Spontaneous Neoplasms in Germfree BALB/cPi Mice (35935)

CARLOS S. SMITH AND H. IRA PILGRIM

Departments of Anatomy and Surgery, University of Utah College of Medicine, Salt Lake City, Utah 84112

Early reports (1) indicated that germfree animals did not develop spontaneous tumors. Since then, however, many reports from various laboratories have indicated that the germfree environment has no apparent effect on the occurrence of spontaneous neoplasms in mice. Ward (2) states that he could find no difference in spontaneous tumor incidence between germfree and conventional mice; but no data were presented. Walburg *et al.* (3) found little difference in leukemia incidence between germfree and conventional RF and ICR mice. This report of little difference in leukemia incidence has been confirmed by several laboratories (4–6). Pilgrim and Labrecque (7) compared the occurrence of mammary tumors in germfree and conventional C3Hf mice, and concluded that there was no difference in incidence.

The BALB/c strain was reported as developing no spontaneous tumors in 4 years of maintenance in a germfree environment (8). This paper presents data which indicate that the germfree state has little or no effect on the development of spontaneous tumors in this strain. This laboratory does not maintain conventional mice. BALB/c mice given a defined flora are maintained here, but they have not been kept for a long enough period of time to provide control data. The reason for publishing these data at this time without adequate conventional controls is because of the marked discrepancy between our results and those of the Notre Dame group (8); with these authors claiming that there are no tumors, while our animals have tumor incidences that are similar to those reported in conventional animals of the same strain. Since both our mice and those used by the Notre Dame group are derived from the same source (Andervont), this discrepancy would not be expected.

Materials and Methods. The germfree BALB/cPi subline was derived from BALB/cAnHf in 1965 by cesarean section (9) and foster nursed on germfree Swiss mice obtained from Carl Miller at the National Institute of Health. The animals have been maintained by brother $\times$ sister mating in autoclavable aluminum free-flow isolators (10). They were given Wayne sterilizable Lab-Blox and canned water *ad libitum*. Retired breeders were maintained in aluminum cages, 4 $\times$ 5 $\times$ 10 in., with 5–6 animals/cage. Animals were sacrificed, when they were moribund or when tumors were noticed. Others were sacrificed at random. Tissues were fixed in formol–acetic–alcohol (11). Paraffin embedded sections were stained with hematoxylin and eosin, and toluidine blue.

Results. The raw data on the occurrence of spontaneous neoplasms in germfree BALB/cPi mice is presented in Table I.

Of 67 aging germfree BALB/cPi mice, sacrificed over a 2 year period, 14/67 (21%) developed primary lung tumors. Reticular neoplasms were noted in 5 (8%) of the 67 germfree BALB/cPi mice. Angiomas were found in 4 (6%) of the 67 animals.

Discussion. Andervont and Dunn (12) found 157 lung tumors in 650 conventional BALB/c mice (24%). Deringer (13) found 44/169 (26%), and Madison *et al.* (14) found a 16% incidence of lung tumors (332/2088). These data are compatible with the observed incidence of 21% which we found in our germfree BALB/cPi mice. The slight differences observed might be accounted for by differences in the age of the sacrifice of the mice. We can conclude that there is probably little difference in the incidence of lung tumors in germfree and conventional BALB/c mice.

Madison *et al.* (14) reported a 5%

TABLE I. Age Distribution of Spontaneous Neoplasms in Germfree BALB/cPi Mice at Time of Sacrifice.
Sample consists of 15 males, 52 females, with a median age of 21 months.

	Age (months)																		Totals			
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	♂	♀	No.	%
Lung tumors	1[b]	1		1			1		1	1	2				1	3	1[c]	1	3	11	14	20.9
Angiomas							2							1			1[d]			4	4	6.0
Mammary tumors	1[b]																1[d]			2	2	3.0[a]
Lymphosarcomas						1							1				1[c]			3	3	4.5
Reticulum cell sarcoma, Type A																	1			1	1	1.5
Type B																		1		1	1	1.5
Carcinoma of the skin													1		1					2	2	3.0
Adrenal cortical carcinomas																		2		2	2	3.0
Carcinoma of ovary																	1[c]			1	1	1.5[a]
Hepatoma											1			1						2	2	3.0
Myoepithelioma					1															1	1	1.5
Mice with tumors	1	1	0	1	1	1	3	0	1	1	3	0	2	2	2	3	3	4	3	26	29	43.3
Mice without tumors	0	1	2	5	0	0	5	1	1	4	1	2	8	0	6	1	1	0	12	26	38	56.7
Total of mice	1	2	2	6	1	1	8	1	2	5	4	2	10	2	8	4	4	4	15	52	67	

[a] Percentage of female mice only. Numbers with the same superscript [b], [c], or [d] indicate that the tumors were in the same mouse.

