A STUDY OF THE EFFECT OF ANALOGS OF NUCLEIC ACID BASES ON IMMUNOGENESIS USING JERNE'S METHOD

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It was shown by a number of authors [1-3, 6, 7, 10, 11, 14, 15] that some analogs of nucleic acid bases (6-mercaptopurine, 6-thioguanine and others) injected into animals during immunization, partially or completely block the production of antibodies. It remains unclear whether this effect is connected with the inhition of the proliferation of antibody-producing cells and their precursors or with the reduction of antibody production by individual cells, or, finally, with the overall hyperplasia of lymphoid tissue. The method recently suggested by Jerne [8], which makes it possible to find the cells which produce antibody among the total population of lymphoid cells, is of particular interest for the study of this question. This method has a number of advantages over other immuno-morphological methods: being strictly specific (in contrast to the plasmocellular reaction) it makes it possible to subject an extremely broad poplulation of lymphoid cells to immuno-morphological analysis, which favorably distinguishes it not only from the "microdrop" [9, 12] and "immuno-adhesion" [9, 13] methods, but even from the immunofluorescent [5] method.

In the present work, the changes in the number of antibody producing cells from the effect of 6-mercaptopurine and 6-thioguanine were studied using Jerne's method.

EXPERIMENTAL METHOD

The experiments were done on adult mice of the CC57BR line of both sexes. The mice were immunized (once) by an intravenous injection of washed sheep erythrocytes (30% suspension of cells in physiological solution, 0.2 ml per mouse). The number of antibody producing cells in the spleen (in some experiments also in the iliac and mesenteric lymph nodes, thymus and blood) were determined 2, 4, and 6 days after immunization.

The treatment of the experimental animals with the nucleic acid base analogs was carried out according to the following plan. The mice which were killed on the 2nd day after immunization were injected with 6-mercaptopurine (or 6-thioguanine) on the day of immunization (immediately after the injection of erythrocytes) and on the following day. In the 4 day and 6 day studies the analogs were injected on the day of immunization and 1, 2, and 3 days afterwards. The analogs were injected intraperitoneally: 75 mg/kg of 6-mercaptopurine and 3 mg/kg of 6-thioguanine.

The control animals either were not injected with the analogs (control I) or received 4 injections of 6-thioguanine in the month before immunization (control II on the general toxicity of the preparation).

In another series of experiments, the mice, immunized once with a 30% suspension of sheep erythrocytes and given the analogs for 4 days, were again injected with erythrocytes (0.2 ml of a 1% suspension) 5-6 weeks after immunization. The number of antibody-producing cells were determined 2, 4, and 6 days after the 2nd injection.

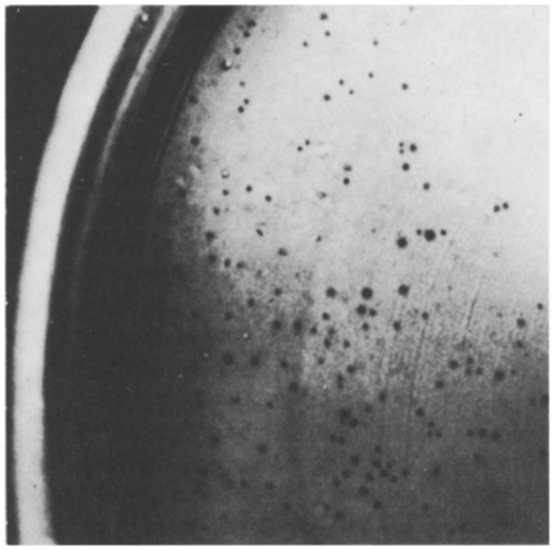

Fig. 1. Hemolytic zones (plaques) in agar on Petri dish. The zones of hemolysis appear as dark spots. Macrophotograph with transverse illumination. Magnified 2.5 ×.

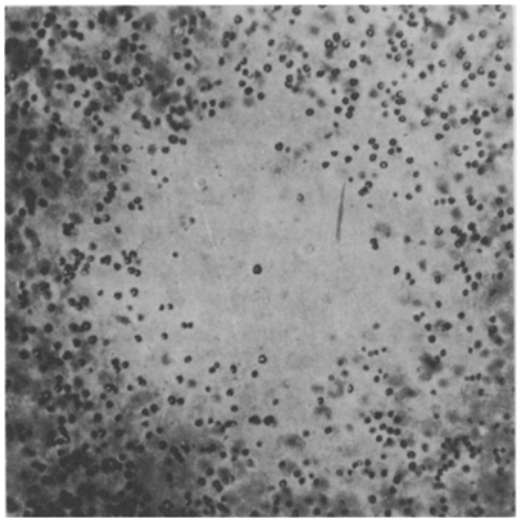

Fig. 2. A single plaque under the microscope. Ob. 10 ×, Oc. Homal 3 ×.

On the day of the experiment, the mice were killed with ether and cell suspensions of the organs being studied made in Hanks' solution (pH 7.0-7.2). The number of antibody-producing cells was determined by the method of Jerne and Nordin [8] with slight modifications. To eliminate the possibility of the presence of complement fixing substances [6], agar in the form of a 2-2.5% gel washed for 5-7 days with running tap water was used. After a final washing in distilled water the agar was melted and an equal volume of doubly concentrated Hanks' solution added to it. The 1-1.25% agar obtained was poured into Petri dishes (18-20 ml per dish).

