

IMMUNODEPRESSIVE ACTIVITY OF SOME RADIOMIMETICS DURING IMMUNIZATION OF MICE WITH SHEEP'S ERYTHROCYTES

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Sarcolysin, degranol, and thio-TEPA effectively suppress the production of antibody-forming cells. The optimal times of injection of these compounds and the duration of their effect on the immunologic reactivity of animals and on hematopoiesis were investigated.

Alkylating agents are known to be effective inhibitors of immunogenesis [12-14]. However, many aspects of the mechanism of their immunodepressive action are still unexplained.

The object of this investigation was to study the immunodepressive activity of certain radiomimetics: novembichin, degranol, sarcolysin, and thio-TEPA. The existing data on this question are few in number [2, 4-10] and do not allow comparison of the effectiveness of these agents. Because of the extensive clinical use of these compounds, the investigation of their immunodepressive activity is of both theoretical and practical interest.

EXPERIMENTAL METHOD

Adult CC57BR and noninbred mice weighing 20-25 g were immunized intravenously with sheep's erythrocytes ($5 \cdot 10^8$ cells).

Novembichin and degranol were injected intravenously the day before immunization and on the day of injection of antigen, in various doses (novembichin 1 mg/ml, degranol 30 mg/kg/day). Thio-TEPA and sarcolysin were injected intraperitoneally, either as a single dose of 16 mg/kg at various times or as four separate daily doses of 8 mg/kg, starting on the day before immunization. These doses corresponded to the upper limit of tolerance. In some experiments the animals received whole-body irradiation on an ÉGO-2 apparatus in the highest tolerated dose (500 R).

The number of antibody-forming cells in the spleen was determined on the 4th day after immunization by the method of Jerne and Nordin [11] with some modifications. The titer of hemolysins and hemagglutinins circulating in the blood was determined in parallel tests by the usual method. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of experiments to study the effect of sarcolysin, thio-TEPA, degranol, and novembichin on the immune response are given in Table 1.

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TABLE 1. Action of Alkylating Compounds on Primary Immune Response

Compound	Day of injection of compound*	Number of mice	Number of cells in spleen ($\cdot 10^6$)	Number of antibody-forming cells in spleen	Titer of antibodies †	
					hemo-lysins	hemag-glutinins
Novembichin	-1,0	28	185 \pm 13,1	43,050 (31 920-58 080)	5,7 \pm 0,2	6,3 \pm 0,2
Degranol	-1,0	51	26,6 \pm 2,4	88 (52-150)	<1	<1
Sarcolysin	-1, 0, +1, +2	20	21,5 \pm 4,1	19 (11-32)	<1	<1
thio-TEPA	-1, 0, +1, +2	68	60 \pm 10	159 (106-237)	<1	1,1 \pm 0,3
Control	0	15	316 \pm 24	115,300 (91 411-145 500)	6,2 \pm 0,7	7,7 \pm 0,5

*Days of injection of compound: -1) on day before immunization; 0) on day of immunization; +1) on day after immunization.

†In logarithms to base 2, initial dilution of serum 1:10.

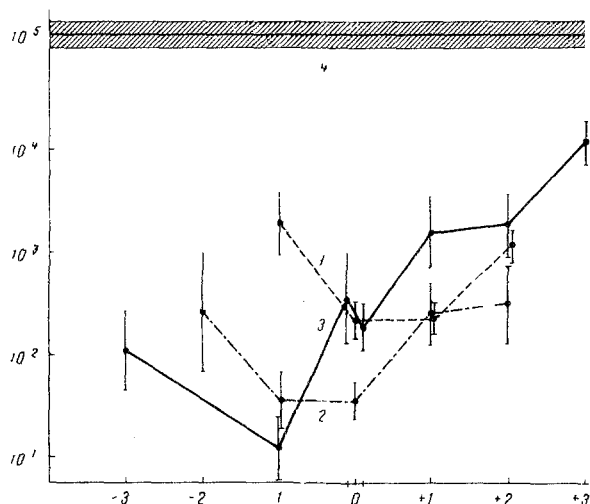


Fig. 1. Effect of whole-body irradiation and of some radiomimetics on immune response depending on times of administration. Abscissa, days of irradiation or injection of radiomimetics before (-3, -2, -1) or after (+1, +2, +3) injection of antigen; ordinate, number of antibody-forming cells in spleen 4 days after immunization; 1) thio-TEPA; 2) sarcolysin; 3) irradiation (500 R); 4) control (immunized mice).

The study of the duration of the aftereffect of these compounds (Fig. 2) showed that 30 days after injection of sarcolysin and degranol the immunologic reactivity was still substantially lowered. After the action of thio-TEPA, however, reactivity was restored more rapidly and had almost reached the control level on the 30th day. Immunologic reactivity was not restored for a very long time after whole-body irradiation (7.3% of the control level on the 30th day).

The number of nucleated cells in the spleen was restored faster after injection of the tested immunodepressive agents than ability to produce antibody-forming cells (Fig. 2).

All the tested compounds depressed hematopoiesis (Fig. 3). The number of leukocytes in the peripheral blood reached its lowest level after 4 days, and during the next 20 days it gradually returned to its

All the test compounds had a strong inhibitory action on the immune response, except novembichin, the effect of which was less marked (Table 1).

Comparison of the changes in the total number of spleen cells and changes in the number of antibody-forming cells after administration of sarcolysin, degranol, and thio-TEPA suggests that these compounds act mainly on the antibody-forming cells or their precursors (by comparison with the other spleen cells). The action of novembichin, on the other hand, is less selective.