(108/2088) incidence of reticular neoplasms and Deringer (13) reported 10% (18/182) in BALB/c mice. We found an 8% incidence in germfree BALB/c mice, and concluded that there is little difference between germfree and conventional mice with regards to the incidence of reticular neoplasms. Of the 5 reticular neoplasms we observed, 3 were lymphosarcomas, which were metastatic to the liver and kidneys, and the other 2 were reticulum cell sarcomas, Type A and Type B (15). One reticulum cell sarcoma, Type A, and one lymphosarcoma was successfully transplanted into conventionalized BALB/cPi mice.

Our incidence of mammary tumors is also compatible with published data. We found two animals out of 52 (3%) germfree BALB/cPi breeding females with mammary tumors. Andervont and Dunn (12) observed a similar low incidence in his conventional breeding BALB/cAn females of 1% (6/650). A variety of tumors (angiomas, hepatomas, adrenal cortical tumors, myoepitheliomas, and ovarian tumors) were found in low incidence by Madison *et al.* (14).

Deringer also observed angiomas and hepatomas in low frequency. We found the following tumors in our germfree mice: 4 angiomas, 2 hepatomas, 2 squamous cell carcinomas, 2 adrenal cortical carcinomas, a transplantable myoepithelioma, and a carcinoma of the ovary.

The following types of nonneoplastic spontaneous lesions were also noted: pericardial calcification (4 mice), uterine and ovarian cysts (2 mice), cystic liver (1 mouse), cystic kidney (1 mouse), cystic inguinal lymph node (1 mouse), cystic mesenteric lymph node (1 mouse), gallstones (3 mice), glomerulonephritis (1 mouse), mesenteric vessel thrombus (1 mouse), calcium deposits in the mesentery (6 mice), and an ulcer at the ileocecal junction (1 mouse).

We concluded that there appears to be little or no difference in the incidence of spontaneous neoplasms between that reported in conventional and our findings in germfree BALB/c mice.

Summary. Of 67 germfree BALB/cPi mice autopsied at a median age of 21 months, 14/67 (21%) had lung tumors; 5/67 (8%), reticular neoplasms; and 4/67 (6%), angiomas. Mammary tumors, squamous cell carcinomas, adrenal cortical carcinomas, hepatomas, an ovary carcinoma, and a myoepithelioma were also observed. There was no discernible difference between the incidence of tumors observed in germfree BALB/cPi mice and that reported in the literature for conventional BALB/c mice.

1. Reyniers, J. A., Ann. N.Y. Acad. Sci. **78**, 397 (1959).

2. Ward, T. G., Fed. Proc. **20**, 150 (1961).

3. Walburg, H. E., Jr., Upton, A. C., Tyndall, R. L., Harris, W. W., and Cosgrove, G. E., Proc. Soc. Exp. Biol. Med. **118**, 11 (1965).

4. Walburg, H. E., Jr., and Cosgrove, G. E., *in* "Germ-Free Biology" (E. A. Mirand, and N. Back, eds.), p. 135. Plenum, New York (1969).

5. Pollard, M., Kajima, M., and Teah, B. A., Proc. Soc. Exp. Biol. Med. **120**, 72 (1965).

6. Pilgrim, H. I., Parks, R. C., and Law, L. W., *in* "Germ-Free Biology" (E. A. Mirand, and N. Back, eds.), p. 125. Plenum, New York (1969).

7. Pilgrim, H. I., and Labrecque, A. D., Cancer Res. **27**, 584 (1967).

8. Pollard, M., Nat. Cancer Inst. Monogr. **20**, 167 (1966).

9. Pilgrim, H. I., and Parks, R. C., Lab. Anim. Care **18**, 346 (1968).

10. Pilgrim, H. I., Lab. Anim. Care **19**, 117 (1969).

11. Fekete, E., *in* "Biology of the Laboratory Mouse" (G. D. Snell, ed.), 1st ed., p. 89. McGraw-Hill, New York (1941).

12. Andervont, H. B., and Dunn, T. V., J. Nat. Cancer Inst. **8**, 235 (1948).

13. Deringer, M. D., J. Nat. Cancer Inst. **35**, 1047 (1965).

14. Madison, R. M., Rabstein, L. S., and Bryan, W. R., J. Nat. Cancer Inst. **40**, 683 (1968).

15. Dunn, T. B., J. Nat. Cancer Inst. **14**, 1281 (1954).

Received March 24, 1971. P.S.E.B.M., 1971, Vol. 138.