EFFECT OF BACTERIAL POLYSACCHARIDE ON THE NUMBER OF ANTIBODY-PRODUCING CELLS IN THE SPLEEN OF IRRADIATED AND UNIRRADIATED MICE

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The effect of bacterial polysaccharide on the number of spleen cells producing antibodies against sheep's red cells was investigated in irradiated and unirradiated mice. The polysaccharide increased the number of antibody-producing cells both in lethally irradiated (γ rays) and in unirradiated mice, unimmunized or immunized with sheep's red cells. This increase was connected with the stimulation of proliferative processes in the recipient by the polysaccharide.

KEY WORDS: antibody-forming cells; bacterial polysaccharide; irradiation.

A polysaccharide from the O-somatic antigen of Salmonella typhi (PS) is known to stimulate various factors of natural and artificial immunity, including ability to form antibodies [1, 2, 4, 7, 8]. An increase in the number of antibody-forming cells could be one of the causes of the increased antibody formation.

The object of this investigation was to study the effect of PS on the number of antibody-forming cells (AFCs) in irradiated and unirradiated recipients.

EXPERIMENTAL METHOD

CBA, CC57BR, and noninbred mice weighing 18-20 g were used. Some of the mice received a single dose of whole-body γ-ray irradiation on the ÉKU-50 apparatus amounting to 650 ($LD_{70/30}$) and 900 ($LD_{100/30}$) rad, with a dose rate of 182 rad/min. An intraperitoneal injection of 50 μg PS was given to the experimental mice 24 h before they were irradiated. The PS was obtained by gradual separation of protein and lipids from the complete antigen of S. ty_2 4446, isolated by the aqueous phenol method in the cold [3]. Some mice were immunized with 5×10^8 sheep's red cells (SRBCs) by a single intravenous injection. The number of AFCs in the spleen of the mice 5 days after injection of the SRBCs was determined by the local hemolysis in gel method [5, 9].

Two series of experiments were carried out on irradiated and unirradiated mice. In the unirradiated mice the number of AFCs was determined: 1) 1 h and 1, 2, 5, and 10 days after injection of the PS; 2) after injection of the PS, followed 1 h and 1, 2, and 5 days later by injection of the SRBCs; the test was carried out always 5 days after injection of the SRBCs; and 3) 5 days after injection of the SRBCs, followed 1 h and 2 and 5 days later by injection of the PS. In mice irradiated in a dose of 650 rad the number of AFCs was determined: 1) 5 days after irradiation, 2) 5 days after injection of the PS and 1 day before irradiation. In mice irradiated in a dose of 900 rad the number of AFCs was determined: 1) 5 days after irradiation, 2) 5 days after injection of the SRBCs; 5 days before immunication the mice received an injection of PS and they were irradiated 1 day later, and 3) 5 days after immunication of the mice with SRBCs having been irradiated 5 days previously. The experimental results were subjected to statistical analysis: the geometric mean was calculated for the number of AFCs in each group, together with its confidence limits ($P < 0.05$) and the significance of differences between the values obtained for the groups to be compared.

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TABLE 1. Effect of Polysaccharide on Number of AFCs in Unirradiated Mice

Treatment	Number of AFCs in mouse spleen		
	noninbred	$CC_{57}BR$	CBA
PS—1 h	244 (197—264) [30]	199 (177—223) [30]	147 (105—177) [20]
PS—1 day	163 (147—181) [30]	195 (166—231) [30]	115 (91—148) [20]
PS—2 days	188 (166—216) [30]	220 (187—263) [30]	142 (122—164) [20]
PS—5 days	171 (149—196) [30]	223 (195—255) [30]	192 (153—240) [20]
PS—10 days	195 (171—222) [30]	213 (175—261) [30]	178 (138—229) [20]
PS—1 h; SRBCs	66 220 (50 230—87 900) [20]	69 820 (59 160—824 10) [20]	—
PS—1 days; SRBCs	71 450 (57 000—90 160) [20]	73 450 (58 480—92 680) [20]	—
PS—2 days; SRBCs	58 210 (49 800—68 230) [20]	66 530 (65 460—67 450) [20]	—
PS—5 days; SRBCs	54 950 (46 990—63 100) [20]	71 290 (70 310—72 280) [20]	—
	45 500 (45 080—46 030) [20]	49 890 (49 430—50 230) [20]	—
SRBCs; PS + 1 h	58 340 (50 400—67 100) [30]	34 200 (27 290—42 850) [30]	—
SRBCs; PS + 1 day	26 240 (22 590—30 550) [30]	27 730 (23 710—32 430) [30]	—
SRBCs; PS + 2 days	24 100 (20 000—29 040) [30]	31 120 (26 550—36 390) [30]	—
SRBCs; PS + 5 days	26 610 (20 320—34 830) [30]	42 070 (32 140—55 080) [30]	—
SRBCs	42 850 (37 760—49 430) [30]	42 270 (36 640—48 870) [30]	—

Note. Column headed "Treatment" shows injection given to mice. PS was injected either into unimmunized mice 1 h to 10 days before the test or in conjunction with SRBCs. In the latter case the time of injection of PS relative to SRBCs is indicated: before (–) or after (+). The number of animals is shown in square brackets next to the number of AFCs.

