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Influence of Genotype of Host on Regression of Solid and Ascitic Forms of Sarcoma 180 and Effect of Chemotherapy on the Solid Form[1]

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SUMMARY

Solid Sarcoma 180 implanted s.c. into Swiss albino mice regularly produces a tumor that spontaneously regresses in a low proportion of hosts. A statistically significant increase in the regression ratio has been observed following therapy with basidiomycete polysaccharide, with polyinosinic-polycytidylic acid at a high dose, and with zymosan. The regression ratio was not increased by Protodyne or chlorphenesin.

In several strains of inbred mice that carry the *H-2*^{d} allele (homozygous or heterozygous), in contrast to Swiss mice, no spontaneous regression of Sarcoma 180 is observed, nor was regression induced in these mice by the chemicals that enhanced the ratio in Swiss mice. In the strains of mice lacking the *H-2*^{d} that were tested, various regression ratios up to 100% were observed.

The ascitic form implanted s.c. produced tumors that regressed in the various strains of mice in ratios similar to those observed after solid-tumor implantation. In contrast, the ascitic form inoculated i.p. produced ascites from which mice uniformly died within 10 to 12 days, regardless of the genotype of the host.

INTRODUCTION

In random-bred Swiss albino mice bearing Sarcoma 180, a certain degree of histoincompatibility is expressed by a spontaneous tumor regression observed in 10 to 20% of hosts. This regression rate can be increased significantly by treatment with certain chemicals or biological products (2-6, 10) or by feeding pyridoxine-deficient diet (7, 9). Some of the observed tumor regression have been attributed to stimulation of immunological defense mechanisms. Such stimulation is thought to be largely dependent upon histocompatibility differences between tumor and host, since regression rate in a compatible host (DBA/2) was not increased by chemical treatment, except by high i.v. doses of zymosan (4).

In the present study Swiss albino mice were reexamined for their suitability for primary screening of chemicals and biological products for enhancement of regression rate of Sarcoma 180, even though this tumor spontaneously regresses in a certain proportion of individuals. In addition, the effect of genotype of inbred murine strains was investigated with respect to enhancement of regression rate under chemotherapy and to median survival time, in response to inoculation of the solid and of the ascitic form of Sarcoma 180 by various routes.

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MATERIALS AND METHODS

The strain of Sarcoma 180 used in the present study has been continuously propagated since 1919 first at Memorial Hospital and later in the Sloan-Kettering Institute (14). It is maintained in female Swiss mice obtained from the Taconic Farms, Germantown, N.Y.

Ascitic form of Sarcoma 180 has been repeatedly induced in Swiss albino mice in this laboratory by i.p. or i.m. (hind leg) inoculation of Sarcoma 180, uniformly producing in all mice, after 8 days of growth, 2 to 3 ml ascitic fluid containing 5 to 8 $\times$ 10^7 cells/ml. In this study the ascitic form was injected either i.p. or s.c., each inoculum consisting of 1 $\times$ 10^7 cells suspended in 0.1 ml 0.85% NaCl solution.

The solid form of Sarcoma 180 was inoculated by trocar s.c. into a single site in the right axillary region of mice. Chemical treatment of such inoculated mice, started as a rule on the day after tumor implantation, consisted of i.p. doses given once a day for 6 days. (Chlorphenesin was administered subdivided into 2 half-doses, given 1 in the morning and 1 in the afternoon 6 hr after the first half-dose.)

Controls consisted of mice of the same lots, either untreated or given injections (on the same schedule as the chemically treated mice) of 0.2 ml 0.85% NaCl solution.

The following inbred mouse strains were used: A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BL/KsJ, DBA/2J, and SJL/J were from The Jackson Laboratory, Bar Harbor, Maine; 1st-generation hybrid strains AKR/J $\times$ DBA/2J F_1, C57BL/6J $\times$ DBA/2J F_1, BALB/cJ $\times$ A/J F_1, and C3H/HeJ $\times$ DBA/2J F_1 (hereafter called AKD2F_1, B6D2F_1, CAF_1, and C3D2F_1, respectively) were obtained from the same source and Swiss-Webster albino mice were from the Taconic Farms.

Chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) and Protodyne, a basic protein from *Escherichia coli*, were

Table 1
Proportion of mice showing regression of Sarcoma 180 after chemotherapy

Swiss-Webster albino female mice were implanted s.c. with Sarcoma 180; therapy was started 24 hr later. Chemicals in 0.2-ml doses were administered i.p. once a day for 6 days.

Chemical					0.85% NaCl solution			Significance of difference	
Name	Dose[a] (mg/kg/day)	No. of tests	Total no. of mice	Regression[b] ratio	No. of tests	Total no. of mice	Regression ratio	Differences between regression ratios	p[c]
Chlorphenesin	50-200	3	84	0.24	3	28	0.21	0.03	N.S.[d]
Protodyne	31.3-125	3	69	0.26	3	23	0.26	0.00	N.S.
Poly(I,C)	1.25-5.0	3	90	0.26				0.09	N.S.
	10	3	30	0.47	3	30	0.17	0.30	<0.01
Basidiomycete polysaccharide	31.3-500	4	125	0.42	4	25	0.24	0.18	<0.01
Zymosan	15.6-250	3	107	0.47	3	23	0.13	0.34	<0.01

[a] Range of doses tested at 2-fold increments, including the maximal tolerated dose. Where the outcome was not dose dependent, the results have been pooled.

[b] Regression ratio: no. of mice alive without tumor at 60 days/ no. of mice alive at 8 days; deaths before Day 8 are attributed to toxicity of chemical.

[c] *p:* probability that observed difference between regression ratios (treated – pooled controls, NaCl solution) is statistically significant at indicated level, by *z* test.

[d] N.S., not significant ($p > 0.05$.)

supplied by Wallace Laboratories, Cranbury, N. J.; mucopolysaccharide, obtained from the extract of the mycelium of a basidiomycete, was provided by Kureha Chemical Industries Co., Ltd., Tokyo, Japan; poly(I,C)[2] was prepared by Dr. L. D. Hamilton, Brookhaven National Laboratory, Brookhaven, N. Y., and zymosan was purchased from Nutritional Biochemical Corporation, Cleveland, Ohio.

All the chemicals were dissolved in 0.85% NaCl solution and adjusted to pH 7.2 with sodium bicarbonate or HCl.

RESULTS

Solid Form of Sarcoma 180. The proportion of Swiss albino female mice showing spontaneous repression after implantation of Sarcoma 180 ranged from 13 to 26% in tests involving 129 control mice (Table 1). Of the 5 chemicals tested the 2 polysaccharides (basidiomycete mucopolysaccharide and zymosan) significantly enhanced the proportion of mice showing regression, independently of dose, over a wide range of doses. Poly(I,C) was ineffective at lower doses, but at the maximally tolerated dose of 10 mg/kg/day it significantly increased the regression rate. Chlorphenesin and Protodyne, at any dose used, had no significant effect on the Sarcoma 180 regression ratio, thus failing to confirm a preliminary report on enhanced regression rate in Sarcoma 180 (13).

In the search for a strain of mice in which Sarcoma 180 would not regress spontaneously, the regression ratio at 60 days and the median survival time of the hosts were examined in 7 inbred, 4 F_1 hybrid, and Swiss albino strains of mice. No significant difference in the outcome of these tests was observed between the female and male mice in 4 strains tested or between the animals treated with 0.85% NaCl solution and those left untreated. Therefore, the results in these pairs of categories have been pooled (Table 2). There were no spontaneous regressions of Sarcoma 180 growing in 3 strains of mice homozygous for *H-2*^{d} or in 4 strains heterozygous for *H-2*^{d}. Four other strains lacking the *H-2*^{d} allele showed regression ratios from 38 to 100%. The clinical picture of regression of Sarcoma 180, consisting of extensive necrosis, ulceration, reduction in size, and finally disappearance of tumor mass, was the same as that

Table 2
Effect of genotype on regression ratio and on survival time of mice after s.c. implantation of Sarcoma 180

Groups of equal size were untreated or were treated with 0.85% NaCl solution[a] (0.2 ml once a day for 6 days, beginning 24 hr after implantation).

