Cytotoxic Action of Normal Human Serum on Ehrlich Ascites and Sarcoma 180 Cells.* (25513)

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Our recent studies(1,2) demonstrated markedly cytotoxic action of normal human serum on HeLa cells and spontaneously transformed U12 cells of human origin, serially propagated in tissue culture. No such effect was demonstrable on newly isolated or untransformed cell strains derived from normal tissue. Similar cytotoxicity of human serum was observed on atypical mouse cells, strain L and the DeBruyn Lymphosarcoma. Fedoroff(3,4) described in detail the effects of certain human sera on strain L, particularly that of schizophrenics. Recently Willheim *et al.* (5,6) have shown that normal human serum is able to digest Ehrlich ascites tumor cells, Sarcoma 180 cells, Krebs ascites tumor cells, and Walker rat ascites tumor cells. These observations demonstrate the presence of naturally occurring cytolysins or cytotoxins, whose presence may be related to natural tumor immunity. Further investigations were undertaken to determine if the cytotoxic effect of human serum on Ehrlich ascites tumor and Sarcoma 180 cells was similar to its action on strains HeLa, U12 and the other atypical human cell strains studied(2).

Materials and methods. Ehrlich ascites tumor† (EA) and Sarcoma 180‡ (Sa 180) were perpetuated by serial intraperitoneal injections of Strain CF-1 mice. Ascitic fluid was aseptically aspirated 10 days after injection. Only bloodless fluid was used. The cells were washed by serial centrifugation and suspended in synthetic cell medium S-15(1). Cell suspensions so prepared were incubated at 37°C for 1 hour with doubling saline dilutions of serum or serum reagents. After incubation, cytotoxicity was determined by Eosin staining method(1,7,8). Degree of cytotoxicity is the per cent of eosin-stained cells counted on a hemocytometer. Equivalent numbers of cells of both strains were incubated 1 hour with serum and heated serum. After washing, equal numbers of each pretreated strain were inoculated intraperitoneally into CF-1 mice to compare their ability to produce ascites tumors. Sera used were pools prepared from 25 to 50 donors, aliquots of which were frozen at –40°C and thawed when needed. In general R1, R2, R3, and R4 were prepared according to the method of Ecker *et al.*(9), the R4 prepared with NH_4OH. RP and RPb were prepared fresh for each experiment according to the method of Pillemer *et al.*(9). Serum was inactivated by heating at 56°C 1 hour (Serum Δ). Complement-fixed (CF) serum, deficient in C′1, C′2 and C′4, was prepared by precipitating in the serum, pneumococcal capsular polysaccharide type III with its type specific rabbit antiserum. Equal volumes of serum were adsorbed with doubling numbers of EA and Sa 180 cells to determine if toxic effects could be removed in this way. Aliquots of the EA and Sa 180 adsorbed sera were then tested on viable EA and Sa 180 cells respectively, using Eosin staining technic. Hemolytic titrations for the 4 components of complement were also performed(10) on aliquots of adsorbed serum, to determine if adsorption out of cytotoxicity was associated with inactivation of any of the components of C′.

Results. Toxicity of pooled human serum on both EA and Sa 180 cells is well shown by Eosin staining method. Allowing for variation in activity from one serum pool to another, susceptibility to injury by both cell strains is of approximately the same order; 1 to 5 x 10^6 cells killed/ml of serum. This is approximately 10 times the toxicity for strain U12 previously studied. These effects are

