Effects of Thymectomy, Splenectomy and 3-Methylcholanthrene on Neoplasia Expression, Incidence and Latency in AKR Mice[1] (36895)

CARRIE E. WHITMIRE, RONALD A. SALERNO, AND LOUISE S. RABSTEIN[2]
Microbiological Associates, Inc., 4733 Bethesda Avenue, Bethesda, Maryland 20014

Recent studies by us (1) and others (2–5) have demonstrated that both leukemia and 3-methylcholanthrene (3MC) induced tumors rarely develop in the same animal. Peters *et al.* (in preparation) studying a large BALB/c mouse population found no spontaneous lymphatic neoplasms concomitantly in the same mouse with non-hematopoietic tumors of mesenchymal origin. These findings suggest the possibility of an interference mechanism in the development of different types of neoplasms within the same animal. We have observed an inverse relationship between spontaneous leukemogenesis and 3MC sarcoma induction in AKR mice (6). We have also reported that mice with high levels of type C RNA virus genome expression are less susceptible to 3MC tumor induction than mice with low levels of genome expression (1, 7).

This study was undertaken to determine if the leukemic expression itself or the high levels of endogenous type C RNA viral genome (8, 9) or other factors are the basis for the apparent mutual exclusion of 3MC sarcomas and leukemia in the AKR mouse. Because thymectomy but not splenectomy reduces the incidence of spontaneous leukemia in AKR mice (10, 11), we studied their effect on sarcoma induction by 3MC. If no enhancement of sarcoma induction occurs in the absence of leukemia as reported by Duran-Reynals for AKR mice painted with 3MC (4), a high endogenous level of type C RNA virus genome expression may at least in part be responsible for the relatively low incidence of sarcoma-induction in the AKR mouse.

Materials and Methods. AKR/J 4 week old female mice were obtained from the Jackson Laboratory, Bar Harbor, Maine and housed, fed, and observed as described previously (1). One third of the mice were maintained as intact controls. The remaining mice were anesthetized with Nembutal (Sodium Nembutal, Abbott Laboratories) and half were splenectomized and the other half thymectomized. At 8 weeks of age, the animals of each group (intact, thymectomized, splenectomized) were randomized and injected subcutaneously (sc) with 0.05 ml trioctanoin (Eastman Organic Chemicals, Rochester, N.Y.) or 150 μg 3MC (Eastman Organic Chemicals) dissolved in 0.05 ml trioctanoin. A different 0.5 ml syringe and 23 gauge 1 in. needle was used for each animal.

The mice were examined daily for 36 weeks. When evidence of leukemia or sc tumors at the site of inoculation was observed, the mice were sacrificed by cervical dislocation. A complete macroscopic examination was made for leukemia, tumors and other abnormalities as well as for confirmation of successful surgical removal of thymus or spleen tissue. No evidence of spleen was found in the splenectomized animals. Grossly observable thymus tissue was found in only 3 thymectomized animals (treated with 3MC). Two of these animals developed thymomas and one developed a sc sarcoma without thymoma. These three animals were ex-

[1] This study was conducted under Contract NIH 70-2068 within the Special Virus-Cancer Program of the National Cancer Institute, National Institutes of Health, Public Health Service.

[2] The authors thank Dr. Russel M. Madison for thymectomizing and splenectomizing mice used in this study, Mr. Horace C. Turner for the complement fixation studies and Mr. Foster McClure for the statistical analysis. We express our appreciation for the excellent technical assistance in the animal studies to Mr. N. Baker, Mr. S. Zelnio, and Mr. H. Ratrie.

cluded from the results presented here since we were primarily interested in the effect of thymectomy on sarcoma-induction. Standard histological preparations were made on all mice[3] (1). The latency periods and tumor incidences were computed as previously described (1).

Procedures for detecting the type C RNA virus *gs* antigen in tumor and normal muscle tissues have been described previously (1). Rat antiserum pool 26 and highly specific guinea pig antiserum were used (1).

Results. As shown in Table I the total incidence of sarcomas both alone and with leukemia was not significantly affected by either thymectomy or splenectomy ($p > 0.20$) as compared with the intact mice treated with 3MC. The incidence of 3MC induced sarcomas in the absence of leukemia was significantly higher in the thymectomized mice than in intact or splenectomized mice ($p < 0.05$). No significant differences in sarcoma incidences in the absence of leukemia occurred between splenectomized and intact mice ($p > 0.20$). Sarcomas in the presence of leukemia occurred only six times in the 3MC treated mice (6/48, 13%), 4 times in the intact mice and 2 times in the splenectomized mice.

