EFFECT OF 6-THIOGUANINE ON IMMUNOLOGIC REACTIVITY OF ANIMALS TO FOREIGN ERYTHROCYTES

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Administration of 6-thioguanine to mice 24-48 h after immunization with sheep erythrocytes sharply inhibits formation of antibody-producing cells. If two small immunizing doses of erythrocytes were given to the mice, 6-thioguanine had no significant effect on the stage of sensitization, but suppressed the secondary response when injected after the second injection of antigen.

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Analogs of bases of the nucleic acids are known to be capable of inhibiting the development of immunity [1, 5]. They are used in surgical practice to overcome the barrier of tissue incompatibility. Obviously, therefore, the study of the mechanism of action of analogs of the bases of nucleic acids is of considerable practical as well as theoretical importance.

Sterzl [7] considers that analogs of bases of the nucleic acids prevent the formation of a new template for antibody synthesis in the antibody-forming cells. Other workers consider that a more probable cause of inhibition of the immune response is the antiproliferative action of these substances [2].

The object of the present investigation was to study the course of immunogenesis after administration of 6-thioguanine in relation to number of doses of the immunizing stimulus, size of dose of the compound, and scheme of its administration. The immune response was recorded by the method of local hemolysis in gel, by which the immunologic activity of extensive cell populations can be assessed quantitatively.

EXPERIMENTAL METHOD

Adult CC57BR mice were immunized intravenously with sheep or rat erythrocytes. 6-Thioguanine was injected intraperitoneally. The doses of antigen and of 6-thioguanine and the schemes of their administration are given in the text. The number of antibody-forming cells in the spleen was determined at various times after immunization by the method of local hemolysis in agar [1, 3]. When sheep erythrocytes were used as antigen, guinea pig complement was used, and when working with rat erythrocytes, rabbit complement was used (rabbit serum diluted 1:2.5 with physiological saline). Complement from guinea pig serum was ineffective in the latter case.

The time taken for the number of antibody-forming cells in the mouse spleen to be doubled was calculated by the formula:

$$T = \frac{0.301 \times t}{\lg N_t - \lg N_0},$$

where T represents the time taken for the number of antibody-forming cells to double after t hours, N_0 the original number of antibody-forming cells, N_t the number of antibody-forming cells after t hours. The results were analyzed by statistical methods.

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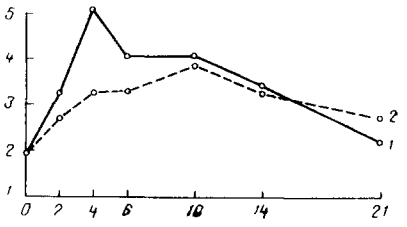

Fig. 1. Effect of 6-thioguanine on immune response of mice injected with sheep erythrocytes. 1) Control; 2) experiment. Ordinate, logarithm of number of antibody-forming cells in spleen; abscissa, days after immunization.

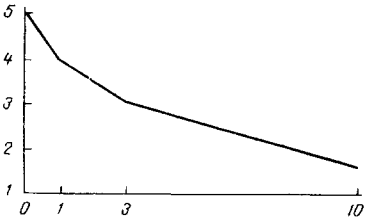

Fig. 2. Relationship between dose and immunodepressive action of 6-thioguanine. Ordinate, logarithm of number of antibody-forming cells in spleen; abscissa, dose of 6-thioguanine (in mg/kg).

EXPERIMENTAL RESULTS

After intravenous injection of $5 \cdot 10^8$ sheep erythrocytes into mice the number of antibody-forming cells in the spleen rose rapidly during the first 4 days (Fig. 1). The mean time for the number of antibody-forming cells to be doubled in this period was 9 h. This corresponds to the generation time of cells of blast type [4]. After 4 days the curve of the number of antibody-forming cells in the mouse spleen gradually fell, although it still remained above its original level as long as 3 weeks after immunization ($P = 0.003$). Injection of 6-thioguanine (in a dose of 3 mg/ kg on the day of immunization and on the following 3 days) did not prevent the appearance of antibody-forming cells in the spleen of the mice, but they were far fewer in number and they appeared more slowly (Fig. 1). Under the influence of 6-thioguanine the peak of the response was postponed by 10 days. The time taken for the number of antibody-forming cells to double after injection of 6-thioguanine increased to 27-32 h. Later (after 10 days) the curve of the number of antibody-forming cells in the spleen began to fall, but much more slowly than in the control animals, so that by the 21st day after immunization the number of antibody-producing cells in the spleen of the experimental mice was significantly higher ($P = 0.001$) than the control level (Fig. 1).

