

HETEROLOGOUS ANTILYMPHOCYTE SERUM HASTENS THE GROWTH OF 7,12-DIMETHYLBENZ(α)ANTHRACENE INDUCED TUMOURS IN MICE

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Summary.—The present paper describes the effects of repeated administration of rabbit anti-mouse lymphocyte serum (ALS) or normal rabbit serum (NRS) on tumours induced in Charles-River mice by 7, 12-dimethylbenz(α)anthracene (DMBA) given at birth. ALS or NRS were given either at the time of DMBA administration and subsequently at weekly intervals for the first 10 weeks of life, or at daily intervals for 7 days during the first, second, third or fourth week of life. The incidence and histology of the tumours were studied. It was found that treatment of very young mice with ALS greatly reduced the mean survival time of mice and significantly increased the incidence of malignant lymphoma. The incidence of lung tumours was found to be significantly increased in the animals injected with ALS during the second week of life.

Treatment with ALS in the other experimental groups gave results essentially similar to those observed in DMBA control and NRS treated mice.

ANTILYMPHOCYTE serum (ALS) and other immunosuppressive agents have been described as factors enhancing tumour incidence, this description being based on evidence of medical therapy of human patients as well (Allison, 1970). There are reports of the effects of ALS on animals treated with chemical (Fisher, Soliman and Fisher, 1969; Balner and Dersjant, 1969; Woods, 1969; Rabbat and Jeejebhoy, 1970; Wagner and Haughton, 1971; Haran-Ghera and Lurie, 1971) or viral carcinogens (Allison, Berman and Levey, 1967; Hirsch and Murphy, 1968; Law, Ting and Allison, 1968; Vandeputte, 1969; Burstein and Allison, 1970).

While there is evidence for an increased susceptibility to the action of oncogenic viruses following ALS treatment, it was not indisputably evident that ALS had the same enhancing effect on chemically induced tumours. In addition, there is relatively little in the literature on the effects of ALS on lymphoma and other tumours induced by 7,12-dimethylbenz-

(α)anthracene (DMBA) given at birth in mice. In a previous paper (Baroni *et al.*, 1972) we described the effects of a single administration of ALS and normal rabbit serum (NRS) on tumours induced by DMBA given at birth in mice. Since both ALS and NRS seemed equally to influence the incidence of the tumours, we thought it worthwhile to study the possible effects of repeated injections of ALS and NRS on tumours induced by DMBA given at birth.

MATERIAL AND METHODS

Charles-River mice from the colony of this Institute were used in the present experiments. They are derived from a stock obtained in 1968 and since then maintained in our laboratory by brother \times sister mating. Newborn mice were injected subcutaneously with 7,12-dimethylbenz(α)anthracene (DMBA) (Eastman Organic Chemicals) according to a procedure already described (Rappaport and Baroni, 1962).

The preparation, titration and characteristics of ALS and NRS used in the present study have been described previously (Baroni, Kimball and Wagar, 1969; Baroni *et al.*, 1972).

The mice were divided in 5 main groups as follows: Group 1 treated with DMBA at birth; Group 2 treated with DMBA at birth and with 10 intraperitoneal injections of 0.10 ml of ALS at weekly intervals (first injection at birth); Group 3 treated with DMBA at birth and with 7 intraperitoneal injections of ALS at daily intervals according to one of the following schedules: 0.05 ml of ALS from birth to Day 7 (Group 3A); 0.1 ml of ALS from Day 8 to Day 15 (Group 3B), 0.1 ml of ALS from Day 16 to Day 21 (Group 3C), or 0.1 ml of ALS from Day 22 to Day 29 (Group 3D); Group 4 treated with DMBA at birth and with NRS given as designed for ALS in Group B to parallel hosts; Group 5 treated with DMBA at birth and with NRS given as designed for ALS in Groups 3 to parallel hosts (Groups 5A, 5B, 5C, 5D).

All mice were checked weekly and observed until death occurred. Few of them were killed when moribund. The incidence and histology of the tumours have been determined.

