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Strain Susceptibility and Resistance to 1,2-Dimethylhydrazine-Induced Enteric Tumors in Germfree Rats[1] (40146)

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Epidemiological observations among various ethnic groups have placed an emphasis upon the role of environmental factors, such as diet, in the etiology of human colonic carcinoma (1). An etiological role was assigned to the intestinal microflora in clinical cases as well as in experimental animal models in regard to the production of secondary bile acids, presumed cocarcinogens (2), and to the activation of indirect carcinogens (3).

The germfree rat is a useful tool for assessing the etiological role of the intestinal microflora in disease. In previous publications (4, 5), it was demonstrated that neoplastic lesions, including malignant adenocarcinomas, could be induced in a relatively short time in the intestinal tract of conventional and of germfree Sprague–Dawley (S–D) rats by the oral administration of 1,2-dimethylhydrazine (DMH). It had been assumed previously that DMH required metabolic activation from the intestinal microflora (6). Since we found germfree S–D rats susceptible to the carcinogenic effects of DMH, it was of interest to test the susceptibility of other strains of germfree rats to DMH. As in the case of mice (7), we here present evidence that genetic resistance to DMH carcinogenesis can be demonstrated in rats.

Methods. Male and female germfree rats representing S–D, Lobund–Wistar (L–W) and Buffalo (Bu) strains were maintained germfree in plastic isolators and fed autoclave-sterilized diet (Teklad L-485) and water *ad lib.* The three strains of rats were derived from the U.S. National Institutes of Health (S–D), the Wistar Institute (L–W) and Microbiological Associates (Bu). The S–D and L–W strains were propagated randomly under the germfree condition, but the Bu strain was inbred. Standard operating procedures established at Lobund Laboratory were used for maintenance and testing of germfree rats (8). The schedule of carcinogen administration was similar to that described in a previous report (4): 1,2-dimethylhydrazine dihydrochloride (Aldrich) was dissolved in physiological saline at 15 mg/ml. The solution was filter-sterilized (Millipore filter 0.22 μm) and administered by gastric intubation at the dose of 30 mg/kg body weight once a week for 10 weeks. At 20 weeks after the onset of DMH administration, the rats were anesthetized by ether, exsanguinated from the heart, and examined at 3X duoloupe magnification for tumors in the opened intestine and rectum. They were examined for metastatic lesions, as in lymph nodes and visceral organs. Individual tumors in the intestine were counted and recorded according to anatomical sites. Intestinal tumors, lymph nodes and other visceral organs were excised and fixed in Bouin's solution and paraffin-embedded sections thereof were stained with hematoxylin and eosin for histological examinations. More than 100 untreated germfree rats of L–W strain, in excess of age 30 months, were examined as controls. Controls also included 100 S–D rats in excess of age 24 months, and 50 Bu rats, in excess of age 14 months. No intestinal tumor was found in them. They were maintained under the same conditions of germfree technology as the rats being subjected to the DMH treatments.

Results. All rats representing the three strains survived to the completion of the experimental DMH protocol. They showed no clinical evidence of toxicity. The diet consumption was not hindered and the rate and extent of weight gain was within expected limits.

The results obtained among the three

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strains of DMH-treated germfree rats are recorded in Table I. S–D rats exhibited the highest susceptibility to the carcinogenic effects of DMH: all of them developed an average of 6.7 tumors/rat. This level is comparable to the tumors induced by DMH in earlier experiments with germfree S–D rats (4). The tumor incidence was 7.3 for five males and 5.3 for three females. The histological observations were also similar in that the spectrum of neoplasms in each rat ranged from benign polyps to malignant adenocarcinomas which penetrated the muscularis mucosa and into the serosal cover of the intestine. Adenocarcinomas were observed in two forms, pedunculated and sessile, some of the former were so large as to fill the colon lumen; and some of them showed bleeding into the lumen. Metastatic foci were noted in the paraaortic lymph nodes in two rats. Tumors were not detected in other visceral organs.

