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Effect of Germ-free Status and Antilymphocyte Serum on Induction of Various Tumours in Mice by a Chemical Carcinogen given at Birth

RECENTLY we reported[1] a dramatic effect of germ-free status (GF) on the induction of hepatomas by 7,12-dimethylbenz(a)anthracene (DMBA) injected subcutaneously at birth into C3H male mice. In the experiments then reported, mice were killed at 30 to 41 weeks, and very few neoplasms apart from hepatomas were encountered. Twenty-two out of thirty-seven DMBA-treated minimum-disease mice had hepatomas and three had pulmonary adenomas, compared with three out of thirty-four DMBA-treated GF mice with hepatomas and two with pulmonary tumours. Here we report studies on the female C3H mice corresponding to the males covered by the previous report. Details of the origin and maintenance of mice, of the genetic relationship between mice kept GF and those kept under minimum disease (MD) conditions, and of the source and method of administration of DMBA, have been reported[1]. Horse anti-mouse lymphocyte serum (ALS) was obtained from Burroughs Wellcome. A preliminary assay of the batch used showed that doses of 0·5 ml. on days 2 and 5 after grafting prolonged the retention of A-strain skin grafts on CBA-strain mice from the normal 11 days to more than 20 days.

Table 1. TUMOUR INDUCTION IN GERM-FREE (GF) AND MINIMUM DISEASE (MD) FEMALE C3H MICE IN RESPONSE TO DMBA AT BIRTH

Treatment at birth	Micro-biological status	No. of mice examined *post mortem*	Age at necropsy (weeks)	Mice with neoplasms
None	GF	13	40	0
None	MD	22	40	0
40 μg DMBA in 0·04 ml. trioctonoin subcutaneously on day 7	GF	10	30–34	2 (Both pulmonary adenomas, one per mouse)
40 μg DMBA in 0·04 ml. trioctonoin subcutaneously on day 7	MD	21	34	6 (All pulmonary adenomas, five mice with one adenoma each, one mouse with two adenomas)

In the absence of ALS treatment, the response of female C3H mice to DMBA treatment in terms of tumours present at necropsy at 30 to 40 weeks seemed to be little affected by GF status (see Table 1). As far as these few data permit any conclusion, they are in line with those for male C3H mice in which GF status appeared to influence only hepatoma incidence and not the incidence of any other type of neoplasm.

The new finding of real interest relates to a comparison of mice injected when less than 24 h old with 20 μg DMBA in 0·02 ml. trioctonoin and subsequently given repeated injections of ALS between days 224 and 269. Details of ALS treatment are as follows: days 224 and 227, 0·5 ml. intraperitoneally, and days 234, 241, 248, 255, 262 and 269, 0·5 ml. subcutaneously. Mice were killed 26 days after the final injection of ALS when they were 42 weeks old.

Three MD-maintained mice and one GF mouse died before or during ALS treatment. Two of the former had tumours (an injection site sarcoma in a mouse that died at 16 weeks and generalized malignant lymphoma of thymic origin in a mouse that died at 27 weeks). No tumours were seen in the remaining MD mouse or in the GF mouse. Details of tumours—all of them histologically confirmed—in mice examined after death at 42 weeks are given in Table 2.

Table 2. EFFECT OF GERM-FREE STATUS ON TUMOUR DEVELOPMENT IN FEMALE C3H MICE GIVEN 20 μG DMBA AT BIRTH AND A COURSE OF ALS INJECTIONS BETWEEN DAYS 226 AND 269

Micro-biological status	No. of mice examined *post mortem* on day 296	No. of mice with tumours	Details of mice with tumours
GF	17	0	—
MD	17	8	Four mice—one pulmonary adenoma each (one of 7 mm diameter) One mouse—hepatoma One mouse—a pulmonary adenoma and a hepatoma One mouse—5 mm diameter inter-abdominal spindle-cell sarcoma and a pulmonary adenoma One mouse—thymic lymphoma

The findings cannot be regarded as other than preliminary because data from certain essential control groups are not available. Clearly GF mice are not entirely resistant to the development of tumours in response to the neonatal injection of DMBA, and the complete absence of tumours in the germ-free ALS-treated mice shown in Table 2 may be spurious. Nevertheless the difference in response between the GF and MD mice in this experiment is striking, especially if the occurrence of two cases of neoplasia earlier in the experiment and the large size of some of the neoplasms encountered in the MD mice are taken into account. The results suggest that, in this particular experiment, GF status and/or ALS treatment markedly influenced tumour yields in response to DMBA given at birth.

If subsequent experiments indicate that ALS treatment enhances tumour development in response to chemical carcinogens, then the indications that immunosuppression permits the growth of otherwise suppressed latent virus-induced neoplasms[2–6] will seem to have even more important and widespread implications than at present.

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