Further Studies of the Immunological Properties of Polysaccharides from *Serratia marcescens (Bacillus prodigiosus)*

III. Passive Immunization Against the Lethal Activity of the Polysaccharides with Fractions of Mouse Antiserum Elicited by a Single Injection of Polysaccharide*

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In previous work (1 to 4), it was demonstrated that mice could be protected against the lethal action of polysaccharides from *Serratia marcescens* by procedures of passive immunization which, in certain instances, did not seem to interfere with the tumor-necrotizing action. Moderate amounts of the serum globulin fractions from rabbits given multiple injections of the polysaccharides afforded mice substantial protection only against the lethal action of polysaccharides from the homologous strain of organism. When greater amounts of the rabbit globulin fractions were used, it was noted that the mice were protected also against the serologically unrelated polysaccharides from a different strain of organism. Globulin fractions from sera of mice given a single injection of polysaccharide were found to afford mice bearing sarcoma 37 considerable protection against the homologous polysaccharide and also, in one test, against the heterologous polysaccharide (3). Active immunization of the mouse with the polysaccharides seemed to confer an entirely non-specific protection (3). Because of the indications of lack of strain specificity in the mouse compared with the rabbit, it was considered of interest to study in greater detail the effects of passive immunization with mouse antisera. As the supplies of these particular preparations of polysaccharides are nearly exhausted and since our plans for future investigations with new preparations are different from those already described, it seemed advisable to present the following results as a concluding report of this phase of the study.

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EXPERIMENTAL

The preparations of the polysaccharide-lipid complex used in this work were P-10, P-10a, and P-20 from the 724 strain of *Serratia marcescens* and P-3.M and P-3.Ls from the G.W. strain. As before, these materials were supplied to us by Dr. M. J. Shear of the Chemotherapy Section of the National Cancer Institute.

Except for one special experiment, 7- to 8-week old Swiss mice from our stock colony were injected intraperitoneally with 100 γ of a preparation of polysaccharide and exsanguinated by heart puncture either 3 or 14 days later. The serum was fractionated with ammonium sulfate into the globulin and albumin components. After dialysis, determination of the protein content by micro Kjeldahl analyses, and adjustment of the protein level to 10 to 15 mg. per cc., a 1-cc. quantity of the protein in physiologic saline was injected intraperitoneally into 7- to 8-week old Swiss mice bearing sarcoma 37 which had grown to a diameter of 12 to 15 mm. Three hours later, these mice were injected intraperitoneally with 0.5 cc. of a saline solution of the amount of polysaccharide which ordinarily kills about 70 per cent of a group of tumor-bearing Swiss mice. Control groups of mice injected only with the polysaccharide, and groups injected with either bovine serum albumin or the globulin fraction from normal Swiss mice prior to the administration of polysaccharide, were included in the experimental series.

RESULTS

The observations noted in the series of tests have been compiled and summarized in the accompanying table. When 600 γ of the polysaccharide P-10a (724 strain) was administered to a

total of 85 tumor-bearing Swiss mice, it was found that 73 per cent of the mice died within 24 hours and 81 per cent died within 96 hours (line 1). This 96-hour mortality rate was assigned a ratio of 1 in the last column of the table. Prior injection of the mice with either bovine serum albumin or with the globulin fraction from blood of normal 8-week old Swiss mice at ratios of protein to polysaccharide of 16 to 25 afforded them little protection against the lethal action of the polysaccharide (lines 2 and 3). A study was also made of the influence of a passive transfer of the serum albumin fraction from mice given a single 100-γ injection of P-10. This fraction isolated from blood obtained 3 days after the injection exerted a slight protective action (line 4); when obtained at 14 days, the albumin fraction was ineffective against the lethal action of P-10a (line 5).

From lines 6 and 7, it is seen that the injection of the globulin fraction (glob. 3/P-10 and glob. 14/P-10) from sera of mice obtained either 3 or 14 days after a single injection of P-10, had a pronounced effect in decreasing the lethal activity of the polysaccharide P-10a. The globulin fraction of sera toward a different preparation (P-20) of polysaccharide from the same strain of organism appeared to be less protective when obtained on the third day than on the fourteenth day (lines 8 and 9). Prior injection with the globulin fraction from sera of mice obtained 3 days after a single 100-γ injection of the P-3.M polysaccharide (G.W. strain) afforded the mice slight protection against the lethal action of P-10a (line 10). An extensive reduction in the mortality rate was noted following the use of the globulin fraction from sera of mice obtained 10 days after the injection of 250 γ and 400 γ of P-20, spaced 7 days apart, into Swiss mice bearing sarcoma 37 (glob. S/P-20, line 11) and after injection of 100 γ and 800 γ of P-20, spaced 4 days apart, into normal Swiss mice (glob. N/P-20, line 12).