The incidence of leukemia was significantly decreased ($p < 0.01$) in the thymectomized animals given either trioctanoin or 3MC as compared to intact mice. Splenectomy did not affect the total incidence of leukemia (alone or with sarcomas) in mice given either trioctanoin or 3MC ($p > 0.2$). The only lymphatic tissue involvement in thymectomized mice occurred in the 35th week as lymphatic leukemia involving only the spleen.

The total incidence of neoplasia in 3MC treated mice was not affected by splenectomy when compared with the intact mice; however in the thymectomized mice where only 1/18 of the mice developed leukemia the total incidence of neoplasia was reduced to 10/18, 9/18 being sarcomas.

As shown in Table II, thymectomy re-

TABLE I. Effects of Splenectomy, Thymectomy and 3-Methylcholanthrene on Neoplastic Expression in AKR Mice.

Treatment		Incidence and type of neoplastic expression													
		Sarcomas						Leukemia							
		Without leukemia		+ With leukemia		= Total sarcomas		Without sarcomas		+ With sarcomas		= Total leukemia		Total neoplasia	
Surgical[a]	Chemical[b]	Tu/T[c]	%	Tu/T	%	Tu/T	%	Tu/T	%	Tu/T	%	Tu/T	%	Tu/T	%
None	Trioctanoin	0/47	0	0/47	0	0/47	0	41/47	87	0/47	0	41/47	87	41/47	87
	150 μg 3MC	5/23	22	4/23	17	9/23	39	14/23	61	4/23	17	18/23	78	23/23	100
Splenectomy	Trioctanoin	0/19	0	0/19	0	0/19	0	13/19	68	0/19	0	13/19	68	13/19	68
	150 μg 3MC	5/25	20	2/25	8	7/25	28	18/25	72	2/25	8	20/25	80	25/25	100
Thymectomy	Trioctanoin	0/27	0	0/27	0	0/27	0	0/27	0	0/27	0	0/27	0	0/27	0
	150 μg 3MC	9/18	50	0/18	0	9/18	50	1/18	6	0/18	0	1/18	6	10/18	56

[a] Surgical procedures were done at 4 weeks of age.
[b] Chemical treatment was done at 8 weeks of age.
[c] Tu/T = total tumored mice/total mice at risk for 9 months.

[3] The histological studies were conducted under Contract PH 43-67-697 within the Special Virus-Cancer Program of the National Cancer Institute, National Institutes of Heath, Public Health Service.

TABLE II. Effects of Splenectomy, Thymectomy and 3-Methylcholanthrene on the Latency Period of Neoplastic Expression in the AKR Mouse.

Treatment		Latency periods (weeks) for neoplastic expression											
		Sarcoma alone			Leukemia alone			Sarcoma + leukemia			Total neoplasia		
Surgical[a]	Chemical[b]	Av[c]	50%[d]	Range	Av	50%	Range	Av	50%	Range	Av	50%	Range
None	Trioctanoin				26	22	16–35				26	22	16–35
	150 μg 3MC	26	21	18–31	26	22	21–35	24	22	20–27	26	22	18–35
Splenectomy	Trioctanoin				27	23	18–35				27	23	18–35
	150 μg 3MC	24	20	18–31	25	22	21–35	21	21	21	24	22	18–35
Thymectomy	150 μg 3MC	22	19	18–28	35		35				24	19	18–35

[a] Surgical procedures were done at 4 weeks of age.
[b] Chemical treatment was done at 8 weeks of age.
[c] Average latency calculated on total incidence.
[d] 50% latency is the average latency period for the first 50% of the mice developing neoplasia.

duced the latency period for sarcoma development by 4 weeks. Splenectomy reduced the average sarcoma latency period by 2 weeks below that of intact animals treated with 3MC. The latency period for the development of leukemia was not altered by either splenectomy or 3MC treatment. Two splenectomized mice developed sarcomas during the 21st week, and at necropsy, were found to have leukemia as well. This occurred 3 weeks earlier than the average latency period for sarcoma development and 4 weeks earlier than the average latency period for leukemia in the splenectomized mice.

The presence of *gs* antigen in the tissues of AKR mice is readily detected by the CF and gel diffusion (gd) tests (1). All spleen, thymus and mesenchymal tissues (muscle and subcutaneous) from both control and tumored mice and tumors from tumored mice were found to contain *gs* antigen at a final dilution of $\geqq$ 1:640 indicating a high level of viral antigens in all tissues. Highly specific *gs*-1 guinea pig antiserum made with electrofocused purified *gs* antigen confirmed the specificity and sensitivity of the murine sarcoma virus rat antiserum (12). Further confirmation of the identity of the *gs*-1 antigen in the 3MC induced tumors was shown by the gd test (1).

