INVESTIGATION OF THE ACTION OF SYNGENEIC SPLEEN CELLS OF MICE TREATED WITH DIUCIFON ON MAGNITUDE OF THE IMMUNE RESPONSE

V. P. Leskov, V. M. Pisarev, and S. S. Arshinova

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KEY WORDS: diucifon; immune response.

It was shown previously that during treatment of patients with chronic pneumonias with the immunostimulant diucifon the relative percentage of autorosette-forming lymphocytes, which secrete a factor with the biological activity of T cell growth factor (TCGF) [2], in their peripheral blood is increased [3]. If mononuclear cells from patients with the ataxia-teleangiectasia syndrome are treated with diucifon *in vitro*, TCGF has been shown to be released into the supernatant [1]. It is possible that the immunostimulating effect of diucifon is linked with an increase in the number of cells releasing TCGF under the influence of the drug.

In this investigation the effect of spleen cells treated with diucifon on the immune response was studied.

EXPERIMENTAL METHOD

CBA mice were obtained from the "Stolbovaya" Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR. Thrice washed spleen cells from intact mice were incubated in glass flasks the bottom of which had an area of 4.4 cm^2, in a concentration of 10×10^6 cells/ml, in a volume of 5 ml for 2 h at 37°C in medium 199 in the presence of diucifon, phytohemagglutinin (PHA, from Difco, USA), concanavalin A (con A, from Sigma, USA), pokeweed mitogen (PWF, from Sigma), or levamisole. After incubation the cells were washed three times with medium 199 and injected intravenously in a dose of 5×10^6 into mice immunized with sheep's red blood cells in a dose of $3 \cdot 10^6$. On the 4th day after immunization the magnitude of the immune response was estimated by determining the number of direct plaque-forming cells (PFC) [6].

For statistical analysis of the results the geometric mean numbers of PFC with confidence intervals at the $P \leq 0.05$ level were used.

EXPERIMENTAL RESULTS

The data in Table 1 show that injection of spleen cells preincubated in medium 199 into mice intensified the immune response fourfold. A tendency for the immune response to be intensified also was maintained in the next four experiments in response to injection of untreated spleen cells. It was therefore correct, from our point of view, to use the immune response of mice into which incubated and washed spleen cells, subsequently designated normal spleen cells (NSC), were injected as the control in this series of experiments.

Table 1 shows that injecting syngeneic spleen cells, treated with diucifon in a concentration of 10 or 100 μg/ml, into mice enhanced the immune response by 3-5 times compared with the action of NSC. Injection of spleen cells treated with con A, a frequently used inducer of TSGF release [5], also intensified the immune response. Spleen cells treated with PHA, PWM, or levamisole did not cause intensification of the immune response.

The intensifying action of cells treated with diucifon or con A may perhaps be due to release of TCGF by them under the influence of these preparations. However, PHA and PWM also induce release of TCGF from cells [5], but spleen cells treated by these substances, un-

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TABLE 1. Effect of Spleen Cells Treated with Various Agents on Immune Response

Expt. No.	Control	Number of PFC in spleen						
		without treatment	diucifon 10 μg/ml	diucifon 100 μg/ml	levamisole 100 μg/ml	con A 10 μg/ml	PHA 10 μg/ml	PWM 10 μg/ml
1	2 249 1318+3837 *n*=8	8 166 5970+11 170 *n*=6	12 650 7 745±20 610 *n*=6	24 380 17 300±34 430 *n*=3	—	29 990 13 240±67 420 *n*=4	—	16 180 7998+32 720 *n*=7
2	9 204 4365+19 410 *n*=8	16 670 6730+41 210 *n*=7	50 000 32 580+76 560 *n*=6	22 750 11 640+44 570 *n*=7	14 190 *n*=7	—	—	—
3	5 212 2447+10 670 *n*=7	9 141 5093+16 370 *n*=7	24 950 16 110+38 640 *n*=7	29 580 22 030+39 810 *n*=7	—	—	—	—
4	5 520 1982+15 380 *n*=5	12 560 7586+20 750 *n*=7	—	33 190 21 430+51 290 *n*=6	—	18 880 12 910+27 610 *n*=7	12 190 8590+17 300 *n*=7	10 520 8072+13 710 *n*=7
5	11 860 8453+16 630 *n*=6	10 160 6776+15 240 *n*=8	19 140 13 650+26 790 *n*=7	—	—	—	—	—
				—	—	—	—	—

Legend. Mice in control received only sheep's red blood cells, mice in experiment received intact spleen cells or spleen cells treated with preparations and sheep's red blood cells. Here and in Table 2, n denotes number of animals.

