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Genetics of Colon Carcinogenesis in Mice Treated with 1,2-Dimethylhydrazine[1]

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SUMMARY

Genetic analysis of colon tumor induction by symmetrical 1,2-dimethylhydrazine (DMH) was undertaken in F_1, F_2, and reciprocal backcross hybrids derived from a cross between two inbred mouse strains, the 100% susceptible ICR/Ha and completely resistant C57BL/Ha. Mice, 12 to 14 weeks old, received 22 successive weekly s.c. injections of 0.35% aqueous solution of DMH buffered to pH 6.5. A dose of 15 mg/kg/mouse/week produced invasive colon adenocarcinomas in all ICR/Ha males and females (60 of 60) within 22 weeks. None of the 90 C57BL/Ha mice developed DMH tumors during 44 weeks of observation. Susceptibility to the carcinogen was dominant, as indicated by 100% colon tumor incidence in reciprocal ICR/Ha × C57BL/Ha F_1 hybrids (68 of 68) and in the susceptible backcross ICR/Ha × F_1 (42 of 42). Tumor yield in F_2 hybrids (94 of 120) was 78%, which is in close agreement with the 3:1 ratio expected if a single dominant DMH susceptibility gene is inherited via the F_1 from the ICR/Ha grandparent. Likewise, tumor yield in resistant backcross mice of genotype C57BL/Ha × F_1 (46 of 117) is not out of line with the anticipated 1:1 ratio in the latter type of test hybrids. Tests with five isozyme markers and two coat color genes have tentatively ruled out linkage of DMH susceptibility on seven autosomes. The 47% tumor incidence among 57 male resistant backcross hybrids, regardless of whether their single X chromosome was inherited from the ICR/Ha or C57BL/Ha strain, provides evidence against sex linkage.

INTRODUCTION

Large bowel tumors in rats and mice, induced by DMH,[2] constitute a histological analog of colon cancer in man. Because the human disease is significantly more frequent under the environmental impact of western civilization, animal research has focused on environmental carcinogens. Recent reports consider dietary factors, intestinal flora (9), bile salt metabolites, and other variables in this complex etiological problem.

Experiments along such lines paid little attention to the genetic makeup of the test animals used. However, the familial incidence of certain large bowel tumors in man that involve inheritance of a single dominant autosomal gene (6) warrants a genetic analysis of DMH carcinogenesis in suitable mice. Two inbred strains (one susceptible, the other resistant to DMH) were available for this study. Colon papillomas and adenocarcinomas developed within 22 weeks in 100% of ICR/Ha (hereafter called ICR) mice given s.c. injections of DMH. No tumors of any kind occurred in C57BL/Ha (hereafter called C57BL) mice, given identical treatment during 44 weeks of observation (2, 3).

MATERIALS AND METHODS

Test Hybrids. Reciprocal matings between susceptible ICR and resistant C57BL mice produced F_1 hybrids under standardized maintenance and dietary conditions. These F_1 hybrids were mated to males and females of both parent strains, yielding susceptible backcross and BCR hybrids. F_2 mice were derived from crosses between F_1 animals. Our mating scheme is shown in Table 1.

Treatment. At the start of DMH treatment, all mice were 12 to 14 weeks old and weighed from 23 to 27 g. Once a week, for 22 consecutive weeks, they received s.c. injections of 0.35% aqueous solution of DMH buffered with EDTA to pH 6.5. The DMH dose base was 15 mg/kg/mouse. The weekly dose averaged 0.37 mg DMH in 0.2 ml. Follow-up observation was up to 44 weeks, at which time the surviving tumor-free mice were 14 months old.

Pathology. All mice were sacrificed after adequate follow-up periods. Complete autopsies were performed. Tumors of the colon were graded for frequency on a 1+ to 4+ scale (Fig. 1). They were then examined in histological sections (2) cut from formalin-fixed specimens stained with hematoxylin and eosin to determine polyposis, invasiveness, and other features of the DMH-induced bowel lesions.

Linkage Studies. ICR and C57BL mice have electrophoretically distinct isozyme alleles for the enzyme loci *Dip-1*, *Gpd-1*, *Es-1*, *Mod-1*, and *Es-3* mapped on Chromosomes 1, 4, 8, 9, and 11, respectively. Isozyme mobility was determined in homogenates of individual kidneys removed from 9 BCR hybrid mice with DMH-induced colon tumors. Starch gel electrophoresis and specific histochemical staining (10) differentiate between backcross mice homozygous for the C57BL isozyme and heterozygotes that carry both the ICR and C57BL allele of the same enzyme. Coincidence of colon tumors with 1 of the ICR-derived enzyme markers would

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[2] The abbreviations used are: DMH, 1,2-dimethyl-hydrazine; BCR, resistant backcross.