Strain	Mice *H-2* alleles	Sex	No. of tests (5 mice each)	Regression ratio[b]	Median survival time (days)
BALB/cJ	*dd*	F	8	0.00	25 ± 1.2[c]
DBA/2J	*dd*	F	8	0.00	22 ± 0.9
C57BL/KsJ	*dd*	F	8	0.00	27 ± 1.1
CAF_1/J	*da*	F	8	0.00	28 ± 1.5
$B6D2F_1/J$	*db*	F + M[d]	16	0.00	30 ± 1.1
$AKD2F_1/J$	*dk*	F + M	16	0.00	32 ± 0.9
$C3D2F_1/J$	*dk*	F	8	0.00	27 ± 0.9
A/J	*aa*	F	8	0.38	38 ± 1.4
AKR/J	*kk*	F	8	1.00	>60
C3H/HeJ	*kk*	F	8	0.38	31 ± 2.8
C57BL/6J	*bb*	F + M	16	0.65	>60
Swiss albino		F + M	16	0.19	28 ± 1.7

[a] Since outcome in mice with and without treatment with 0.85% NaCl solution showed no significant difference, the results were pooled.

[b] See Table 1, Footnote *b*.

[c] Median ± S.E.

[d] No sex-related difference in outcome was observed; results from an equal number of male and of female mice were pooled.

[2] The abbreviation used is: poly(I,C), polyinosinic-polycytidylic acid.

described by Bradner *et al.* (3), except in the AKR/J mice, in which the regressing tumors frequently did not break through the skin or extensively necrotize but gradually became smaller under the intact skin and disappeared. Regressions occurred during the 3rd to 5th weeks of tumor growth. There was no reappearance of tumors at the site of inoculation during the subsequent observation period extending to the 60th day of tumor growth.

In all but 2 of the 12 strains studied, including Swiss albino, the median survival time of mice bearing Sarcoma 180 ranged from 22 to 38 days with a narrow distribution of days of death within a strain. Median survival time was strikingly greater for AKR/J and C57BL/6J mice, the majority of which survived 60 days without tumors.

The 2 polysaccharides that had enhanced the regression ratio of Sarcoma 180 in Swiss albino mice were administered to mice of $AKD2F_1/J$ and CAF_1/J strains, heterozygous for *H-2*^{d} in which no spontaneous regression of Sarcoma 180 had been observed. Treatment with zymosan, basidiomycete polysaccharide, or poly(I,C) by i.p., s.c., or i.v. route, using several dose schedules, did not lead to regression of Sarcoma 180 in any case.

Ascitic Form (Sarcoma 180A). Female mice of 6 inbred strains and Swiss albino mice were inoculated s.c. or i.p. with 1×10^7 tumor cells. The tumor that developed at the site of each s.c. inoculation in all mice did not regress in any of the BALB/c mice (Table 3); in the 6 other strains, the regression ratio varied from 6 to 94%

The median survival time of mice bearing solid tumors obtained by s.c. inoculation of the ascitic form of Sarcoma 180 varied from 14 to 23 days in 4 strains of mice and exceeded 42 days in 3 other strains.

In contrast, all mice of all 7 strains, bearing Sarcoma 180A developed ascites with a median survival time varying from 10 to 12 days, showing a narrow distribution of time of individual deaths.

DISCUSSION

Results of the present study justify the use of Swiss albino mice for the primary screening of chemicals and biological products for their ability to enhance the regression rate of Sarcoma 180. All mice of this noninbred strain initially support growth of Sarcoma 180 after implantation, although from 10 to 20% of them subsequently show spontaneous regression. Under chemotherapy, a statistically significant increase of the tumor regression ratio could be demonstrated following treatment with 2 polysaccharides (*Basidiomycetes* mucopolysaccharide and zymosan) and by the maximal tolerated dose of poly(I,C). Perhaps in these mice the borderline state with regard to spontaneous regression reflects an instability necessary for demonstration of enhancement of regression by certain agents. No enhancement of regression followed therapy with a bacterial protein, Protodyne, or a synthetic chemical, chlorphenesin.