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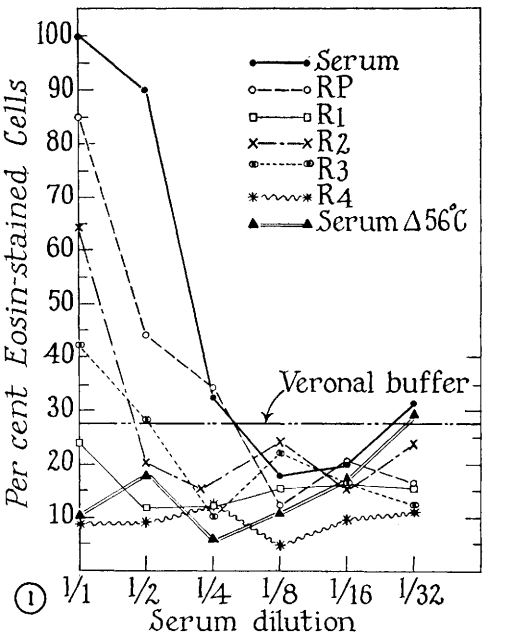
† EA tumor obtained from Atomic Energy Research Project, Western Reserve Univ. School of Med.

‡ Sa 180 obtained from Sloan-Kettering Inst. by Dr. Austin Weisberger.



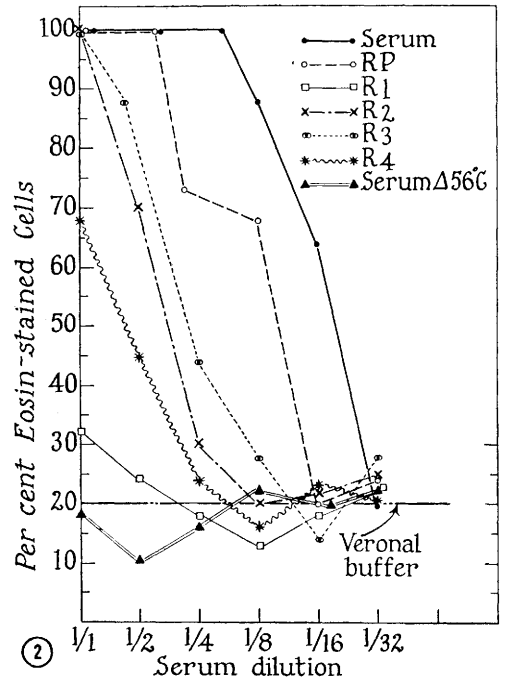


FIG. 1. Cytotoxicity of normal human serum and serum reagents of C′ on EA cells. 4.5×10^5 EA cells/ml of serum, serum reagents, or dilutions thereof, were used. Chart shows lack of cytotoxicity in Serum △, R1, R3, and R4. RP and R2 more closely approximate activity of native serum.

FIG. 2. Cytotoxicity of normal human serum and serum reagents of C′ on Sa 180 cells. 3.6×10^5 Sa 180 cells/ml of serum, serum reagent, or dilutions thereof, were used. Chart shows lack of toxicity mainly in Serum △ and R1. Toxicity of R4 is somewhat greater, while RP, R2, and R3 more closely approximate activity of native serum.

largely abolished by heating or complement-fixation of the serum.

With EA cells, the activity of R1, R3, and R4 is much less than native serum. The activity of RP and R2 is almost that of native serum. Addition of purified human properdin§ (0-30 u/ml) does not enhance activity of RP or RPb. Sa 180 cells are least affected by R1, while R2, R3, R4 and RP were closer to activity of native serum. Some of these observations are summarized in Fig. 1 and 2.

Treatment of EA and Sa 180 cells with serum prior to intraperitoneal inoculation in mice markedly inhibits formation of ascites tumor. 14-21 days following injection, mice so treated develop at most only a minimal accumulation of a parvicellular massive ascites within 1 week (Table I, Fig. 3).

Adsorption of serum with large numbers of cells produces a marked diminution in cytotoxicity for the adsorbing cell strain. Loss of serum toxicity by adsorption with EA and Sa 180 cells was not associated with significant inactivation of any of the components of C′. Results of a typical experiment are embodied in Table II.

Discussion. Heat-labile cytotoxins are present in normal human serum for Ehrlich ascites tumor and Sarcoma 180 cells, similar in some respects to cytotoxins active against human strains U12 and HeLa. A unit volume of serum kills approximately 10 times more EA and Sa 180 cells than U12 cells. Strains Sa 180, EA, U12 and HeLa show a similar ability to detoxify human serum by adsorption.

