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Inhibition of antibody production by thioinosine. 3. Studies on antibody biosynthesis

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Abstract

The effect of thioinosine on antibody response of immunized rabbits has been studied. When given simultaneously with the antigen, thioinosine profoundly suppresses the formation of humoral antibody.

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INHIBITION OF ANTIBODY PRODUCTION BY THIOINOSINE PART III. STUDIES ON ANTIBODY BIOSYNTHESIS

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Chemoimmunosuppressive agents (1), alkylating agents (nitrogen mustard and cyclophosphamide), purine antagonists (6 mercapto purine (2—4) and azothioprine (5)) and folic acid analog (amethopterin), are widely used as drugs for therapy of "autoimmune" diseases such as progressive hepatitis (6) and rheumatoid-arthritis (7).

As these agents are mostly antimetabolites (8), they are much toxic to the patients. Therefore, it is necessary to select less toxic chemicals, yet maintaining their immunosuppressive activity.

Thioinosine, 6 mercaptopurine riboside (MW, 284.29) having 6-mercaptopurine (6-MP) residue in its chemical structure is considered to have immunosuppressive effect. As to the toxicity of thioinosine, LD₅₀ for mice is about 2500 mg/kg by intraperitoneal injection (9) and in comparison with that of 6-mercaptopurine, 380 mg/kg, the toxicity of thioinosine is extremely low. However, there is no report concerning immunosuppressive effect of thioinosine.

We investigated the effect of thioinosine on antibody production of rabbit injected with bovine serum albumin. This report describes briefly our results.

MATERIALS AND METHODS

Fifteen white New Zealand strain rabbits weighing about 3 kg and kept in a stock ad libitum diet were used. On *Experiment 1*, 3 rabbits were used as control group, 4 as thioinosine treated group, and 4 as 6-MP treated group. On *Experiment 2*, 2 rabbits were used as control, and 2 were as thioinosine treated group. All animals were injected intramuscularly with 70 mg of crystalline bovine albumin (Nutritional Biochemicals Corporation), on *Experiment 1*, and with 100 mg on *Experiment 2*, 3 times a week during 2 weeks. The experimental animals were given 6-MP (Zellstoffabrik, "Waldhof"), 3.0 mg/kg/day, or thioinosine 5.4 mg/kg/day (mole equivalent) intramuscularly according to the schedule. Blood was drawn from a marginal ear vein at appropriate intervals. Antibody titer was measured by antigen antibody dilution method.

RESULTS

Experiment 1 : Rabbits were given thioinosine from 0 day to 23 days, showed no detectable antibody during the 23 days of the study as shown in Table 1, one rabbit out of 4 was dead during the experiment. In the

TABLE 1. COURSE OF THE FIRST IMMUNE RESPONSE IN CONTROL, AND THIOINOSINE AND 6-MP TREATED RABBITS. (EXPRESSED AS ANTIBODY TITER)

method	date (D/M) days after first injection	16/2	27/2	4/3	10/3
control rabbits	No. 1	0	0	0	4
	No. 2	0	0	0	8
	No. 3	0	0	0	1
thioinosine treated rabbits	No. 4	0	0	0	0
	No. 5	0	0	0	0
	No. 6	0	0	0	0
	No. 7	0	dead*	dead	dead
6-MP treated rabbits	No. 8	0	0	0	0
	No. 9	0	0	dead	dead
	No. 10**	0	0	0	0
	No. 11	0	0	dead	dead

Antigen (bovine serum albumin 70 mg/once) was injected on zero, third, fifth, eighth, tenth and twelfth respectively. Thioinosine (5.4 mg/kg/day) or 6-MP (3.0 mg/kg/day) was injected every day.

* died on February 25th. ** died on March 12th.

case of 6-MP treated rabbits by the same schedule, antibody production was not detected. In this case, 2 rabbits out of 4 were dead. Three control rabbits injected with only bovine serum albumin showed 1 : 1, 1 : 4 and 1 : 8 of antibody titer respectively 23 days after the first injection of antigen.

Experiment 2 : Rabbits were injected with bovine serum albumin, 5 times every day, and 31 days after the first injection average antibody titer showed 1 : 8 as shown in Fig. 1. Then antibody titer decreased to 1 : 4 and 1 : 2 on 40 days after the first injection of bovine serum albumin. Rabbits were injected with bovine serum albumin 3 times at 2 days' interval from 43 to 47 days and thioinosine was administered from 31 to 51 days after the first injection. Average antibody titer in the serum of thioinosine treated rabbits shows only 1 : 1 on 53th day. In contrast to this, the control group without being treated by thioinosine showed 1 : 32 of average antibody titer.

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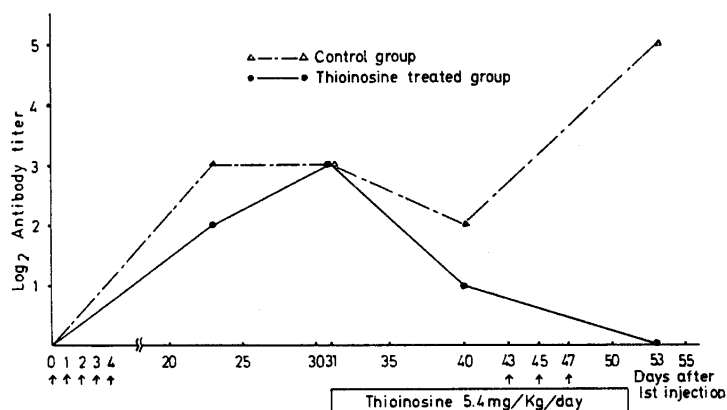


Fig. 1 Course of immune response in control and thioinosine treated rabbits. Each vertical arrow represents antigen injection. Time of administration of thioinosine is represented by the clear block. Antibody titer of 0 day is below 1 : 1.

DISCUSSION

The data described herein demonstrate that thioinosine, its 6-MP residue, convinced us of the powerful nucleic acid antimetabolite, abolishes the humoral antibody production in the rabbits to bovine serum albumin when given simultaneously with the antigen. As to the site of action of thioinosine with respect to antibody formation, suppression of protein synthesis in immunoblasts or plasmacytes or that of RNA synthesis in memory cells can be considered to be present.

SCHWARTZ and DAMESHEK (3) described the drug induced immunological tolerance. On the other hand, GOH *et al.* (10) reported that it was not possible to produce immunological tolerance in rabbits pretreated with 6-MP by injection of human serum γ -globulin, and ARGANO and TOBIN (4) described similar phenomena to GOH with 6-MP by injection of human serum albumin. The results of GOH and ARGANO suggest that the effect of 6-MP on antibody synthesis will be described as "temporary suppression of antibody synthesis". It is inferred that the mechanism of thioinosine to antibody synthesis will be similar to 6-MP from its chemical structure, though its metabolic pathway* should be investigated. Studies to determine the mechanism of thioinosine with relation to 6-MP as well as its

* It is considered that 6-MP competes with hypoxanthine for inosinic acid pyrophosphorylase and itself reacted with PRPP and converted to thioinosinic acid (TIMF) (11). This nucleotide inhibits several reactions involving inosinic acid: the conversion to xanthylic acid (XMP) and the conversion to adenylic acid (AMP) via adenylosuccinate (SAMP) (12). However the mechanisms of thioinosine on nucleic acid biosynthesis is not completely clear.

effects on the secondary response and on the immune response to cellular antibody are in progress.

CONCLUSION

The effect of thioinosine on antibody response of immunized rabbits has been studied. When given simultaneously with the antigen, thioinosine profoundly suppresses the formation of humoral antibody.

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