After allowance for the effect of mortality, the *chi*-squared distribution on one degree of freedom has been calculated in order to assess the possible difference in tumour incidence among the various experimental groups. Since spontaneous incidence of tumours is remarkably low in our colony of Charles-River mice, these data are not reported.

RESULTS

Table I shows tumour crops and survival rates. Treatment of very young mice with ALS (Group 3A) or chronic administration of the serum for 10 weeks (Group 2) apparently reduced the mean survival time. On the other hand, administration of ALS in the other experimental groups had less effect on mortality. Table II presents the expected malignant lymphoma, lung and subcutaneous tumour crops assuming that DMBA, ALS and NRS treatments are equally carcinogenic. In Table III are shown the observed and expected tumours for all periods considered in Tables

I and II. In Table IV the various experimental groups are compared with each other after allowance for the effect of mortality. It is now clear that treatment with ALS increases the number of malignant lymphomata only if the antiserum is administered during the first week of life (Group 3A). In fact, the comparison of Group 1 with Group 3A and of Group 3A with Group 5A indicates that the increased number of malignant lymphomata observed in Group 3A is statistically significant and due to the ALS treatment. A statistically significant increased lung tumour count was noticed in the group injected with ALS during the second week of life (Group 3B).

Administration of ALS in the other experimental groups did not influence the incidence of tumours induced by DMBA.

DISCUSSION

We have reported in a previous paper that a single injection of ALS was clearly incapable either of preventing or increasing the appearance of tumours induced in mice by DMBA given at birth (Baroni *et al.*, 1972). We anticipated that these negative results could depend on timing, dosage and number of injections of ALS. In the present set of experiments we observed a markedly increased number of lymphoma bearing mice in the group given the antiserum for the first 7 days of life. The fact that such increase of lymphoma induction was obtained only in the group given ALS during the first week of life could be explained, assuming that malignant cellular transformation in the lymphoid tissue starts immediately after DMBA injection (Rappaport and Baroni, 1962). Thus, ALS given at the same time and shortly after DMBA treatment (Group 3A), *e.g.*, during the very initial phases of malignant transformation (Rappaport and Baroni, 1962), could be expected to exert rapid and long-lasting effects on the immunocompetence of the host, allowing unimpeded growth of tumour cells.

TABLE I.—*Tumour Crops Discovered at Autopsy in the Various Experimental Groups*

Group	Periods																	
	1 (0-5 weeks)			2 (6-10 weeks)			3 (11-15 weeks)			4 (16-20 weeks)			5 (21-25 weeks)			6 (26-30 weeks)		
	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST
1	0/16	0/16	0/16	0/4	0/4	0/4	2/0	0/2	0/2	2/4	4/2	0/6	1/6	2/5	1/6	3/1	1/3	1/3
2	0/18	0/18	0/18	4/8	3/9	0/12	0/2	2/0	2/0	3/3	3/3	1/5	4/5	5/4	1/8	2/2	1/3	1/3
3A	0/23	0/23	0/23	1/6	4/3	0/7	1/0	0/1	0/1	3/2	1/4	2/3	1/0	1/0	0/1	2/0	1/1	0/2
3B	0/28	1/27	0/28	0/7	1/6	1/6	1/0	0/1	0/1	0/0	0/0	0/0	4/4	7/1	0/8	2/7	9/0	2/7
3C	0/10	0/10	0/10	2/2	2/2	0/4	2/2	0/4	0/4	0/0	0/0	0/0	0/4	2/2	2/2	2/1	2/1	0/3
3D	0/10	0/10	0/10	0/0	0/0	0/0	1/0	0/1	0/1	0/0	0/0	0/0	3/6	5/4	0/9	1/5	3/3	2/4
4	0/14	0/14	0/14	0/3	2/1	0/3	1/4	2/3	1/4	2/2	3/1	0/4	1/3	1/3	0/4	0/1	0/1	0/1
5A	0/15	0/15	0/15	0/1	0/1	0/1	0/4	0/4	0/4	0/2	2/0	0/2	0/0	0/0	0/0	2/3	3/2	0/5
5B	0/10	0/10	0/10	0/0	0/0	0/0	0/4	3/1	1/3	3/4	7/0	0/7	2/2	3/1	0/4	4/0	1/3	1/3
5C	0/12	0/12	0/12	0/1	0/1	0/1	0/3	2/1	0/3	4/5	3/6	0/9	0/2	2/0	1/1	1/0	0/1	1/0
5D	0/12	0/12	0/12	0/1	0/1	0/1	2/4	2/4	1/5	1/1	2/0	0/2	2/2	3/1	0/4	0/1	1/0	0/1