In all these situations, serum activity bears some relationship to serum complement, since heating and complement-fixation of serum eliminates the toxicity for all cells studied. Behavior of EA cells is more like strain U12 and HeLa in its reactions with the R′ reagents of C′, than Sa 180. Sa 180 deviates strikingly from the pattern, with only R1 being significantly less toxic than native serum. Adsorption of serum cytotoxicity with EA and Sa

§ Purified human properdin obtained from Dr. I. H. Lepow.

TABLE I. Inhibition of Ascites Tumor Formation by Sa 180 and EA Cells Pretreated with Human Serum.

		No. mice	Ascites tumor formation 0	+	++	+++	% ascites takes
Sa 180	Serum	16	16	0	0	0	0
	" △	16	0	2	3	11	100
EA	Serum	19	16	3	0	0	18.75
	" △	12	3	0	0	9	75

180 cells is not associated with significant inactivation of any of the components of C′, whereas with strain U12, this was associated with inactivation of C′2 and particularly C′4 (*2*). In most respects our observations on EA cells are in close agreement with those of Willheim *et al.*(5,6). The major exception is that these workers report marked loss of activity in RP, and hence conclude that properdin may be of importance in the reaction. Our data do not support this contention, since RP and RPb were only slightly less active than native serum and addition of properdin to these reagents did not enhance their activity on EA cells.

These and previous observations reflect several varieties of naturally-occurring cytotoxins in human serum, whose function is the destruction of cells genetically alien to the host.

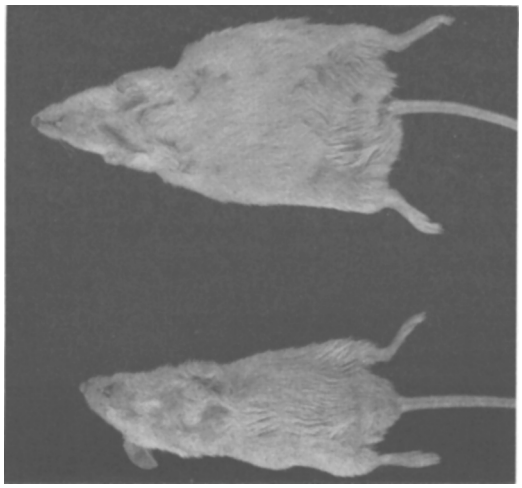


FIG. 3. Effect of prior treatment of Sa 180 cells with Serum and Serum △ on ability to produce ascites tumors. The large mouse had been inoculated 14 days previously with Sa 180 cells preincubated with Serum △. Autopsy revealed massive ascites tumor. The other mouse had been simultaneously inoculated with the same number of cells which had been incubated with normal human serum. Autopsy at 14 days revealed no evidence of ascites tumor.

TABLE II. Effect of Adsorption of Serum with Sa 180 Cells on Cytotoxicity for Sa 180 Cells and Components of C′.

No. adsorbing cells/ml serum	Cytotoxicity (%)*	C′ component titers (units/ml serum) C′1	C′2	C′3	C′4
0 (control)	100	1920	120	120	480
$.045 \times 10^7$	"	960	60	"	320
.090 "	"	"	"	"	"
.18 "	"	"	"	"	"
.37 "	98	"	"	"	"
.75 "	80	"	"	"	"
1.5 "	70	"	60	80	240
3.0 "	42	"	"	80	"
6.0 "	34	"	40	120	"
12.0 "	24	1920	"	80	160

* % of eosin-stained cells.

Summary. (1) Thermolabile, non-dialyzable cytotoxins are present in normal, human serum for Ehrlich ascites and Sarcoma 180 cells. (2) Similarities and differences of these cytotoxins and those active against atypical human cell strains U12 and HeLa are described. (3) Data are presented showing relationship of these cytotoxins to serum complement.

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