Discussion. Although thymectomy virtually eliminated the development of leukemia in the AKR mouse, the total incidence of sarcomas was not significantly increased over that in splenectomized or intact mice treated with 3MC. This is in agreement with the recent findings of Duran-Reynals (4) that thymectomy had no effect on tumorigenesis of 3MC skin painting in AKR mice. The effect(s) of thymectomy and splenectomy on sarcoma induction by 3MC was primarily a shorter average latency period required for tumor development. Thymectomy was the most effective, causing a 4 week reduction in latency while splenectomy decreased the latency period by 2 weeks compared with intact animals treated with 3MC. Both thymectomy (13–15) and splenectomy (16, 17) have an immunosuppressive effect which may at least in part be the reason for the early tumor development in these animals. The

effect of thymectomy on sarcomagenesis may be due to the absence of leukemia in these animals.

Earlier studies have indicated that the sensitivity to sarcoma induction by 3MC is related to the low endogenous levels of virus genome expression (1, 7). Since thymectomy decreased the leukemia incidence but did not significantly alter either 3MC sarcoma induction or the high endogenous level of *gs* antigen in all tissues of the non-tumored or tumored AKR mice, the primary interfering phenomenon between chemical carcinogenesis and leukemia may be the level of endogenous type C RNA virus genome expression. The nature of this interference by high endogenous virus genome expression is not clear. Some antibody production may occur in the mouse acting against the initially 3MC transformed cells and prevent sarcoma induction from proceeding beyond the early stages. This hypothesis is reinforced by the phenomenon demonstrated by Barbieri *et al.* (18) that the tumorigenicity of transformed cells is reduced by super-infection with leukemia viruses. This is apparently due to a new immunogenic stimulation occurring from additional tumor-specific transforming antigens thus interrupting the formation of visible tumors at the site of 3MC inoculation.

The inhibitory effects of super-infection with leukemogenic viruses, either naturally (8, 9) or experimentally induced (3), on sarcoma induction may reflect a preferential tissue tropism of these oncogenic viruses for the reticuloendothelial rather than the mesenchymal tissues, thus resulting in a predominant leukemia expression. The oncogenes for leukemia may be different than those for sarcoma and occur in larger numbers in the same host. Sarcoma viruses represent a mixture of viruses with a higher level of leukemia viruses than sarcoma viruses being present (19).

No effects of 3MC on the latency period of leukemia or sarcoma development occurred in these studies (Table II). Previous studies (6) in our laboratory have demonstrated the effects of 3MC on the latency of leukemia development are dependent on the age of the AKR mice at the time of treatment. When 150 and 300 μg 3MC were given sc to NB mice, leukemia developed 6 and 11 weeks earlier, respectively, than in control mice. When mice were 4, 8, or 16 weeks of age at the time of treatment, leukemia developed at 30 to 34 weeks of age independent of the 3MC dose and at the same time of development in the vehicle control mice. The results reported in the present study that 3MC did not accelerate leukemogenesis appear to be in disagreement with reports in the literature that various chemical carcinogens accelerate leukemia, however there is considerable difference in the age of mice treated, the dose of carcinogen given, the route of administration as well as the carcinogen used. Miyoshi (20) reported 500 μg 3MC to accelerate leukemia, subcutaneous sarcoma and lung adenoma development in newborn mice which is in agreement with our previously reported studies in NB mice (6). Furth and Barnes (21) demonstrated the acceleration of neoplasia when 3 to 8 week old mice were painted or subcutaneously injected with high doses (8 to 34 mg) of 3MC. This dose is considerably higher than the 150 μg used in the present studies and would be expected to react differently. Toth and Shubik demonstrated that NB AKR mice injected with 200 μg benzo(*a*)pyrene developed lymphomas more rapidly than control mice (22). Karvamoto *et al.* (23) demonstrated AKR WN mice treated with 11 mg/g body weight urethan developed the same incidence of leukemia as control mice, however the mean age of death from leukemia was reduced from 261 to 243 days. Engelbreth-Holm and Lefevre (24) demonstrated 9,10-dimethyl-1,2-benzanthracene accelerated the development of leukemia when 4 to 8 month old AKR mice were treated subcutaneously with 0.5 to 1.0 mg doses. These results demonstrate the difficulty of comparing the various studies reported in the literature since the parameters for each study are different. Another difference we have found to be of great significance is the substrain of mice used. Although studies are undertaken with a specific strain of mice, as the AKR, we have demonstrated significant differences in C57BL/6 mice obtained from three sources (25). There is no reason to be-

lieve this same difference does not occur in all strains of mice since the breeding practices used by breeders can vary extensively even with inbred colonies.