TABLE 2. Effect of Polysaccharide on Number of AFCs in Irradiated Mice

No. of group of animals	Treatment	Number of AFCs in mouse spleen			
		noninbred		$CC^{57}BR$	
		650 rad	900 rad	650 rad	900 rad
1	—	38 (28—51) [18]	4,4 (3,8—5,1) [15]	14,2 (14,1—14,3) [15]	1,3 (1,1—1,6) [15]
2	PS	97,8 (97,3—98,3) [19]	20,6 (20,2—20,7) [20]	76,5 (76,2—77,1) [20]	8,7 (8,6—8,8) [20]
3	PS +SRBCs		35,0 (34,4—35,4) [20]	—	20,4 (20,3—20,9) [16]
4	SRBCs		7,8 (6,3—9,7) [16]	—	3,4 (3,3—3,5) [21]

Note. All mice were irradiated. PS was injected into the mice of groups 2 and 3 24 h before irradiation. SRBCs were injected into the mice group 3 (on the 4th day after irradiation) and group 4 (on the 5th day after irradiation). The mice of group 1 were killed on the 5th day after irradiation, those of group 2 on the 4th day after irradiation, and those of groups 3 and 4 on the 5th day after injection of SRBCs. The number of animals is shown in square brackets below the number of AFCs.

EXPERIMENTAL RESULTS

Comparison of the number of AFCs in the unirradiated noninbred and inbred mice (Table 1) showed that the number was less in intact CBA mice than in noninbred and CC57BR mice. Injection of the PS increased the number of AFCs in the inbred mice. A tendency toward an increase in the number of cells also was observed in the noninbred mice. Observations lasting 10 days showed that throughout this period the number of AFCs in the mice receiving PS was greater than in animals not receiving PS. After immunization with SRBCs the number of AFCs in the spleens of the mice rose. Injection of PS at different times (1 h, 1, 2, and 5 days) before immunization of the mice with SRBCs had a stimulant effect: at all times the increase in the number of AFCs after injection of PS was statistically significant regardless of the interval between the injections of PS and SRBCs. Different results were obtained if PS was injected after immunization of the mice with SRBCs. In that case injection of PS did not increase the number of AFCs. The PS thus increased the number of AFCs in the unirradiated mice, whether unimmunized or immunized with SRBCs, provided that the PS was injected before the SRBCs and not after. Irradiation sharply reduced the number of AFCs in the mice (Table 2). This decrease depended on the dose of irradiation. After irradiation in a dose of 950 rad ($LD_{100/30}$) only single AFCs were observed in the spleen. Injection of PS 24 h before irradiation considerably increased the number of AFCs in the spleens of the mice irradiated in a dose of 950 rad. This effect of PS may be due either to the radioprotective action of PS [7, 10], preventing injury to the cell, or to its stimulant action, manifested as more intensive proliferation [2]. The increase in the number of AFCs under the influence of PS was therefore greater in relation to doses of irradiation not causing death of 100% of the animals, i.e., doses permitting the preservation of a certain number of cells undamaged by radiation in the animal. After irradiation in an absolutely lethal dose the number of cells protected by the preliminary injection of the radioprotector PS was less than after irradiation in a dose not causing death of 100% of the animals. However, on account of the stimulant action of PS, even after an absolutely lethal dose of irradiation the number of AFCs in the spleens of the irradiated mice increased considerably. Immunization of mice irradiated in an absolutely lethal dose with SRBCs increased the number of AFCs only slightly. If, however, irradiated mice which had received PS previously were immunized, the number of AFCs increased considerably, even if a dose of $LD_{100/30}$ was given. Hence PS increased the number of AFCs also in those irradiated animals whose spleen contained extremely few AFCs without the injection of PS. Although PS has a radioprotective action and its mechanism is like that of the chemical radioprotectors [6], its radioprotective effect is also brought about through intensification of the proliferative processes, leading to an increase in the number of cells in the body. Under these circumstances more cells remain viable after irradiation than in an animal not stimulated by PS. This property of PS results in an increase in the number of AFCs. However, the reason for the intensification of proliferative processes under the influence of PS and for the associated increase in the number of AFCs remains unexplained.

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