The degree of depression of the immune response was increased with an increase in the dose of 6-thioguanine (Fig. 2).

After intravenous injection of $5 \cdot 10^8$ rat erythrocytes, the immune response of the mice was characterized by a rapid decrease in the number of antibody-forming cells in the spleen immediately after the peak (on the 4th day). Under these conditions the action of 6-thioguanine was expressed simply by a sharp decrease in the absolute number of antibody-forming cells.

In the next series of experiments we studied the immunologic depressive activity of 6-thioguanine as a function of the time of its administration. Mice immunized with $5 \cdot 10^8$ sheep erythrocytes received an injection of 12 mg/kg 6-thioguanine on the day of immunization or on one of the following days. Four days after injection of antigen the number of antibody-forming cells in the spleen was determined and the titers of circulating antibodies in the blood serum found (Table 1).

The results in Table 1 show that 6-thioguanine had a marked immunologic depressive action when administered 1-2 days after injection of the antigen. At other times its administration was effective.

In the next series of experiments we studied the effect of 6-thioguanine on formation of the secondary immune response. Mice were injected with $1 \cdot 10^6$ sheep erythrocytes twice at an interval of 6 days. 6-Thioguanine (3 mg/kg per diem, 4 times altogether) was injected on days from 0 to +3 or from +6 to +9 after the primary immunization.* Analysis of the results (Table 2) shows that administration of 6-thioguanine against the background of primary immunization did not prevent sensitization of the immunologically competent cells: a second injection of antigen produced a typical secondary response, although it was less marked than in the control. If, however, 6-thioguanine was injected after the second antigenic stimulus, the immune response was virtually absent (Table 2).

*Day 0, day of immunization; day +1, 1st day after immunization, and so on.

TABLE 1. Relationship between Immunologic Depressive Action of 6-Thioguanine and Time of Administration

Day of injection of 6-thioguanine after immu.	Number of animals	Total number of cells in spleen ($\cdot 10^6$)	Number of antibody-forming cells in spleen		Titer of antibodies†	
			per million cells	total number ($\cdot 10^3$)	hemolysis	hemagglutinins
0	10	159±12	709±67	108,4±2,8	6,8±0,3	4,9±0,2
1	10	140±9	10±3	1,5±0,5	0,6±0,2	1,2±0,3
2	10	131±5	14±2	1,9±0,3	1,5±0,5	2,9±0,3
3	5	172±27	521±79	86,1±11,8	5,6±0,2	5,5±0,3
—*	9	218±20	725±79	174,7±26,0	6,3±0,3	4,4±0,3

*No 6-thioguanine given.

†In $\log_2$ values; scale origin (0) corresponds to a titer < 1:10.

TABLE 2. Effect of 6-Thioguanine at Different Stages of Immunization

Group of animals	Day of experiment											Number of rats	Number of antibody-forming cells in spleen of mice ($\cdot 10^3$), M ±m
	0	1-й	2-й	3-й	4-й	5-й	6-й	7-й	8-й	9-й	10-й		
1	A						A				X	48	65,4±10,0
2	AT	T	T	T			A				X	34	29,7±5,0
3	A						AT	T	T	T	X	11	0,7±0,1
4	A										X	15	1,5±0,2
5							A				X	40	0,6±0,2

Legend: A denotes injection of antigen, T injection of 6-thioguanine, and X performance of Jerne's test.

The results of these experiments show that 6-thioguanine had no significant effect on the first stages of the immune response (Stages $x \rightarrow y$ in the terminology of Sercarz and Coons [6]). Conversely, the next phase $y \rightarrow z$ of the immune response was depressed highly effectively by the analog. In the doses given, 6-thioguanine had no direct cytotoxic action on the antibody-forming cells, judging by the relatively weak effect of its injection on the 3rd day after immunization and also by the fact that the size of the patches in animals receiving 6-thioguanine and in the controls was the same on the 4th day after immunization (diameter of zones of hemolysis 0.33 ± 0.01 and 0.34 ± 0.01 mm respectively). Considering the dynamics of the immune response after injection of 6-thioguanine and also the sensitivity to the action of the compound of those phases of the immune response which are closely associated with proliferation of antibody-forming cells [6], it may be postulated that the immunodepressive action of 6-thioguanine is due primarily to its antimitotic properties.

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