Summary. The effects of thymectomy, splenectomy and 3MC treatment on neoplastic expressions were studied in AKR mice. In the intact and splenectomized mice, 3MC increased the total incidence of neoplastic expression due to the development of sc tumors at the site of 3MC treatment and not to an increase in incidence of leukemia. Thymectomy eliminates leukemia during the nine-month observation period in the trioctanoin vehicle control mice and reduced the incidence of leukemia to 1/18 in the 3MC treated mice. The incidence of 3MC induced sarcomas was not increased by thymectomy even in the absence of leukemia, therefore the total incidence of neoplasia was reduced from 100% to 56%. Both thymectomy and splenectomy reduced the latency period for 3MC sarcoma induction.

1. Whitmire, C. E., Salerno, R. A., Rabstein, L. S., Huebner, R. J., and Turner, H. C., J. Nat. Cancer Inst. **47**, 1255 (1971).

2. Meier, H., and Myers, D. D., *in* "Comparative Leukemia Research 1971" (R. M. Dutcher, ed.), Bibl. haemat., 37, Karger, Basel/Munchen/New York, in press.

3. Duran-Reynals, M. L., and Lilly, F., Trans. Proc. **3**, 1243 (1971).

4. Duran-Reynals, M. L., J. Nat. Cancer Inst. **48**, 95 (1972).

5. Lilly, F., and Duran-Reynals, M. L., J. Nat. Cancer Inst. **48**, 195 (1972).

6. Whitmire, C. E., Salerno, R. A., Merold, V. A., and Rabstein, L. S., J. Nat. Cancer Inst. **49**, 221 (1972).

7. Whitmire, C. E., Salerno, R. A., Rabstein, L. S., and Huebner, R. J., *in* "Comparative Leukemia Research 1971" (R. M. Dutcher, ed.), Bibl. haemat., 37, Karger, Basel/Munchen/New York, in press.

8. Huebner, R. J., and Todaro, G. J., Proc. Nat. Acad. Sci. **64**, 1087 (1969).

9. Huebner, R. J., Todaro, G. J., Sarma, P., Hartley, J. W., Freeman, A. E., Peters, R. L., Whitmire, C. E., Meier, H., and Gilden, R. B., *in* "Defectiveness, Rescue and Stimulation of Oncogenic Viruses" p. 33. Editions du Centre National da la Recherche Scientifique, Paris (1970).

10. McEndy, D. P., Boon, M. C., and Furth, J., Cancer Res. **4**, 377 (1944).

11. Law, L. W., and Miller, J. H., J. Nat. Cancer Inst. **11**, 253 (1950).

12. Oroszlan, S., Fischer, C. L., Stanley, T. B., and Gilden, R. V., J. Gen. Virol. **8**, 1 (1970).

13. Hays, E. F., Cancer Res. **28**, 21 (1968).

14. Miller, J. F. A. P., Grant, G. A., and Roe, R. J. C., Nature (London) **199**, 920 (1963).

15. Miller, J. F. A. P., Ting, R. C., and Law, L. W., Proc. Soc. Exp. Biol. Med. **116**, 323 (1964).

16. Moller, E., J. Nat. Cancer Inst. **35**, 1053 (1965).

17. Ferrer, J. F., and Mihick, E., Cancer Res. **28**, 116 (1968).

18. Barbieri, D., Belehradek, J., and Barski, G., Int. J. Cancer **7**, 364 (1971).

19. Huebner, R. J., Proc. Nat. Acad. Sci. **58**, 835 (1967).

20. Miyoshi, I., Acta Med. Okayama **17**, 51 (1963).

21. Furth, J., and Barnes, W. A., Cancer Res. **1**, 17 (1941).

22. Toth, B., and Shubik, P., Cancer Res. **27**, 43 (1967).

23. Kawamoto, S., Kirschbaum, A., Ibanez, M. L., Trentin, J. J., and Taylor, H. G., Cancer Res. **21**, 71 (1961).

24. Engelbreth-Holm, J., and Lefevre, H., Cancer Res. **1**, 102 (1941).

25. Whitmire, C. E., and Salerno, R. A., Cancer Res. **32**, 1129 (1972).

Received June 5, 1972. P.S.E.B.M., 1972, Vol. 141.