EFFECT OF SOME IMMUNODEPRESSANTS ON ANTIBODY FORMATION AGAINST SHEEP'S ERYTHROCYTES

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Experiments carried out on mice to study the immunodepressive action of certain antimetabolites of nuclear metabolism (6-thioguanine, imuran, compound KI-43) and alkylating agents (cyclophosphane, dipin, chlorambucil) have shown that cyclophosphane is the most active immunodepressant. It sharply depresses the primary and secondary response of antibody formation to sheep's erythrocytes, and has a less marked effect on the formation of immunologic memory to this antigen. The state of decreased immunologic reactivity after injection of cyclophosphane persists for 2-6 weeks (depending on the injected dose), although in the active form the compound is present in the body for not more than 3 h after its injection.

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The search for methods of suppressing immunologic reactivity of the body is an urgent task at the present time in connection with the transplantation of organs and tissues and the treatment of autoimmune diseases. One such method is to use artificially synthesized substances possessing immunodepressive action. In recent years many such immunodepressants have been studied, the most active of which have been found to be certain antimetabolites and alkylating agents [7, 14].

An important stage in the investigation of these immunodepressants is the determination of the comparative effectiveness of their action on antibody formation. Besides its practical interest, this problem is also theoretically important, because the use of agents which differ in their chemical structure and character of action on the immune response can yield additional information concerning the process of antibody formation itself. A no less important matter is the study of the action of immunodepressants on various forms of immune response (primary and secondary response, the formation of immunologic memory).

The object of this investigation was to compare the immunodepressive action of various substances belonging to two classes of compounds: analogs of the nucleic acid bases and alkylating agents. As well as the better known immunodepressants [6-thioguanine, imuran, cyclophosphane (cyclophosphamide)], other substances were used, whose effect on immunogenesis has received little study (compound KI-43, dipin,* chlorambucil).

EXPERIMENTAL METHOD

Adult noninbred albino mice and CC57BR mice of both sexes were used in the experiments, Sheep's erythrocytes were used for immunization as a single intravenous injection in a dose of 500 million, and as two injections, each of 1 million, to investigate the secondary immune response. The number of antibody-

* Tetraethyleneimido-piperazine-N,N'-diphosphoric acid.

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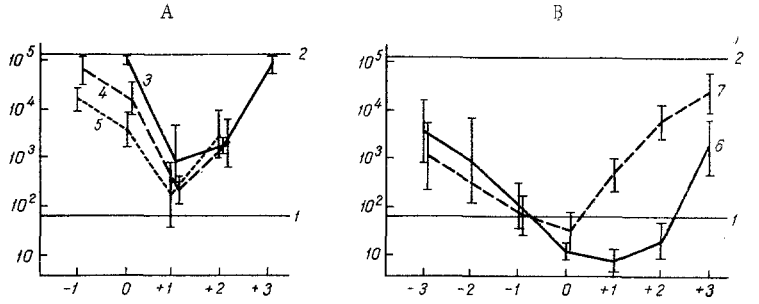

Fig. 1. Effect of some antimetabolites of nuclear metabolism (A) and alkylating agents (B) on the primary immune response. 1) Level of antibody-forming cells in control unimmunized mice; 2) in control immunized mice; 3) 6-thioguanine; 4) KI-43; 5) imuran (440 mg/kg); 6) cyclophosphane; 7) dipin. Abscissa, days of injection of immunodepressants relative to day of immunization (day 0); ordinate: number of antibody-forming cells in spleen.

forming cells in the spleen was formed on the 4th day after immunization by means of the reaction of local hemolysis in gel [2, 12].

Analogs of the nucleic acid bases (6-thioguanine, imuran, compound KI-43) and alkylating agents (cyclophosphane, dipin, chlorambucil) were injected intramuscularly in the maximal tolerated doses: 6-thioguanine—12 mg/kg, imuran—110 mg/kg, KI-43—40 mg/kg, dipin—100 mg/kg, and chlorambucil—15 mg/kg body weight. The exception was cyclophosphane, the dose of which in most experiments was about half the maximal tolerated dose (200 mg/kg). Imuran was also used in a lethal dose (440 mg/kg). Immunodepressants were injected at different times relative to the day of injection of the antigen.

