Cytotoxic Activity of Isoantibody on Sarcoma 1.* (27762)

I. CHOUROULINKOV,† E. A. BOYSE, AND L. J. OLD (Introduced by C. A. Stetson)
Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division, Cornell Medical College, and Department of Pathology, New York University School of Medicine, New York City

The purpose of this report is to record the sensitivity of the A-strain tumor Sarcoma 1 to the cytotoxic effects of H-2 isoantibody in the mouse. With recent modifications of the cytotoxic test *in vitro* it has been possible to demonstrate the complete sensitivity of certain tumors which previously were thought to contain a proportion of cells resistant to isoantibody(1,2). Sarcoma 1 has been regarded as completely resistant to isoantibody both *in vitro* and *in vivo*(3-9). This finding has played a prominent part in the prevailing impression that many examples of homograft rejection cannot be attributed entirely to formation of specific isoantibody(3,10,11).

Materials and methods. Tumors. Two ascites lines of Sarcoma 1 have been examined.‡ The history of this A-strain tumor is recorded elsewhere(12).

Antisera. Immune sera were prepared by conventional methods in either C57BL/6 or I strain mice: Sarcoma 1 was inoculated 3-4 times in increasing dosage over a period of 2-3 months, the last inoculum (i.p.) containing 50-100 million cells.

Cytotoxic test in vitro. Ascites cells were harvested at 5 or 6 days at which time there is little contamination with erythrocytes and the viscous material that is characteristic of this tumor during its later growth phase. Following gentle centrifugation of the ascites fluid the cells were resuspended in Medium 199 (Microbiological Associates, Bethesda) at a desired cell concentration.

The cytotoxic test described by Gorer and O'Gorman(13) was used in a modified form(1,2). Antiserum dilutions and cells were incubated in small tubes for 30 minutes in a water bath at 37°C. Complement (½ dilution of pooled guinea pig serum) was then added, followed by further incubation for 60 minutes. The volume used was 0.05 ml for each of the 3 constituents. After incubation the tubes were kept in an ice bath during the time necessary to read the test. 0.05 ml of trypan blue solution(2) was added immediately prior to examination.

Results. Table I shows the results of a cytotoxic test with 4 sera on the same cell suspension. Serum controls, omitting complement, were invariably negative and have therefore not been included. Although the serum prepared in I mice was evidently more potent, C57BL/6 antisera also showed high cytotoxic activity. These observations have been repeated several times and no difference in sensitivity has been found between the 2 sublines of Sarcoma 1.

Table II records a more detailed study of the I anti-Sa 1 serum using a series of Sa 1 cell dilutions. The behavior of this tumor under these conditions is similar to that of other sarcomas(2): as the concentration of cells is increased, the proportion of stained cells declines, despite the availability of excess antibody.

The result of a passive immunity experiment in C57BL/6 males is illustrated in Table III. Complete suppression of growth was observed in 3 of 5 mice receiving 0.5 ml of antiserum intraperitoneally 4 hours before subcutaneous inoculation of 50,000 Sa 1 cells. It is probable that inhibition of this sort can be obtained only with small inocula. With Sa 1 the balance between suppression and enhancement must be extremely delicate: in fact, 2 of 5 mice injected with antiserum showed enhanced growth. Similar suppression has been observed in I mice treated with I anti-Sa 1 serum.

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† Visiting fellow from Institut de Recherches sur le Cancer, Villejuif, Seine, France.

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TABLE I. Simultaneous Cytotoxic Titrations of 4 Different Sera with Sarcoma 1 Ascites Cells.*

	Immune serum	Antiserum dilution 1/2	1/4	1/8	1/16	1/32	1/64	1/128	C′ controls (no immune serum)
1	I ♀ anti-Sa 1	79†	98	100	99	97	97	95	12
2	C57BL/6 ♀ anti-Sa 1	74	85	98	97	92	77	59	14
3	*Idem*	73	88	91	86	66	48	34	16
4	"	71	84	81	66	51	41	30	14

* 2.5×10^6 ascites cells/ml. (C′ source—guinea pig serum 1/2 throughout.)
† % cells stained by trypan blue.

TABLE II. Simultaneous Cytotoxic Titrations of I Anti-Sa 1 Serum against Various Concentrations of Sa 1 Ascites Cells.

Cell conc. 10^6/ml	Antiserum dilution 1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	C′ controls (no immune serum)
32	40*	55	46	41	20	11	—	—	—	—	3
16	87	86	78	78	52	25	10	—	—	—	4
8	91	97	95	90	80	33	26	11	—	—	6
4	93	99	97	96	96	89	54	28	—	—	4
2	92	97	99	99	96	96	87	66	24	—	5

* % cells stained by trypan blue. (C′ source—guinea pig serum 1/2 throughout.)
— less than 10% stained.

