crosses of the C57BL/6 and DBA/2 mouse strains. In crosses between other, similar, strains there is evidence of additional complexities[5]. Indeed, the toxicity of 2378-TCDD to the liver, assessed by the induction of an hepatic porphyria, has been shown to

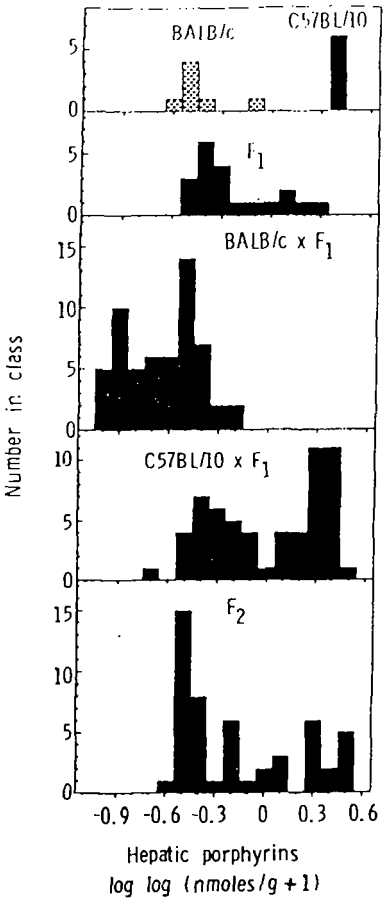

Figure Histograms representing the numbers of animals with hepatic porphyrin levels falling within a particular range of values. The generation is indicated on each histogram. All mice were females treated with 75 μg/kg of 2378-TCDD.

vary between different *Ah* non-responsive strains[6]. Crosses of the DBA/2 and AKR strains revealed that there was involvement of genes other than *Ah* in the determination of the degree of toxicity[6].

EXPERIMENTAL PROCEDURES

Groups of female mice of the *Ah* responsive BALB/c and C57BL/10 strains, the F_1 and F_2 hybrids and backcrosses derived from these strains, as well as female mice of the C57Bl/6By, BALB/cBy, CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ and CXBK recombinant inbred (RI) strains, were dosed with 2378-TCDD (75 or 150 μg/kg, p.o., dissolved in corn oil). The mice were kept in a negative-pressure plastic-film isolator for 5 weeks and were then killed with CO_2. Livers were removed and portions taken for histology and for analysis of porphyrin levels[6,7].

RESULTS and DISCUSSION

C57BL/10 mice of either sex respond to 2378-TCDD with the development of hepatic porphyria[7]. However, BALB/c male mice are far more resistant to 2378-TCDD and the BALB/c female mice show virtually no response[6]. Since there was such a clear-cut difference between female mice of the two inbred strains it was decided to investigate the nature of the genetic control of this strain difference. A cross of C57BL/10 and BALB/c mice produced F_1 hybrids, of which some became experimental animals whilst others were either interbred to produce an F_2 generation or were crossed to mice of the parental strains to produce the two backcross generations. Measurement of the liver levels of porphyrins was made after dosing with 75 μg/kg of 2378-TCDD. The hepatic porphyrin levels are extremely variable, as has been noted previously[6], and the values (v_n, nmoles/g liver) were each transformed logarithmically:

$$f_n = \log_{10}(\log_{10}(v_n + 1))$$

to render the distribution of the parental strains more homogeneous.

The transformed values were classified according to the range in which they fell and the numbers in each class for each cross are plotted as histograms (Figure).

The Figure shows the clear separation of the parental strains and, although the spread of the F_1 animals is wide, there is a preponderance of values falling in the range corresponding to the parental BALB/c values. All the mice (N = 57) of the backcross to the BALB/c strain fell in, or below, the range of the parental BALB/c mice (Figure).

The backcross to the C57BL/10 parent strain produced a histogram which was bimodal, with an antinode at a value equivalent to 9 nmoles porphyrins/g liver (Figure). The ratio of animals with levels of liver porphyrins which fell above and below the antinode was 31:28. This was consistent with the 1:1 ratio expected for the action of a single gene. The distribution of values in the F_2 generation was more ragged (Figure). The ratio, 17:33, of numbers falling above and below the same antinode was not significantly different from the 1:3 ratio expected of the single gene hypothesis.

Attempts to confirm the hypothesis of single gene action by applying either a likelihood analysis[8] or a non-parametric analysis[9] to the results indicated that there was, in fact, polygenic control of the action of 2378-TCDD in the livers of mice.

Confirmation of this finding was sought by an investigation of the action of 2378-TCDD in mice of the set of RI strains derived from C57BL/6 and BALB/c mice. These show an *Ah* locus allelism which is expressed as a structural variation of the *Ah* receptor protein – the 2378-TCDD binding protein[10].

Treatment of females of the RI strains with 2378-TCDD (150 μg/kg) revealed some striking differences in response. Both the parental strains developed porphyria, although to only a minor extent in the BALB/cBy strain. Only the CXBI strain showed a mean level of hepatic porphyrins greater than that of the BALB/cBy strain. Indeed, the CXBE and CXBK strains had porphyrin levels induced by 2378-TCDD which were lower than the control levels of many of the other strains. Neither of the two strains which carry the C57BL/6-derived Ah^{b-1} allele showed a greatly increased porphyrin content following 2378-TCDD treatment.

CONCLUSIONS

- Allelism at the *Ah* locus of the C57BL/6 (C57BL/10) and BALB/c *Ah* responsive mouse strains is not responsible for the differing hepatic sensitivity of the strains to 2378-TCDD.
- At least two genes control this strain difference.
- The major effect of the alleles of these loci present in the BALB/c strain is to reduce the sensitivity of the liver of the mice to the porphyrinogenic action of 2378-TCDD.

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