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Table 1

Matings and hybrid mice produced for testing the genetic segregation of susceptibility to DMH-induced colon cancer

Cross	Test hybrids
ICR × C57BL	F_1 type I
C57BL × ICR	F_1 type II
F_1 I or II (F or M) × F_1 I or II (F or M)	F_2
F_1 I or II (F or M) × ICR (F or M)	BCS[a]
F_1 I or II (F or M) × C57BL (F or M)	BCR

[a] Susceptible backcross.

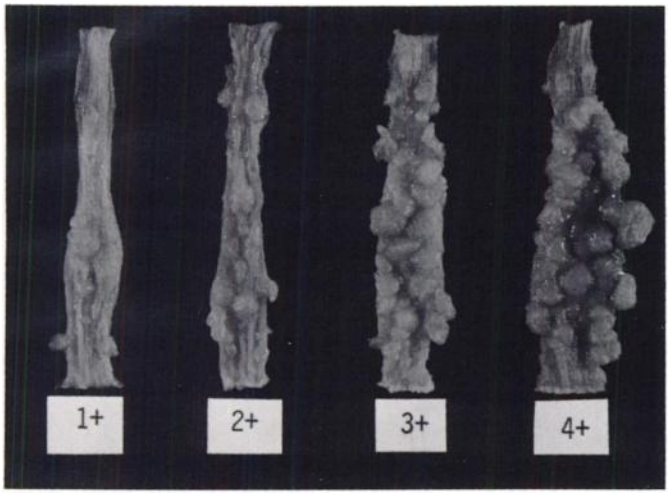

Fig. 1. Whole mounts of the distal 5-cm interior of large bowels from 4 DMH-treated F_2 hybrid mice of genotype ICR/Ha × C57BL/Ha F_1 female × F_1 male. The 1+ to 4+ scoring system is useful for classifying the extent of colorectal carcinogenesis in susceptible mice.

indicate linkage of DMH susceptibility on the corresponding chromosome.

In 2 coat color markers, *albino* and *agouti*, the ICR strain (c/c,A/A) differs from C57BL strain (C/C,a/a) on Chromosomes 7 and 2. These color phenotypes segregate in the F_2 and BCR hybrids permitting correlation with tumor incidence. Sex linkage of DMH response was evaluated in reciprocal BCR males that carried either the ICR or the C57BL X chromosome.

RESULTS

Genetics of Colon Tumor Susceptibility. The percentages of colon tumors developing in the parental and hybrid mice are summarized in Table 2. The 100% tumor incidence in the large bowels of F_1 and susceptible backcross hybrids indicates dominance of the ICR-derived susceptibility to DMH carcinogenesis. Findings in the F_2 and BCR are mutually supportive and in agreement with the respective 75% and 50% tumor incidences expected if a single dominant susceptibility gene is inherited from the ICR grandparent. The low 32% tumor incidence in BCR females (Table 2, Line 8) may be due to an age effect, which is not apparent in males (8).

Colorectal Lesion Patterns. Against the background of the entire ICR genome, this locus appears to maximize DMH metabolism to an active carcinogenic metabolite, promoting the 4+ and 3+ tumor yields shown in Fig. 1. The homogenous F_1 genome contains an equal chromosome contribution from the ICR and C57BL parents. Accordingly, the F_1 colonic lesions are mostly in the 3+ or 2+ category. The broad genetic recombination in the F_2 population is reflected in the wide variation of DMH oncogeny, ranging from 1+ (a few papillomas) to 4+ (multiple invasive adenocarcinomas). Minor modifying genes inherited from the resistant C57BL parent may account for the least severe lesion pattern (below 2+) in the BCR hybrids. Photomicrographs of sections from typical DMH colon tumors were shown in an earlier paper (2).

Linkage Tests. Autosomal linkage of the ICR susceptibility gene was tested by means of 5 enzyme loci: *Dip-1* on Chromosome 1; *Gdp-1* on 4; *Es-1* on 8; *Mod-1* on 9; and *Es-3* on 11. Our ICR and C57BL mice carry different alleles for 2 coat color genes: *albino* on Chromosome 7 and *agouti* on Chromosome 2. If there were linkage with any 1 of these 7 markers, a significant majority of the DMH colon tumors would coincide with heterozygosis for the ICR allelic marker, whereas most of the homozygotes for the corresponding C57BL allele would remain tumor free.

The colon tumor incidence among the *A/a* agouti-colored F_2 mice (83 ± 4.4%) and the F_2 albinos (77 ± 8.1%) does not differ significantly from the expected 3:1 ratio. Hence, linkage on Autosomes 2 and 7 is excluded.

The enzyme data (Table 3) are based on starch gel elec-

Table 2

DMH-induced colon tumor incidence in 2 inbred mouse strains and 4 types of hybrids showing inheritance of major dominant susceptibility gene

Genotype	No. with tumor/no. tested	% with tumor	
		Observed	Expected
ICR (M and F)	60/60	100	100
C57BL (M and F)	0/90	0	0
F_1 (M and F)	68/68	100	100
BCS[a] (M and F)	42/42	100	100
F_2 (M and F)	94/120	78	75[b]
BCR (M and F)	46/117	39	50[b]
BCR (males only)	27/57	47	50[b]
BCR (females only)	19/60	32	50

[a] Susceptible backcross.