Enhancement of regression of Sarcoma 180 by chemotherapy is probably mediated by increased immune responses of the host to transplantation antigens of the tumor other than those determined by the *H-2* histocompatibility loci, since (*a*) no spontaneous regression of Sarcoma 180 has been observed in mice homozygous or heterozygous with respect to the *H-2*^{d} allele of the *H-2* locus and (*b*) chemicals capable of enhancement of the regression ratio of Sarcoma 180 in Swiss albino mice were inactive against Sarcoma 180 growing in $AKD2F_1/J$ or CAF_1/J mice that are heterozygous with respect to the *H-2*^{d} allele.

Snell *et al.* (11) have reported that Sarcoma 180 shows a definite affinity for mice of the *H-2*^{d} lines in which it does not regress spontaneously. Snell and Stimpfling (12) have pointed out that the histocompatibility between the host and the graft which is determined by the matching of their *H-2* loci is a necessary but not entirely sufficient condition for the progressive growth of the graft. Differences in "minor" histocompatibility loci existing between Sarcoma 180 and its hosts determine the specificities of "weaker" antigens and could account for the difference in the proportion of spontaneous regression in AKR/J and C3H/HeJ mice that both have the *H-2*^{k}/*H-2*^{k} genotype.

Antigens from several microbial species can interfere with the activity of cytotoxic factors directed against the mammalian cells, and these effects do not appear to be

Table 3
Effect of genotype on regression and on survival time of mice after s.c. or i.p. inoculation of Sarcoma 180A

Female mice were inoculated with 1×10^7 cells of Sarcoma 180A; no treatment. They were observed for 6 weeks.

Mice			s.c. inoculation		i.p. inoculation	
Strain	*H-2* alleles	No. of tests (8 mice each)	Regression ratio[a]	Median survival time (days)	No. of tests (8 mice each)	Median survival time (days)
BALB/cJ	*dd*	2	0.00	23 ± 1.9[b]	2	10 ± 0.3
A/J	*aa*	2	0.06	14 ± 0.9	2	10 ± 0.4
AKR/J	*kk*	2	0.94	>42	2	10 ± 0.4
C3H/HeJ	*kk*	2	0.57	>42	2	12 ± 0.4
C57BL/6J	*bb*	2	0.31	16 ± 1.0	2	10 ± 0.9
SJL/J	*ss*	2	0.81	>42	2	11 ± 0.4
Swiss albino		2	0.38	>42	2	12 ± 1.6

[a] See Table 1, Footnote *b*.
[b] Median ± S.E.

directed against any particular *H-2* specificity (1). Recent comparison of the peptide composition of 2 *H-2* alloantigens obtained from mouse tumor cells (15) has indicated that the antigenic specificity of these glycoproteins is determined primarily by primary amino acid structure, removal of the sugar residues results in no loss of antigenic reactivity, and the specificity of alloantigens does not seem to be the result of the differential association of sugars to a common peptide backbone. These observations might, at least in part, explain the inability of microbial polysaccharides to disrupt the histocompatibility between the $H\text{-}2^d$ mice and Sarcoma 180.

After s.c. inoculation, the compatibility of Sarcoma 180 and Sarcoma 180A was essentially the same, with a few exceptions, with regard to regression ratio as well as to median survival time. In contrast, ascitic tumors developing after i.p. inoculation of Sarcoma 180A killed the murine hosts regularly in 10 to 12 days, regardless of genotype. Evidently, the ascitic form produces the type 2 response described by Gorer (8) in which the tumors grown s.c. do not evoke the formation of the fibrovascular stroma by the host but virtually vascularize themselves. In noncompatible mice the immune response of the host to the s.c. inocula of Sarcoma 180A is then directed against the stroma and blood vessels that have developed from the inoculated tumor cells.

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