TABLE 2. Effect of Spleen Cells Treated with Diucifon and of NSC on Immune Response when Injected at Different Times after Immunization

Day of injection of spleen cells after immunization	Immune response of mice		
	injection of NSC	injection of spleen cells treated with diucifon	no cells injected (control)
0	5 662 (3162—10 120) *n*=15	15 420 (11 070—21 480) *n*=11	3767 (2382—5957) *n*=22
1-й	6 166 (3475—10 960) *n*=12	3 673 (1754—7691) *n*=13	1644 (1089—2483) *n*=25
2-й	2 553 (1219—5346) *n*=7	2 371 (1349—4159) *n*=8	2455 (1476—4074) *n*=16
3-й	10 860 (7311—15 140) *n*=10	4 592 (2118—9954) *n*=12	2275 (1667—3105) *n*=26

like those treated with diucifon and con A, do not intensify the immune response. The reason is probably that mitogens and, in particular, PHA, during their action on cells induce two opposite processes: the formation of T helper cells and secretion of intensifying factors (TCGF, for example), and also the formation of T suppressors and release of suppressor factors [7, 8]. The relations between these processes depend on the dose of mitogen, duration of incubation of the cells with mitogen, and the conditions of incubation [7]. The experimental conditions which were chosen may perhaps also have had the result that equilibrium was established among cells treated with PHA and PWM between suppressors and helpers, and injection of these cells did not affect the immune response.

Since we postulated that the action of cells treated with diucifon on the immune response is linked at least in part with TCGF release, and since injection of TCGF has its strongest action on the initial stages of the immune response, it was logical to study the action of cells treated with diucifon on different stages of the immune response.

It will be clear from Table 2 that injection of spleen cells treated with diucifon in a concentration of 100 μg/ml on the day of immunization considerably potentiated the immune response. Injection of NSC on the day of immunization did not affect the magnitude of the immune response. Spleen cells treated with diucifon, and NSC potentiate the immune response if injected one day after immunization. Neither NSC nor spleen cells treated with diucifon had

any effect on the magnitude of the immune response if injected two days after immunization. Injection of NSC on the third day after immunization considrably potentiated the immune response. Cells treated with diucifon at this same time reduced the immune response compared with that after injection of NSC.

Spleen cells treated with diucifon thus had the strongest action on the initial stages of the immune response. NSC have their maximal action if injected in the final stages of the immune response, i.e., they have an effect that is similar to the action of a stimulator of antibody producers [4]. Treatment of the cells with diucifon abolishes the immunostimulating action of NSC when injected in the final stages of the immune response. The impression is created that diucifon, which facilitates release of TSGF — a factor intensifying proliferation — prevents release of factors facilitating differentiation of cells into antibody producers. It is possible that simultaneous injection of cells treated with diucifon and NSC will have the strongest action on the magnitude of the immune response. The results may be further justification for the use of immunostimulating therapy by injection of autologous cells treated with diucifon or with other preparations, and also of cells incubated under suitable conditions, making use of the principles of plasmaphaeresis.

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EXOCYTOSIS OF CYTOTOXIC T LYMPHOCYTES STUDIED BY FREEZE-FRACTURING AND STEREOPHOTOGRAMMETRY

A. A. Shevelev and S. N. Bykovskaya

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Rejection of a graft of normal or tumor tissue takes place mainly through the activity of cytolytic T lymphocytes (CTL), whose mechanism of action has not yet been explained. To study this problem model systems have been developed *in vivo* and *in vitro*, and with them it has become possible to investigate interaction between CTL and target cells (TC). On the basis of the evidence so far obtained several hypotheses, explaining the cytolytic effect of T killer cells, have been put forward [1, 2]. The present writers have suggested a secretory mechanism of action of CTL [2-6]. Electron-microscopic investigations have revealed activation of the secretory apparatus of CTL during interaction with TC. In the zone of contact between the lymphocyte and TC an amorphous substance, corresponding in electron-optical density to the contents of secretory vacuoles demonstrable in the cytoplasm of CTL, has been found. However, discharge of secretory vacuoles was not observed.

The aim of this investigation was to detect exocytosis and to describe secretory vacuoles in the zone of contact between the lymphocyte and TC, using techniques of freeze-fracturing and stereophotogrammetry [7, 8].

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.)

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