Group	Periods																	
	7 (31-35 weeks)			8 (36-40 weeks)			9 (41-45 weeks)			10 (46-50 weeks)			11 (51-55 weeks)			12 (56-60 weeks)		
	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST
1	2/0	0/2	1/1	0/6	4/2	0/6	1/0	1/0	0/1	0/1	1/0	0/1	0/0	0/0	0/0	0/1	0/1	0/1
2	2/4	4/2	1/5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3A	1/0	1/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3B	1/0	1/0	0/1	0/1	0/1	0/1	0/2	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
3C	1/2	3/0	0/3	0/1	0/1	0/1	0/3	0/3	0/3	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0
3D	1/4	4/1	0/5	0/0	0/0	0/0	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0
4	2/3	2/3	1/4	0/0	0/0	0/0	0/2	0/2	0/2	0/1	0/1	0/1	0/0	0/0	0/0	0/1	0/1	0/1
5A	3/4	3/4	2/5	2/1	0/3	0/3	0/0	0/0	0/0	0/2	0/2	0/2	0/0	0/0	0/0	0/0	0/0	0/0
5B	0/6	2/4	0/6	0/3	0/3	0/3	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/1
5C	1/2	1/2	0/3	0/0	0/0	0/0	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
5D	1/7	5/3	1/7	0/0	0/0	0/0	0/3	0/3	0/3	0/2	0/2	0/2	0/1	0/1	0/1	0/0	0/0	0/0

ML = Malignant lymphoma.
 LT = Lung tumours.
 ST = Subcutaneous tumours.

KEY: a/b: a = No. of these autopsies at which tumour(s) were found.
 b = No. of autopsies of animals which did not have a tumour diagnosed before death and which did not die of a tumour.

TABLE II.—*Expected Tumour Crops Assuming that All Treatments are Equally Carcinogenic (From Table I)*

Group	Periods																	
	1 (0-5 weeks)			2 (6-10 weeks)			3 (11-15 weeks)			4 (16-20 weeks)			5 (21-25 weeks)			6 (26-30 weeks)		
	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST
1	0.00	0.09	0.00	0.84	1.71	0.10	0.00	1.00	0.35	3.13	3.12	0.47	3.17	7.38	0.63	0.90	3.66	0.75
2	0.00	0.10	0.00	1.69	3.85	0.30	0.86	0.00	0.00	2.34	4.68	0.39	2.64	5.90	0.85	1.80	3.66	0.75
3A	0.00	0.13	0.00	1.27	1.28	0.17	0.00	0.50	0.17	1.56	6.25	0.23	0.00	0.00	0.10	0.00	1.22	0.50
3B	0.00	0.16	0.00	1.48	2.57	0.15	0.00	0.50	0.17	0.00	0.00	0.00	2.11	1.47	0.85	6.33	0.00	1.75
3C	0.00	0.05	0.00	0.42	0.85	0.10	0.86	2.00	0.71	0.00	0.00	0.00	2.11	2.95	0.21	0.90	1.22	0.75
3D	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.50	0.17	0.00	0.00	0.00	3.17	5.90	0.95	4.52	3.66	1.00
4	0.00	0.08	0.00	0.63	0.42	0.07	1.73	1.50	0.71	1.56	1.56	0.31	1.58	4.42	0.42	0.90	1.22	0.25
5A	0.00	0.08	0.00	0.21	0.42	0.02	1.73	2.00	0.71	1.56	0.00	0.15	0.00	0.00	0.00	2.71	2.44	1.25
5B	0.00	0.05	0.00	0.00	0.00	0.00	1.73	0.50	0.53	3.13	0.00	0.55	1.05	1.47	0.42	0.00	3.66	0.75
5C	0.00	0.07	0.00	0.21	0.42	0.02	1.30	0.50	0.53	3.91	9.37	0.71	1.05	0.00	0.10	0.00	1.22	0.00
5D	0.00	0.07	0.00	0.21	0.42	0.02	1.73	2.00	0.89	0.78	0.00	0.15	1.05	1.47	0.42	0.90	0.00	0.25

