SENSITIVITY OF IMMUNOCOMPETENT SPLEEN CELLS OF MICE OF DIFFERENT GENOTYPES TO ALKYLATING AGENTS

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The writers previously [3] described significant differences in mice of different lines in their sensitivity to the immunodepressive action of alkylating agents: cyclophosphamide (CP), thiophosphamide (thiotepa), and sarcolysin (SL). Irrespective of the type of immunodepressant, DBA/2 mice were most sensitive to immunodepressive action, BALB/c mice were relatively resistant, and C3H/Sn mice occupied an intermediate position. It has been suggested that the differences between mice of these lines are mainly due to the character of interaction between immunocompetent target cells and the immunodepressants.

The aim of this investigation was to study sensitivity of spleen cells of mice of different lines to the action of alkylating immunodepressants.

EXPERIMENTAL METHOD

Male mice weighing 18-25 g belonging to three inbred lines were used: BALB/c JLacSto, C3H/Sn Rap, and DBA/JSto. As immunodepressants, CP (Cyclophosphan, produced by the Saransk Medical Preparations Factory), thiotepa (synthesized at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute), and SL (synthesized in the Chemical Technology Laboratory, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) were used. Since metabolic activation of CP is essential for the compound to exert its biological action in vivo [7-9], the in vivo activated immunodepressant [5] was used in experiments to study the action of CP on cells in vitro: CP (200 mg/kg) was injected intraperitoneally into BALB/c mice, the mice were killed 30 min later, and the "active serum," containing active metabolites of CP, was obtained. Sheep's red blood cells (SRBC) were used as the antigen.

To study the action of the immunodepressants in vitro on mouse spleen cells, a cell suspension was prepared in medium 199 with antibiotics, which was incubated with the agent to be tested ("active" serum, SL, or thiotepa) for 1 h at 37°C. The incubation mixture contained (in 1 ml): 0.5 ml of "active" serum, 4 μg SL, or 12.5 μg thiotepa. After incubation the cells were washed three times with cold (4°C) medium 199 and injected intravenously into syngeneic recipient mice in a dose of $5 \cdot 10^7$ cells together with $4 \cdot 10^8$ SRBC. The recipients were given an intraperitoneal injection of 200 mg/kg CP 3-4 h before the cells were injected, to suppress their intrinsic immunoreactivity [1]. The number of antibody-forming cells (AFC) in the recipients' spleen was determined 5 days after transfer by the method in [10]

To study the effect of CP on subpopulations of spleen cells from BALB/c and DBA/2 mice the technique in [6] was used: The principle of it is that intact T or B cells are added to cells treated with CP in vitro. The degree of restoration of the immune response was judged from whether CP attacked mainly T or B lymphocytes. Syngeneic spleen cells treated with rabbit anti-T- or anti-B-serum served as the source of intact T and B cells. Antisera were obtained and their activity and specificity studied by the method in [4].

The experiments were set up as follows. Spleen cells from intact BALB/c and DBA/2 mice, numbering $2.4 \cdot 10^8$, were incubated for 45 min at room temperature with 0.5 ml of anti-T-serum or anti-B-globulin, isolated from anti-B-serum, and 0.8 ml of complement (guinea pig serum diluted 1:2). The volume of the incubation mixture was 8 ml. After incubation the cells were washed, resuspended in medium 199, and injected in a dose of $2 \cdot 10^7$ cells intravenously into recipient mice treated with CP, along with $4 \cdot 10^8$ SRBC. Spleen

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TABLE 1. Sensitivity of Spleen Cells of Different Lines of Mice to Alkylating Agents

Line of mice donating cells	Immune response, % of control		
	CP	Thiotepa	SL
BALB/c	13,1 (9,1—19,0) n=12 P=0,028	17,9 (9,1—34,7) n=33 P>0,5	37,6 (26,9—52,6) n=27 P<0,0001
C3H/Sn	6,5 (3,7—11,3) n=11 P=0,0003	15,5 (8,3—29,2) n=20 P=0,012	11,8 (7,2—19,1) n=13 P=0,32
DBA/2	1,4 (0,8—2,4) n=12	6,5 (4,4—9,5) n=29	8,2 (4,3—15,4) n=13

Legend. Here and in Table 2, confidence intervals at P = 0.5 level shown in parentheses; n) number of animals.