To 0.8-1% agar, also prepared in Hanks' solution, melted and cooled to 39-40°, were added sheep erythrocytes containing 50 million cells per ml and a cell suspension of the organs being tested (1.5 million nucleated cells per ml). The mixture was carefully stirred with a magnetic stirrer, 2 ml poured onto the surface of the solidified agar in the plates and spread over the surface (by tilting the plate) in a thin layer. After the agar solidified, the plates were placed in an incubator at 37° for an hour. At the end of the incubation the surface of the agar was dried a little by opening the plate for 10 min, and 3 ml of complement (guinea pig serum) diluted 1:5 poured into it. The result was determined after 30 min of incubation at 37°.

The zones of hemolysis in the form of clear round spots (plaques) with a diameter of 0.1-0.7 mg (Fig. 1) around the antibody producing cells were easily distinguished by the naked eye. Under the microscope the plaque is a round zone free of erythrocytes or containing a small number of cells. In its center a nucleated cell is usually seen (Fig. 2).

With a small number of plaques (up to 100-120 per plate), they were counted completely. If there were more plaques, they were randomly counted in 20 squares with an area of 0.25 cm^2, then converted for the whole surface of the plate and the number of plaques per 1 million added cells (or in the whole spleen) calculated.

Spleen cells of nonimmunized mice were used as the control (control for nonspecific hemolysis). As a rule, in this case plaques did not develop.

The data obtained was treated statistically.

RESULTS

It was established in preliminary experiments that after intravenous immunization of the mice antibody producing cells are found only in the spleen and are absent from the lymph nodes, thymus and blood.

The results of counting the number of cells which produce hemolysis (plaques) in the spleens of animals of the various groups after initial immunization are presented in the table.

As seen from the table, under normal conditions, the number of antibody producing cells 2 days after immunization is small (control I). It increases sharply on the 4th day and then again falls on the 6th day.

Number of Antibody Producing Cells (in 1 Million Spleen Cells) Determined by the Jerne Method

Treatment of animals	Time after immunization (in days)		
	2	4	6
Immunization (control I)	8,6±2,8 (11)	341,3±48,2 (11)	54,1±13,5 (10)
Immunization a month after injection of 6-thioguanine (control II)	9,0±3,6 (5)	249,3±60,2 (5)	26,1±10,2 (5)
Immunization + 6-mercaptopurine	3,4±1,4 (5)	104,6±36,1 (6)	39,5±6,7 (5)
Immunization + 6-thioguanine	2,2±0,5 (6)	7,0±1,8 (6)	17,5±8,5 (5)

Note. The number of animals examined is indicated in brackets.

The preliminary (the month before immunization) injection of 6-thioguanine leads to some decrease (not statistically significant) in the number of antibody producing cells (control II).

6-Mercaptopurine, introduced during the initial immunization and in the days following it, causes a statistically significant decrease in the number of plaques on the 4th day after immunization (R = 0.001). On the 2nd and 6th days the number of plaques is also decreased in comparison with control I, but it is not statistically significant (R = 0.1-0.4).

The introduction of 6-thioguanine during the initial immunization causes a sharp decrease in the number of antibody producing cells in all the periods after immunization. This decrease is greatest on the 4th day, when the number of antibody producing cells is almost 50 times less than in the control. An even sharper difference is obtained if the absolute number of antibody producing cells in the spleen is calculated. Thus, on the 4th day the spleens of animals who received 6-thioguanine contained on the average only 900 antibody producing cells, in the controls it was 100 times more (92,000).

The data presented shows that the introduction of nucleic acid base analogs during initial immunization leads to a decrease in the number of antibody-producing cells. 6-Thioguanine has an especially strong effect in this respect. The hemolytic zones (plaques) around the antibody producing cells of the experimental (received analogs of nucleic acid bases) and control animals did not differ essentially in external appearance and size. In the animals subjected to the action of the analogs of nucleic acid bases, general hypoplasia of the lymphoid tissue was noted: the number of nucleated cells in the spleen decreased an average of two times in comparison with the number in the control. Both of the preparations tested caused this effect to the same degree.

In series II of the experiments in which the effect of the analogs injected during the initial immunization, with the 2nd injection of antigen, was studied different results were obtained. No significant difference between the number of antibody-producing cells in the control and experimental animals was found. However, these experiments were done on a comparatively small number of animals, and therefore, it will only be possible to draw conclusions after additional studies.

The results obtained make it possible to conclude that the drop in the immunological reactivity of the body from the use of analogs of nucleic acid bases is caused by a decrease in the number of antibody producing cells. The decrease is not only absolute (in calculating for the whole spleen) but relative (in calculating per 1 million cells), that is, it does not depend on the general hypoplasia of the lymphoid tissue. This allows the assumption that the analogs selectively inhibit the proliferation of the antibody-producing cells or their precursors.

The experiments which were done also show that the method of determining the number of antibody-producing cells in a gel can be successfully used to study the laws of antibody production. An analysis of the data obtained shows that in the early stages of immunogenesis the number of cells producing antibodies is extremely small, but

later rapidly increases. This fact, as well as the decrease in the number of antibody-producing cells after the injection of nucleic analogs, agrees with one of the assumptions of the clonal-selection theory on the close connection of antibody production with cellular proliferation processes [4].

SUMMARY

A study was made in dynamics on the number of spleen cells producing antibodies following immunization of mice with erythrocytes. It was found that shortly after immunization the number of these cells was extremely small and subsequently grew rapidly. In animals given injections of 6-mercaptopurine or 6-thioguanine during immunization the number of antibody forming cells reduced considerably. Upon use of 6-thioguanine this effect was the most pronounced. The findings obtained are considered in the light of the clonal-selection theory.

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