Group	Periods																	
	7 (31-35 weeks)			8 (36-40 weeks)			9 (41-45 weeks)			10 (46-50 weeks)			11 (51-55 weeks)			12 (56-60 weeks)		
	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST	ML	LT	ST
1	0.00	2.47	0.14	1.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.87	2.47	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3A	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3B	0.00	0.00	0.14	0.16	0.40	0.00	0.15	0.15	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3C	0.93	0.00	0.43	0.16	0.40	0.00	0.23	0.23	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3D	1.87	1.23	0.73	0.00	0.00	0.00	0.07	0.07	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	1.40	3.71	0.58	0.00	0.00	0.00	0.15	0.15	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5A	1.87	4.95	0.73	0.16	1.20	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5B	2.81	4.95	0.87	0.50	1.20	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5C	0.93	2.47	0.43	0.00	0.00	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5D	3.28	3.71	1.02	0.00	0.00	0.00	0.23	0.23	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00

ML = Malignant lymphoma.
 LT = Lung tumours.
 ST = Subcutaneous tumours.

TABLE III.—*Observed and Expected Tumours for All Periods (From Tables I and II)*

Group	Overall observed			Overall expected			Ratio		
	ML	LT	ST	ML	LT	ST	ML	LT	ST
1	11	13	3	9.04	20.23	2.44	1.21	0.64	1.22
2	15	18	6	11.20	20.66	3.02	1.33	0.87	1.98
3A	9	8	2	2.83	9.38	1.31	3.18	0.85	1.52
3B	8	19	3	10.23	5.37	3.06	0.78	3.53	0.98
3C	7	9	2	5.61	7.82	2.20	1.24	1.15	0.90
3D	6	12	2	9.63	11.53	2.85	0.62	1.04	0.70
4	6	10	2	7.95	13.18	2.34	0.75	0.75	0.85
5A	7	8	2	8.24	11.34	2.86	0.84	0.70	0.69
5B	9	16	2	9.29	11.90	3.12	0.96	1.34	0.63
5C	6	8	2	7.47	14.12	1.79	0.80	0.56	1.11
5D	6	13	2	8.18	8.15	2.75	0.73	1.59	0.72

ML = Malignant lymphoma.

LT = Lung tumours.

ST = Subcutaneous tumours.

TABLE IV.—*Influence of ALS and NRS on Tumour Induction*

Groups compared	Chi-squared for		
	Lymphoma	Lung tumours	Subcutaneous tumours
1-2	1.713	2.925	3.068
1-4	0.902	3.350	0.177
2-4	1.767	1.109	2.990
1-3A	13.875	2.786	0.491
1-5A	0.610	3.566	0.386
3A-5A	13.637	1.186	0.621
1-3B	0.910	37.178	0.129
1-5B	0.433	3.995	0.529
3B-5B	0.495	36.007	0.402
1-3C	0.768	2.761	0.146
1-5C	0.713	5.235	0.152
3C-5C	0.633	2.830	0.042
1-3D	1.792	2.602	0.266
1-5D	1.004	5.469	0.332
3D-5D	1.948	2.905	0.342

1 degree of freedom: 5% = 3.841

1% = 6.635

The present results have also demonstrated that treatment with ALS after DMBA does not significantly affect lymphoma induction. These data suggest that ALS fails to exert an immunosuppressive effect upon that part of the immune system already committed by the newly formed malignant lymphoma tumour specific transplantation antigens.

A noteworthy feature was also the increased incidence of lung tumours observed in the group given ALS during the second week of life (Group 3B). This finding could be tentatively explained assuming that lung cells are less sus-

ceptible to the action of DMBA than lymphoid cells, and consequently lung cellular malignant transformation starts later. If so, the immunodepression following treatment with ALS during the second week of life could account for the increased number of lung tumours observed in this group. This in spite of the fact that in the lung the concentration of the carcinogen could be expected to be higher than in lymphoid tissues receiving their blood supply *via* the greater circulation, since the lung is the first tissue reached by DMBA injected subcutaneously.