The results obtained were analyzed by statistical methods (calculation of the geometric mean and confidence intervals at $P < 0.05$).

EXPERIMENTAL RESULTS

In the experiments of series I, conducted on 240 mice, the effect of immunodepressants was studied on the primary immune response to injection of 500 million sheep's erythrocytes. The compounds were injected in a single dose 3, 2, and 1 day before immunization (days -3, -2, and -1, respectively), on the day of injection of the antigen (day 0), and 1, 2, and 3 days after immunization (days + 1, + 2, and + 3). The experimental results are summarized in Fig. 1, showing that all analogs of the nucleic acid bases used were maximally effective when injected after immunization (6-thioguanine on days + 1 and + 2, compound KI-43 and imuran in a dose of 440 mg/kg on day + 1). The number of antibody-producing cells in the spleen was reduced by 2-3 orders compared with the control. Imuran, if used in the maximal tolerated dose, was ineffective, and only in a lethal dose did it have a marked immunodepressive effect on the animals surviving for 5 days.

Alkylating agents differed in their activity. Chlorambucil had a slight immunodepressive action. Dipin and cyclophosphane, on the other hand, depressed the immune response extremely strongly. In contrast to the analogs of the nucleic acid bases, both these agents gave a marked depressive effect when injected before immunization. However, whereas the best results were obtained by injection of dipin at day -1 or 0, cyclophosphane gave its strongest action when given at days 0, + 1, and + 2. In this way the character of the action of cyclophosphane on the immune response bore some resemblance to that of the nuclear antimetabolites, although the effect of cyclophosphane was much stronger (Fig. 1).

Because of these features characterizing the effect of cyclophosphane on the immune response, this compound was investigated in more detail.

In the next series of experiments, conducted on 40 mice, the action of cyclophosphane on the formation of the immunologic memory to sheep's erythrocytes and on the response to a second injection of the antigen was studied. The mice were injected intravenously with sheep's erythrocytes twice in doses of 1

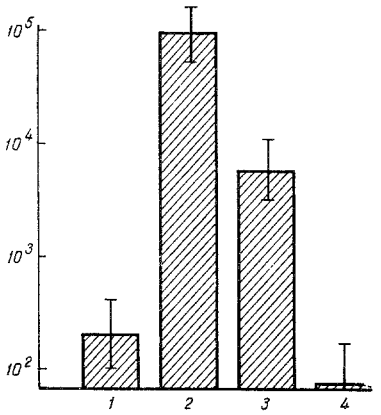

Fig. 2.

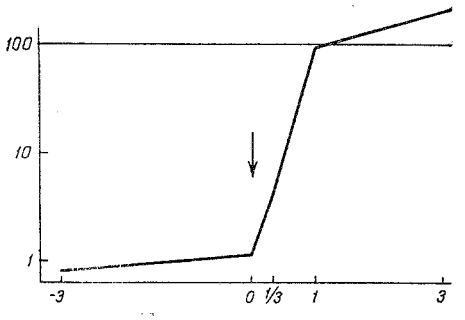

Fig. 3.

Fig. 2. Effect of cyclophosphane on formation and realization of immunologic memory. 1) Single injection of antigen; 2) two injections of antigen; 3) injection of cyclophosphane together with first injection of antigen; 4) injection of cyclophosphane together with second injection of antigen. Ordinate: number of antibody-forming cells in spleen.

Fig. 3. Immune response of spleen cells transplanted into mice receiving cyclophosphane. Abscissa, time of transplantation of cells (in h) relative to time of injection of cyclophosphane (arrow); ordinate, number of antibody-forming cells (in percent of control).

million each time, at an interval of 8-30 weeks. Cyclophosphane was given in a dose of 400 mg/kg (200 mg/kg on two successive days). The compound was injected with either the first or the second injection of antigen (day -1 and 0). The results are shown in Fig. 2.