Germ-free DMH-treated Bu rats had smaller numbers and sizes of tumors in the intestine. Ten of fifteen rats had intestinal tumors at a frequency of 1.7/rat (Table I). Nine male rats had an average tumor incidence of 1.4/rat and six female rats had a tumor incidence of 2.0/rat. The Bu rats had superficial polyps and papillomas in the intestine, with no evidence of extension into the underlying muscularis mucosa. No metastatic lesion was observed in them. Tumors were not found in eight female germfree L–W rats treated with DMH. As noted above, the untreated control S–D, L–W and Bu germfree rats were free of detectable intestinal tumors.

Discussion. This report again demonstrated that intestinal microflora was not essential to the induction of intestinal tumors by DMH in S–D and Bu strains of rats. In contrast the same regimen of DMH administration failed to induce a single tumor in the germfree L–W rats. An intermediate tumor incidence was demonstrated in Bu rats. Though small in experimental numbers, the results are drastically different. The results demonstrate a strain dependent differential susceptibility to DMH-induced enteric tumors. The results are all the more significant because the determinations were observed under germfree conditions by which experimental variables from the environment were excluded. Furthermore preliminary data indicate a similar difference in susceptibility to DMH-induced enteric tumors between conventional S–D and L–W strain rats. It is conceded that microbial flora can play an etiological role in experimental colo-rectal cancer, as exemplified by cycasin (10); however, the role is not exclusive.

Strain susceptibility to chemical carcinogenesis has been reported (9). In experiments on colon carcinogenesis, four strains of conventional mice differed in their susceptibilities to subcutaneously injected DMH (7), which is consistent with the findings reported here in rats. In this regard, the Glaxo strain of Wistar rat was susceptible to the toxic and the carcinogenic effects of injected DMH (10, 11), which emphasizes the unique characteristic of the L–W rats. With conventional animals the differential susceptibility and resistance to DMH might be attributed to differences in the diet, and to its effect on the microbial flora. The results recorded here under controlled conditions indicate that genetic factors do play dominant role(s) in determining host responses to DMH. The differential susceptibility must be attributed to differences in inherent cellular or metabolic processes.

This report should emphasize that in as-

TABLE I. STRAIN DEPENDENT DIFFERENTIAL SUSCEPTIBILITY TO DMH-INDUCED ENTERIC NEOPLASMS AMONG THREE STRAINS OF GERMFREE RATS[a]

Strain of rats	Number of rats with tumors/Number of rats examined	Tumor Incidence (per rat)							
						Colon			
		Duodenum	Jejunum	Ileum	Cecum	Upper ½	Lower ½	Rectum	Total
S-D	8/8	0.5	0.12	0	0	1.6	4.1	0.4	6.7
Bu	10/15	0.13	0.06	0	0	0.93	0.60	0	1.7
L-W	0/8	0	0	0	0	0	0	0	0

[a] Diet L-485 (Teklad) was used. Germfree rats of 1.5–2.0 months of age were used. DMH was administered orally at 30 mg/kg once a week for 10 weeks. Rats were sacrificed 20 weeks after the initial DMH administration.

sessing the role(s) of intestinal flora and of genetic factors in colon carcinogenesis, close attention must be paid to the strain of experimental animals used because of their inherent susceptibility or resistance. If DMH has been tested by the protocol noted above only in L–W rats, it would have been considered "safe". The comparative analysis of the three strains of germfree rats may offer a unique opportunity to define some important mechanism involved in DMH-induced colon carcinogenesis. Spontaneous colon tumors have not yet been observed in germfree rats, including those of S–D strain, and including rats in the age range of 1–3.5 years. This suggests that, excluding humans with polyposis, a population of susceptible animals will remain free of intestinal neoplasms unless they are exposed to the chemical agent(s) which would induce them; and that some strains of animals exhibit inherent resistance to exposure to such agents.

Summary. Three strains of germfree rats (Sprague–Dawley, Lobund-Wistar, Buffalo) were tested for their susceptibility to DMH-induced enteric neoplasms; Sprague–Dawley rats were most susceptible, Lobund–Wistar rats were not susceptible, and Buffalo rats were intermediate in susceptibility.

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