These observations were confirmed in a separate series of tests in which a 500-γ amount of P-10a was used as the lethal dose. Of the serum globulins obtained from the mice 3 days after a single injection of polysaccharide, it is seen that the globulin fraction of antiserum elicited by P-10 was highly effective and that by P-20 was only moderately effective, whereas the globulins from sera of mice injected with P-3.M showed no protective action against P-10a (lines 13 to 16).

In another series of tests in which 300 γ of the polysaccharide P-3.Ls from the G.W. strain was employed as the lethal dose, it was observed that the globulin fraction of mouse sera obtained 3 days after a single injection of 100 γ of P-3.M conferred a substantial degree of protection on the mice (lines 18 and 19). The globulin fraction of mouse sera obtained 14 days after a single injection of 100 γ of P-10 or P-20 had no significant effect (lines 20 and 21).

These results substantiate and extend our earlier findings (3, 4) that protective antibodies were elicited rapidly in the mouse by a single injection of polysaccharide. They also demonstrate that globulin fractions obtained from mouse sera following a

TABLE 1

PASSIVE IMMUNIZATION OF SWISS MICE BEARING SARCOMA 37 USING FRACTIONS OF SERA FROM SWISS MICE INJECTED WITH POLYSACCHARIDE

Line	Mouse serum protein	Poly.	Amt. in γ	Ratio: protein/poly.	No. of mice	Per cent deaths In 24 hrs.	Per cent deaths In 96 hrs.	Ratio (96 hr. mortality): expt./control
1		P-10a	600	0	85	73	81	1.0
2	Glob./normal	"	"	16–25	46	50	61	0.75
3	Bov. ser. alb.	"	"	"	34	47	65	0.80
4	Alb. 3/P-10	"	"	20	18	50	56	0.69
5	Alb. 14/P-10	"	"	"	21	57	76	0.94
6	Glob. 3/P-10	"	"	16–25	23	17	26	0.32
7	Glob. 14/P-10	"	"	"	24	17	21	0.26
8	Glob. 3/P-20	"	"	"	28	25	43	0.53
9	Glob. 14/P-20	"	"	"	31	19	19	0.23
10	Glob. 3/P-3.M	"	"	"	42	43	48	0.59
11	Glob. S/P-20	"	"	16	10	10	10	0.12
12	Glob. N/P-20	"	"	"	20	10	15	0.19
13		"	500	0	16	31	50	1.0
14	Glob. 3/P-10	"	"	20	9	0	0	0
15	Glob. 3/P-20	"	"	20	8	0	25	0.5
16	Glob. 3/P-3.M	"	"	20	16	38	50	1.0
17		P-3.Ls	300	0	30	63	77	1.0
18	Glob. 3/P-3.M	"	"	50	14	29	29	0.38
19	Glob. 3/P-3.M	"	"	100	10	30	30	0.39
20	Glob. 14/P-10	"	"	50	8	50	63	0.82
21	Glob. 14/P-20	"	"	50	9	67	67	0.87

single injection of P-10 polysaccharide afforded mice a high degree of protection against the lethal action of that polysaccharide at relatively low globulin to polysaccharide ratios of 16 to 25. Similar extents of protection against P-10 were noted previously only with globulin to polysaccharide ratios of about 100 when the γ-globulin fractions of rabbit antisera elicited by a series of injections of P-10 were employed (3).

In addition, the serum globulin fractions from mice given a single injection of the polysaccharides exhibited a degree of specificity similar to that observed with globulin fractions from rabbit antisera elicited by multiple injections (2, 3). Thus, at low globulin to polysaccharide ratios, there was no significant cross protection between the polysaccharides from the 2 strains (lines 10, 16, 20, and 21) whereas at higher ratios of about 100, there was cross protection (3, Table 3) with mouse as well as rabbit globulin fractions from antisera toward the polysaccharides (2, 3).

SUMMARY

It has been found that passive immunization with relatively small amounts of the globulin fractions obtained from sera of mice either 3 or 14 days after a single injection of polysaccharides from *Serratia marcescens* afforded mice bearing sarcoma 37 pronounced protection against the lethal action of the homologous polysaccharide but not against that of the polysaccharides from a different strain of the organism.

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