In the experiments of series II, on 216 animals, the effect of the time of administration of thio-TEPA, sarcolysin, and ionizing radiation on their immunodepressive activity was investigated (Fig. 1). These experiments showed that sarcolysin possesses the strongest immunodepressive action if injected on the day before immunization or on the same day, while thio-TEPA has the strongest action if given on the day of immunization or the following day. Ionizing radiation produces maximum inhibition of antibody formation if the antigen is injected 24 h after irradiation. Irradiation on the day of injection of antigen or on the following days is less effective. However, irradiation 2 h after injection of antigen inhibited the immune response more strongly than irradiation 2 h before injection of antigen.

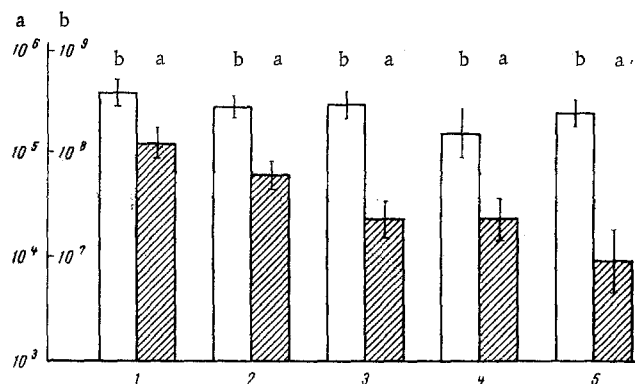


Fig. 2. Immunologic reactivity of animals 30 days after injection of radiomimetics and after whole-body irradiation. 1) Control; 2) thio-TEPA; 3) sarcylisin; 4) degranol; 5) irradiation (500 R); a) number of antibody-forming cells in spleen 4 days after immunization; b) total number of spleen cells.

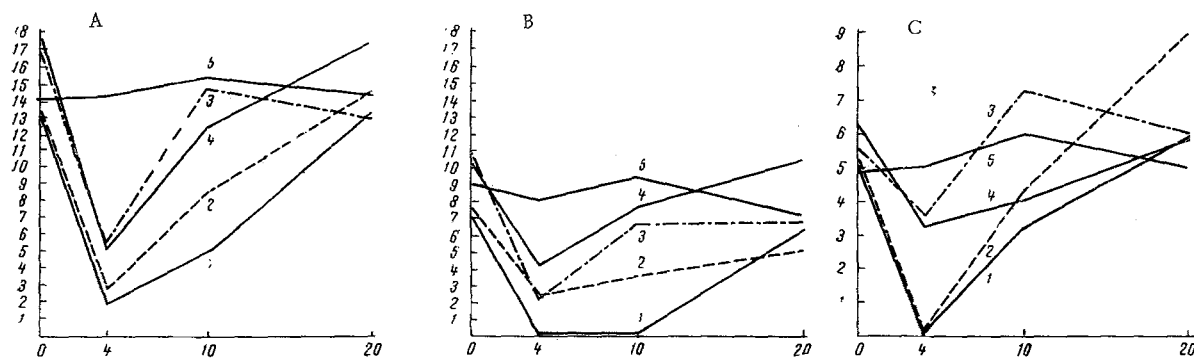


Fig. 3. Effect of some radiomimetics on number of leukocytes (a), lymphocytes (b), and polymorphs (c) in circulating blood. Abscissa, days after injection of compound; ordinate, number of cells (in thousands/mm³): 1) sarcylisin; 2) thio-TEPA; 3) degranol; 4) novembichin; 5) control.

normal level. After administration of these compounds the numbers of both lymphocytes and polymorphs were reduced. The most marked lymphocytopenic action was shown by sarcylisin (Fig. 3b), which caused practically the complete disappearance of lymphocytes from the blood in the period between the 4th and 10th days after injection. The lymphocyte count in the circulating blood was restored much more slowly than the polymorph count after administration of all these compounds except novembichin. However, by the 20th day the lymphocyte count was close to its initial level, or sometimes it was actually higher.

Analysis of these results shows that all the tested compounds (except novembichin) very strongly suppressed immunologic reactivity. So far as sarcylisin and, to some extent, thio-TEPA are concerned, this result is confirmed by the work of other investigators [2, 4-10].

Some immunomorphologic parallels are interesting. As might be expected, the immunodepressive activity of the various compounds is directly dependent on their ability to depress lymphopoiesis.* However, the immunologic reactivity of animals receiving radiomimetics is restored much later than the number of cells in the spleen and the number of lymphocytes in the blood. It may therefore be supposed that immunologic "maturation" of the regenerating lymphoid tissue is delayed compared with normalization of purely morphological indices. This fact has also been observed in relation to regeneration of the spleen after surgical trauma [3].

*Unlike some other workers [1], the writer did not observe any selective depression of lymphopoiesis by novembichin.

All the compounds investigated belong to the group of radiomimetics. It was therefore naturally expected that the times at which their injection produced the maximal effect would coincide with the times of maximal effect of irradiation, or would be earlier (because of persistence of the compound in the body). However, the corresponding curves for sarcolysin and, in particular, for thio-TEPA were shifted slightly toward later periods compared with the curves for irradiation. The reason for this shift is not yet clear.

Both sarcolysin and irradiation gave the maximal effect when given before injection of antigen or simultaneously with its injection. It may therefore be postulated that the chief target of their action consists of potentially immunogenic cells which have not yet started to undergo the cycle of cell conversion associated with the immune response.

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