ROLE OF MACROPHAGES IN NATURAL DEFENSE OF OF MUSCLES AGAINST DYSENTERIC INFECTION

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Antimacrophagal and, to a lesser degree, normal rabbit sera have an immunodepressant action when injected into intact mice along with a living culture of Shigella sonnei. The stronger immunodepressive effect of the antimacrophagal serum is connected with its toxic action on the macrophages.

As yet little is known of the role of macrophages in intestinal infections and, in particular, in bacillary dysentery. There is some evidence that natural resistance to certain infections is due to the macrophagal system [2, 4] and the effect of antimacrophagal sera is to reduce the resistance of animals to certain viruses [3] and to streptococci [1].

Since mice have well-marked natural immunity to dysentery bacilli, it could be useful to study the role of macrophages in this resistance with the aid of antimacrophagal sera.

EXPERIMENTAL METHOD

Antimacrophagal sera were obtained by intravenous immunization of rabbits weighing 3-3.5 kg with cells of the peritoneal exudate of noninbred albino mice obtained 2 days after intraperitoneal injection of 1% peptone solution. The exudate contained 60-80% of cells of macrophagal type. Rabbits were immunized by 4-6 intravenous injections, in doses of 10 million-30 million cells, with intervals of 3-7 days between injections.

Another group of rabbits was immunized with peritoneal exudate macrophages after preliminary culture for 1-4 days in medium No. 199 with the addition of 20% bovine serum and antibiotics. The cell monolayer was washed with medium No. 199 and then carefully removed from the surface of the slide with a rubber-tipped metal needle. The cells were injected intravenously in doses of 20,000-3 million, also 4-6 times.

One week after the end of immunization the animals were exsanguinated, and the sera were inactivated at 56°C for 30 min and adsorbed with mouse erythrocytes to remove hemaglutinins.

The sera were titrated in Dausset's leuko-agglutination test with a suspension of peritoneal exudate cells. The titer of antimacrophagal sera varied between 1:64 and 1:256. Adsorption of the sera by erythrocytes hardly reduced their titer.

The antimacrophagal sera were injected intravenously or intraperitoneally in a dose of 0.3 ml into noninbred albino mice weighing 16-18 g.

A reference strain of Shigella sonnei No. 9090, giving 80-90% of colonies in the S-form when seeded on nutrient agar, was used as the infecting culture. CLD for an 18-h culture of this strain of Sh. sonnei, injected intraperitoneally into animals with physiological saline, was 250 million bacterial cells.

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TABLE 1. Mortality among Mice after Simultaneous Intraperitoneal Injection of Antimacrophagal and Normal Rabbit Sera with a Culture of Shigella sonnei

Sera	Serum No.	Dilutions of sera	Overall mortality among mice*	Index of immunodepressive action†
Antimacrophagal	895	1:5	6/1	6
		1:25	1/1	1
	989	1:5	6/1	6
		1:25	2/1	2
	309	1:5	8/1	8
		1:25	5/1	5
	312	1:5	7/1	7
		1:25	1/1	1
	971	1:25	3/3	1
	986	1:25	7/8	0,9
	982	1:25	7/8	0,9
Normal	101	1:2	9/6	1,5
	105	Undiluted	5/3	1,7
	106	»	9/6	1,5
	107	»	10/6	1,7
	109	1:2	8/3	2,7
	806	Undiluted	10/6	1,7

*Numerator, number of mice dying in experimental group; denominator, number dying in control group.
† Ratio between number of mice dying in experimental group and number dying in control group.

After injection of the antimacrophagal sera or at the same time the mice were infected with sublethal doses of the Sh. sonnei culture, consisting of 31 million, 62 million, and 125 million bacterial cells and the total mortality among the mice from all 3 doses was determined during the next 3 days. The action of the antimacrophagal sera was assessed by comparing the overall mortality among the mice in the experimental group and in the control (mice infected with Sh. sonnei but not receiving antimacrophagal sera).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all the antimacrophagal sera in a dilution of 1:5 and some of these sera in a dilution of 1:25, if injected intraperitoneally simultaneously with the Sh. sonnei culture, increased the mortality among the animals compared with the control by 2-8 times, i.e., suppressed the natural defenses of the mice.

The results given in Table 1 also show that normal rabbit sera, undiluted or diluted 1:2, also had an immunosuppressive action. However, the index of immunodepressive action in this case did not exceed 1.5-2.7.

The antimacrophagal rabbit sera thus had a much stronger immunodepressive action than normal sera. Undiluted rabbit sera, both normal and antimacrophagal, if injected intraperitoneally as a single dose did not themselves (without injection of the Sh. sonnei culture) cause death or disease among the animals.

If normal rabbit sera were injected intraperitoneally or intravenously into mice 24 h before infection, even if undiluted, not only did they not depress the defenses of the mice, but actually stimulated them. If rabbit sera were injected intravenously 30 min-1.5 h before infection, only some of the antimacrophagal sera exhibited their immunodepressive action (undiluted or in a dilution of 1:2), by contrast with the activation of the natural defenses of the mice by normal sera.

The results indicate that antimacrophagal sera have no lethal effect on macrophages and only temporarily depress their functional activity if injected once only. The sera, when injected intraperitoneally

and, in particular, intravenously, are very quickly destroyed in the mouse body, so that their immunodepressive action is maximal if they are injected simultaneously with the infective culture. The increase in the natural resistance of the mice 24 h after injection of the sera can be explained by nonspecific stimulation of the reticuloendothelial system by protein breakdown products of these sera.

Tests of the action of rabbit sera in 6-day cultures of peritoneal exudate macrophages showed that, regardless of the presence or absence of fresh guinea pig complement, the antimacrophagal sera exhibited cytotoxic properties in dilutions of 1:5 and 1:25. A mixture of normal rabbit sera, diluted 1:5, did not have this property.

The stronger suppression of the natural resistance of mice by antimacrophagal sera than by normal rabbit sera is presumably connected with the toxic action of these sera on the macrophages. Depression of the natural immunity of animals to Shigella sonnei as a result of treatment with antimacrophagal sera is evidence of the important role of macrophages in the natural defenses against dysenteric infection.

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