Although administration of cyclophosphane to the animals during sensitization reduced the number of antibody-forming cells after the secondary antigenic stimulus, the response resembles in character the response of typical revaccination. However, injection of cyclophosphane together with the second injection of erythrocytes led to sharp inhibition of the immune response similar to inhibition of the primary response to 500 million erythrocytes. Half the dose of cyclophosphane (200 mg/kg), when given on days -1 (these results are not shown in Fig. 2), also effectively inhibited the secondary response (76 antibody-forming cells per spleen in a dose of 400 mg/kg, 102 cells with a dose of 200 mg/kg, and about 100,000 cells per spleen in the control).

These results demonstrate that the formation of the immunologic memory is more resistant to the action of cyclophosphane than the process of antibody formation.

It was interesting to discover how long the state of lowered immunologic reactivity persists after injection of cyclophosphane. The compound was injected into mice in doses of 200 and 400 (200×2) mg/kg, followed after different intervals by intravenous injection of 500 million sheep's erythrocytes, and the number of antibody-forming cells in the spleen was determined 4 days later. From 8 to 20 animals were taken at each time, and in some cases as many as 50 mice (altogether about 300 animals were used).

The results of these experiments showed that after injection of cyclophosphane in a dose of 200 mg/kg, the immunologic reactivity of the animals was largely restored within 7 days, although even after two weeks it was lower than normally by a statistically significant degree (3rd day 1.1%, 7th day 23.4%, 14th day 43% of the control level). Complete recovery did not occur until after 3 weeks. When cyclophosphane was given in a dose of 400 mg/kg, its depressive effect was more marked still: even after 1.5 months the level of the immunologic response was only 26.7% of the control.

In connection with these results, the length of time during which cyclophosphane remains in the body in an active form has to be considered. To solve this problem, intact mice were injected intravenously with the compound in a dose of 200 mg/kg, after various time intervals, spleen cells from sensitized syngenic mice (100 million nucleated cells per mouse) were injected intravenously along with 1 million sheep's

erythrocytes. On the 5th day after injection the number of hemolysin-producing cells in the spleen of the recipient mice was investigated.

Cyclophosphane effectively depressed the immune response of the transplanted cells (Fig. 3) if it was injected 3 h after or simultaneously with transplantation. Conversely, the spleen cells of sensitized donor mice gave a normal immune response in the recipient's body if they were transplanted 1 or 3 h after injection of cyclophosphane. These results show that by 1 h after injection, cyclophosphane or its active metabolic products [1, 4, 9] are no longer capable of acting on the cells transplanted at this time. Other workers [8] have obtained similar results.

The following conclusion can be drawn from these experiments. To begin with, the very different effects of the immunodepressant investigated must be noted. Cyclophosphane was most effective, even when used in a relatively smaller dose than the other compounds. A special feature of cyclophosphane is that it has an immunodepressive action both before and after immunization (see also [6, 14]), but it is most active in the latter case.

The results obtained conflict with the views [5, 15, 17] that a special phase of the immune response exists which is most sensitive to all manner of different immunodepressive agents. As Fig. 1 shows, the nuclear antimetabolites and dipin selectively attack different phases of the immune response. The effect of cyclophosphane on the immune response differs in this sense from that of dipin and of irradiation [5, 18], although cyclophosphane is usually classed among the radiomimetic agents [7].

It may be considered that the observed differences are connected with the mode of action of different preparations on immunocompetent cells. Nuclear antimetabolites, for example, are most effective in that stage of immunogenesis during which intensive proliferation of antibody-forming cells and their precursors is observed; in the period when the cells do not synthesize DNA and RNA, their action is only slight. Conversely, dipin acts on the cells also in the period preceding their proliferation, thus explaining its high effectiveness when injected on days -1 and 0. The special position of cyclophosphane may be due to its increased activity relative to proliferating cells. This suggestion is confirmed by data in the literature [10, 13]. The formation of immunologic memory, in which the role of proliferative processes is not so evident, is also affected by cyclophosphane to a lesser degree. Similar results were obtained previously during the investigation of 6-thioguanine [3]. These results do not support the view that the phase of primary contact between cells and antigen is more sensitive to various influences [11, 16].

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