In conclusion, the present results agree with those of other investigators (Balner and Dersjant, 1969; Rabbat and Jeejebhoy, 1970) and indicate that, under certain experimental conditions, treatment with ALS is capable of enhancing tumours induced by DMBA given at birth.

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REFERENCES

- ALLISON, A. C. (1970) Tumour Development following Immunosuppression. *Proc. R. Soc. Med.*, **63**, 1077.
 ALLISON, A. C., BERMAN, L. D. & LEVEY, R. H. (1967) Increased Tumour Induction by Adenovirus Type 12 in Thymectomized Mice and

- Mice Treated with Anti-lymphocyte Serum. *Nature, Lond.*, **215**, 185.
- BALNER, H. & DERSJANT, H. (1969) Increased Oncogenic Effect of Methylcholanthrene after Treatment with Anti-lymphocyte Serum. *Nature, Lond.*, **224**, 376.
- BARONI, C. D., KIMBALL, J. W. & WAGAR, R. D. (1969) A Histological and Immunological Study of the Effect of Rabbit Anti-mouse Lymphocyte Serum (ALS) in Balb/c Mice. *Transplantation*, **7**, 303.
- BARONI, C. D., MINGAZZINI, P., PESANDO, P., CAVALLERO, A., UCCINI, S. & SCELSEI, R. (1972) Immunodepressione e Cancerogenesi Chimica. I. Effetti di un'unica Dose di Siero di Coniglio Antilinfociti di topo sui Tumori Indotti nel topo dal 7,12-dimetilbenz(α)antracene (DMBA) Somministrato alla Nascita. *Tumori*, **58**, 397.
- BURSTEIN, N. A. & ALLISON, A. C. (1970) Effect of Antilymphocytic Serum on the Appearance of Reticular Neoplasms in SJL/J Mice. *Nature, Lond.*, **225**, 1139.
- FISHER, E. R., SOLIMAN, O. & FISHER, B. (1969) Effect of Antilymphocyte Serum on Parameters of Growth of MCA-induced Tumours. *Nature, Lond.*, **221**, 287.
- HABAN-GHERA, N. & LURIE, M. (1971) Effect of Heterologous Antithymocyte Serum on Mouse Skin Tumorigenesis. *J. natn. Cancer Inst.*, **46**, 106.
- HIRSCH, M. S. & MURPHY, F. A. (1968) Effects of Anti-thymocyte Serum on Rauscher Virus Infection of Mice. *Nature, Lond.*, **218**, 478.
- LAW, L. W., TING, R. C. & ALLISON, A. C. (1968) Effects of Antilymphocyte Serum on Induction of Tumours and Leukaemia by Murine Sarcoma Virus. *Nature, Lond.*, **220**, 611.
- RABBAT, A. G. & JEEJEBOY, H. F. (1970) Heterologous Antilymphocyte Serum (ALS) Hastens the Appearance of Methylcholanthrene-induced Tumors in Mice. *Transplantation*, **9**, 164.
- RAPPAPORT, H. & BARONI, C. D. (1962) A Study of the Pathogenesis of Malignant Lymphomas Induced in the Swiss Mouse by 7,12-dimethylbenz-(α)anthracene Injected at Birth. *Cancer Res.*, **22**, 1067.
- VANDEPUTTE, M. (1969) Antilymphocytic Serum and Polyoma Oncogenesis in Rats. *Transplantn. Proc.*, **1**, 100.
- WAGNER, J. L. & HAUGHTON, G. (1971) Immunosuppression by Antilymphocyte Serum and its Effect on Tumors Induced by 3-Methylcholanthrene in Mice. *J. natn. Cancer Inst.*, **46**, 1.
- WOODS, D. A. (1969) Influence of Antilymphocyte Serum on DMBA Induction of Oral Carcinomas. *Nature, Lond.*, **224**, 276.