[b] χ^2 test for goodness of fit to 1-gene expectation: F_2 $\chi^2 = 0.70$, $p > 0.4$; male BCR $\chi^2 = 0.16$, $p > 0.6$. The combined BCR data fall somewhat short of the expected tumor incidence because of the incomplete penetrance in BCR females (see "Discussion").

Table 3

Segregation of DMH colon carcinogenesis and 5 autosomal enzyme markers, for which the susceptible ICR/Ha and resistant C57BL/Ha carry different alleles, rules out close linkage on Autosomes 1, 4, 8, 9, and 11

Enzyme locus	Chromosome no.	DMH tumor mice	
		Homozygotes for C57BL enzyme	Heterozygotes for ICR and C57BL enzyme forms
Dip-1	1	5	4
Gpd-1	4	5	4
Es-1	8	4	5
Mod-1	9	5	4
Es-3	11	5	4

trophoresis of isozyme proteins, performed on homogenates of individual kidneys that were removed from 9 BCR hybrids with DMH-induced colon tumors. Since tumors occurred with about equal frequency in mice homozygous for the C57BL isozymes and in heterozygotes for the ICR alleles (Table 3, Column 4), the dominant DMH response gene does not appear to be linked closely to any of the 5 enzyme markers on Chromosomes 1, 4, 8, 9, or 11.

Tumor yield in 2 groups totaling 57 male BCR mice (Table 2, Line 7) was close to the expected 50%, whether they had inherited their single X chromosome from the DMH-susceptible ICR or from the resistant C57BL strain. This constitutes evidence against sex linkage and permits the conclusion that DMH colon carcinogenesis in the 100% susceptible inbred ICR strain, subline 1 (5), is controlled by a dominant autosomal gene.

DISCUSSION

Familial polyposis of the human colon resembles our ICR/Ha × C57BL/Ha hybrid model in its dominant autosomal mode of inheritance, its progressive pathology, and the specificity of the target tissue (6). Penetrance of the large bowel response to remote s.c. DMH treatment is 100% in the F_1 test and 75% in the F_2 test. Full dominance may be overridden by minor modifiers from the C57BL genotype, depending on the genomic frame of reference within which the major DMH-activating gene of ICR origin operates. For example, 50% of our female BCR hybrids were expected to develop colon lesions, but only 32% did; this fits a 2-gene segregation better than the single-gene hypothesis upheld by the BCR males (Table 2, Lines 7 and 8). The tumor deficit in the BCR females may be age related, as observed by Moon *et al.* (8) in BD IX female rats (but much less so in males) treated with DMH after they were 3 months old. The age of our experimental mice was 3 to 3.5 months at the beginning of treatment.

The choice of uniformly responsive animal strains is important for reproducible research in chemical carcinogenesis. Generalized statements in the literature that claim the noncarcinogenicity of a test compound for an entire mammalian species are precarious. The DMH response of 7 inbred mouse strains tested thus far ranges from 0 to 100%. It is 0% in DBA/LiHa, C57BL/Ha (2, 3), C57BL/6J, and AKR/J (1); >60% in SWR/J; >80% in P/J (1); and 100% in ICR/Ha subline 1 (2). The genetics and the derivation of the latter strain from the 67% DMH-responsive random-bred HaICR stock (2), as well as its relation to SWR mice, have been described in detail (5).

DMH is an alkylating agent and hence potentially mutagenic. It is among the fewer than 10% of known carcinogens that were negative in the Ames *Salmonella*/microsome mutagenicity screen, although this sensitive test detects the DMH metabolite methylazoxymethanol (7). That DMH may be activated by enzymes of the intestinal microflora is suggested by the experiments of Reddy *et al.* (9) with germ-free Fischer rats. Such animals develop significantly fewer DMH colon tumors (21%) than those (93%) found in conventional controls. These results provide convincing evidence for the potential modifying role of gut bacteria in DMH carcinogenesis. However, the mating system used in our mouse work (Table 1) would favor inadvertent acquisition of a uniform intestinal microflora through prolonged cage contact between parents and their hybrid offspring. Therefore, bacterial activation of DMH cannot explain the rather precise 3:1 and 1:1 Mendelian segregation of colorectal tumor yield in our test hybrids.

The fact remains that germ-free rats can metabolize DMH to an active carcinogenic derivative. Normal tissues of certain strains of conventional mice should have the same capacity unaided by the microflora. The search for the enzyme system involved in DMH activation will no doubt be accelerated by the considerable chemical knowledge about DMH metabolism (4, 11) and by the apparent single gene control of DMH oncogeny reported here.

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