Discussion. Previous experience with Sa 1 has seemed to indicate that this tumor is entirely resistant to the cytotoxic effects of isoantibody. However, under the experimental conditions described here, complete sensitivity can be demonstrated. Earlier negative results with Sa 1 *in vitro* must be attributed mainly to technical circumstances in the performance of the test, rather than to special types of immunization, since the sera used here were prepared by the usual methods.

TABLE III. Passive Immunity against A-Strain Sarcoma 1 with C57BL/6 Anti-Sa 1 Serum in C57-BL/6 ♂ Mice.*

Vol of antiserum i.p.	Mouse No.	Tumor size (avg of 2 diameters in mm) on day 5	7	10	13	17	19	21	24
0.5 ml	1	—	—	—	—	—	—	—	—
"	2	—	—	—	—	—	—	—	—
"	3	—	—	—	—	—	—	—	—
"	4	—	—	1	2	4	5	7	8†
"	5	2	5	6	9	9	10	10	10†
None	1	5	7	9	8	5	4	—	—
"	2	7	7	7	6	4	3	—	—
"	3	5	7	6	5	2	+‡	—	—
"	4	4	5	3	—	—	—	—	—
"	5	6	7	8	6	3	+	—	—
"	6	4	5	4	3	+	—	—	—
"	7	5	5	6	5	2	—	—	—
"	8	6	6	6	4	+	—	—	—

* Inoculum — 50,000 Sa 1 ascites cells in 0.1 ml subcut. into shaved skin of flank.
† Enhanced.
‡ Palpable nodule.
— No tumor.

It is not possible to point to any particular feature of the revised cytotoxic test to account for its increased sensitivity. However, the use of a more potent trypan blue preparation is probably important since this greatly facilitates the use of more dilute cell suspensions. Again, the choice of a favorable strain in which to prepare the immune serum may sometimes be critical, since the spectrum of antibodies formed will vary from strain to strain. In this connection it may be noted that the I strain yielded excellent antisera against Sa 1.

Experimental results based on the use of Sa 1 and other tumors have sometimes been used to support the concept of an immune mechanism for tissue rejection entirely separate from antibody formation. Such data, whether apparently showing activity by sensitized cells alone, or their synergic or antagonistic interaction with antibody, are bound to remain indecisive when the target tissue is demonstrably sensitive to isoantibody. Such results will always be open to reasonable interpretation solely in terms of antibody activity.

Summary. Under favorable experimental conditions Sa 1 is completely sensitive to cy-

totoxic isoantibody *in vitro*. Similar results may also be obtained with small inocula of Sa 1 *in vivo*. In view of these results, it no longer seems possible to retain the past distinctions based on differences in isoantibody sensitivity between tumors of different origins.

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Passive Transfer of Immunity to Sarcoma I with Serum.* (27763)

MILDRED E. PHILLIPS AND CHANDLER A. STETSON
Department of Pathology, New York University School of Medicine, New York City

It has not previously been possible to demonstrate passive serum transfer of immunity to Sarcoma I, a transplantable tumor derived from the inbred A strain of mice. When transplanted into these mice or into F_1 hybrids of this strain, this tumor grows progressively and kills the animals, but when transplanted as an allogeneic homograft into mice of other strains it grows only for a limited time and then is rejected by a typical homograft reaction(1,2). Mice which have rejected this tumor are generally found to be specifically immune to subsequent implantation(3). Although the rejection is accompanied by a brisk humoral antibody response (4), previous attempts to demonstrate an *in vivo* or *in vitro* cytotoxic effect of these antibodies on the tumor cells have been negative; in fact, antisera obtained from mice immune to Sarcoma I frequently have been found to produce "enhancement" of growth of this tumor in passive transfer experiments(1-5). This has led to the concept that cells of Sarcoma I are qualitatively "antibody-resistant" and that the homograft reaction against such cells must be mediated by some aspect of the immune response other than isoantibody(1-7).

Improvements in technic, however, have permitted the recent demonstration(8-10) that some cell lines previously thought to be resistant to the cytotoxic effect of isoantibody and complement are, in fact, fully susceptible. It has indeed become of some consequence to determine whether any cell is truly "antibody-resistant," and in the present study the effect of isoantibody on the cells of Sarcoma I has been reinvestigated.

Materials and methods. Male mice of the strains A/Jax, A/HeJ and of the cross (C57Bl/6 × DBA/2), and female mice of the C57Bl/6 strain, were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor. All mice were vaccinated against ectromelia one to 2 weeks before use and were caged in groups of 10 or less before and during experiments. Mice of approximately 25 g in weight were used after randomization into experimental and control groups; all were individually numbered by coded ear punches.

The Sarcoma I was obtained in the ascites form from Dr. George Snell, and was main-

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