TABLE 2. Effect of T and B Lymphocytes on Immune Response of Spleen Cells Treated with CP (immune response, in % of control)

Cells studied	BALB/c	DBA/2
1. H-cells (control)	100 (82,2—121,6) n=22	100 (71,6—139,6) n=23
2. CP cells	35,3 (31,2—31,9) n=22	10,8 (8,1—14,6) n=20
3. CP cells + T cells	80,7 (59,2—109,8) n=24	18,0 (13.2—24,6) n=21
4. CP cells + B cells	56,5 (42,2—75,5) n=23	18,1 (11,6—28,3) n=21

Legend. BALB/c: $P_{2-3} < 0.0001$, $P_{2-4} = 0.004$; DBA/2: $P_{2-3} = 0.019$, $P_{2-4} = 0.054$ ($P_U = 0.017$).

cells subjected to the action of "active" serum were then injected intravenously. Mice receiving normal, untreated spleen cells (H cells) or spleen cells treated with one or other serum preparation ("active" serum, anti-T-serum, anti-B-globulin – described as CP-cells, B-cells, and T-cells respectively), served as the control. On the 5th day after transfer of the cells and immunization the number of AFC was determined in the recipients' spleen. The degree of recovery of the immune response was judged by comparing the number of AFC in the spleen of recipients belonging to the experimental and the various control groups.

Statistical analysis of the results was undertaken by the Fisher–Student t test and the Wilcoxon–Mann–Whitney U test [2]. Differences were considered to be significant at the $P < 0.05$ level. The number of AFC in the control animals was taken as 100% and the immune response of mice of the experimental groups was expressed as a percentage of the control.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of the alkylating agents on spleen cells of BALB/c, C3H/Sn, and DBA/2 mice was studied. The results of these experiments (Table 1) showed that spleen cells of DBA/2 mice have significantly higher sensitivity than cells of BALB/c mice to all three alkylating agents. The C3H/Sn mice occupied an intermediate position as regards sensitivity.

These results, together with previous data [3], suggest that the sensitivity of immunocomponent cells is a genetically controlled feature, which, if not the only factor determining the degree of sensitivity or resistance of the immune response of mice of different lines to alkylating agents, at least plays and important role.

For a more detailed study of the mechanisms of the genetic differences in sensitivity to alkylating agents at the cell level, the effect of CP on T and B cells of BALB/c and DBA/2 mice, reacting differently to this immunodepressant (Table 2), was investigated. The results showed that suppression of the antibody-forming function of the spleen cells by CP was expressed much less strongly in the BALB/c than in the DBA/2 mice (to 35.3 and 10.8% respectively of the control level, $P < 0.0001$), in agreement with the results obtained in the previous part of the work. In the BALB/c mice the immune response of the CP cells was significantly lower than that of both T and B cells; the T cells had the stronger effect: The number of AFC did not differ significantly from their number in the control ($P = 0.24$). Partial restoration of the immune response of the CP cells in DBA/2 mice was attributable to the T cells, whereas the B cells gave a borderline effect (when compared with CP cells, $P = 0.054$ by the t test, a significant difference was revealed only by the U test, $P = 0.017$). It can be concluded from these results that both T and B cells are damaged by CP in mice of both lines, with predominant inhibition of T cells. In BALB/c mice, with greater resistance to the action of CP, the T and B cells were able to restore, largely or completely, the immune response of cells treated with the immunodepressant, whereas in DBA/2 mice, which are highly sensitive to the action of CP, T and B cells restored the immune response by a lesser degree.

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