FORMATION OF PRECIPITATION ZONES DURING IMMUNIZATION WITH ALKYLATING COMPOUNDS LONIN-3 AND K-4

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After administration of the chloroethylamine preparations lonin-3 and K-4 to rabbits their serum acquired the property of forming precipitation zones in gel with liver and spleen extracts from mice, rats, rabbits, and dogs. These zones differ in their external appearance from the bands formed in gel during the antigen – antibody reaction.

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In the chemotherapy of malignant tumors we still do not know all the ways by which adaptive reactions may take place to the toxic action of an alkylating compound introduced into the body. It has been shown that the chloroethylamine preparation lonin-3[chemical name p-(di-2-chloroethylamino)-phenylacetamide], if introduced into the body several times in the form of vaccination injections with Freund's deponent, yields sera precipitating in agar with extracts of normal homologous and heterologous organs [2].

The object of the present investigation was to study some of the conditions for obtaining precipitation zones during vaccination with the compounds lonin-3 and K-4 [p-(di-2-chloropropylamino)-phenylacetyl-DL-methionine ethyl ester].

EXPERIMENTAL METHOD

The method used in the investigation was Ouchterlony's gel-precipitation reaction [11], and its micromodification. The following antigens were used: 1-4 mg of lonin-3 in 1 ml 5-20% physiological saline, saline extracts of the spleen, liver, and kidney of healthy mice, rats, rabbits, and dogs, and pharmaceutical γ-globulin. The antibodies used were antisera obtained by immunizing rabbits with compounds lonin-3 and K-4, γ-globulin, and γ-globulin treated with the compound lonin-3, etc. Freund's deponent was used.

Depending on the experimental conditions, lonin-3 was given for 1 immunizing injection in doses of 1, 4, and 10 mg, and compound K-4 in doses of 15 and 30 mg (the doses were determined by the toxicity of the preparation). Altogether 26 experimental sera were prepared: 10 "anti-lonin," 6 "anti-K-4," 2 anti-γ-globulin, 4 anti-γ-globulin sera after treatment of the protein before immunization with the compound lonin-3, and 4 mouse anti-liver sera after treatment of liver homogenate with compounds lonin-3 and K-4.

Two rabbits of the "anti-lonin" group received only the preparation lonin-3 in a dose of 10 mg, without Freund's deponent. The rabbits of control group 1 (5 animals) received Freund's deponent subcutaneously only. The rabbits of control group 2 (7 animals) received no treatment whatever. The sera of 10 healthy rabbits likewise were tested once. As an additional control, 4 rabbit sera against mouse tissues (2 anti-liver and 2 anti-spleen) and 2 rabbit sera against Ehrlich's mouse carcinoma were investigated. Native sera were studied on the 7th, 14th, and 21st days after the end of the immunization cycle.

Altogether 54 sera (26 experimental and 28 control) were investigated in the gel-precipitation reaction.

The rabbits were immunized in two cycles. Each cycle lasted 3 weeks, and in its course each animal was immunized 6 times with equal doses of the preparations as described above. The first injection of the corresponding preparation in the corresponding dose, as a suspension in 1 ml physiological saline, was

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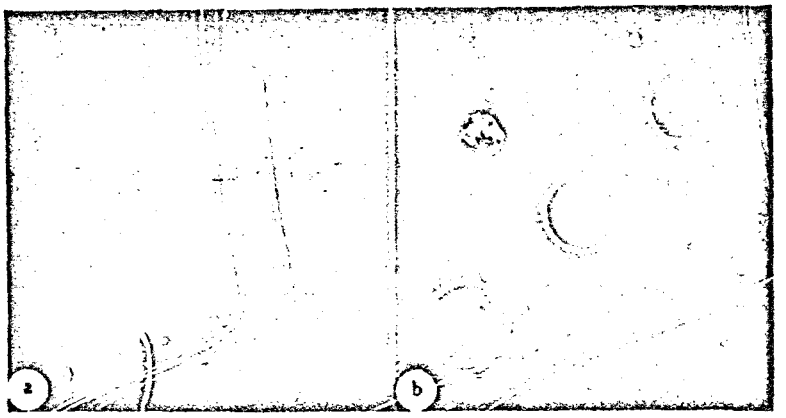

Fig. 1. Gel-precipitation reaction of "anti-lonin-3" (rabbits immunized with lonin-3 in a dose of 1 mg) (L) and "anti-K-4" sera (rabbits immunized with K-4 in a dose of 30 mg) (K^1) with 10-20% and more highly diluted extracts of dog (2) and rabbit (4) spleen. a, b) Variants of the reaction.

given subcutaneously together with Freund's adjuvant (1 ml anhydrous lanolin, 2 ml mineral oil, 2 ml killed BCG). All other preparations were injected intraperitoneally. The intervals between separate cycles were of 1.5-2 months.

The tissue material in the appropriate experiments was injected in a dose of 500 mg per injection [4]. The rabbits of the γ-globulin group received pharmaceutical γ-globulin in a dose of 0.5 ml per injection – either the pure γ-globulin or treated for 1 h with compound lonin-3 in a dose of 2000 and 8000 mg/1000 ml γ-globulin. At the end of 1 h the γ-globulin, together with the compound, was injected into rabbits by the method described above. Each rabbit thus received per injection 0.5 ml of treated γ-globulin and 1 or 4 mg of the compound lonin-3 depending on the experiment. In these experiments two cycles of immunization were given. The first injection in each cycle was given with Freund's deponent.

The rabbits of the "adjuvant" group received Freund's deponent twice in the dose given above, once at the beginning of each cycle of immunization.

EXPERIMENTAL RESULTS

"Anti-lonin-3" and "anti-K-4" precipitating sera were formed in 87.5% of cases. The sera obtained by immunization with lonin-3 in a dose of 1 mg, together with Freund's deponent for one vaccinating injection, did not react with saline extracts of normal tissues of the experimental animals.

Saline extracts of mouse, rat, and rabbit liver and, in particular, spleen gave diffuse precipitation zones with the sera of rabbits immunized with lonin-3 in doses of 4 and 10 mg together with Freund's deponent (Fig. 1a). Similar diffuse precipitation zones were obtained by the reaction of saline extracts of liver and spleen of these experimental animals with the sera of rabbits immunized with K-4 in doses of 15 and 30 mg (Fig. 1b). On dilution of the extracts, separate precipitation bands appeared (Fig. 2a). However, clear bands of the type which appear in the ordinary gel-precipitation reaction when working with antisera against organs, cancer tissues, and γ-globulin (Fig. 2b) were never obtained. On the other hand, in experiments with anti-liver and anti-γ-globulin sera obtained by immunization with the corresponding material treated with lonin-3, precipitation zones were never obtained (Fig. 2b). Anti-lonin-3 sera obtained by immunization with the compound lonin-3 without Freund's deponent gave neither precipitation zones nor bands with saline extracts of the tissues of normal experimental animals in gel.

In control group 1, during the reaction between extracts of normal mouse, rat, rabbit, and dog tissues in gel with the sera of rabbits immunized with Freund's deponent only, neither precipitation zones nor bands likewise were found.

In control group 2, when extracts of normal tissues of the abovementioned animals were tested in the precipitation reaction with sera of unimmunized rabbits, neither precipitation zones nor bands likewise were observed.

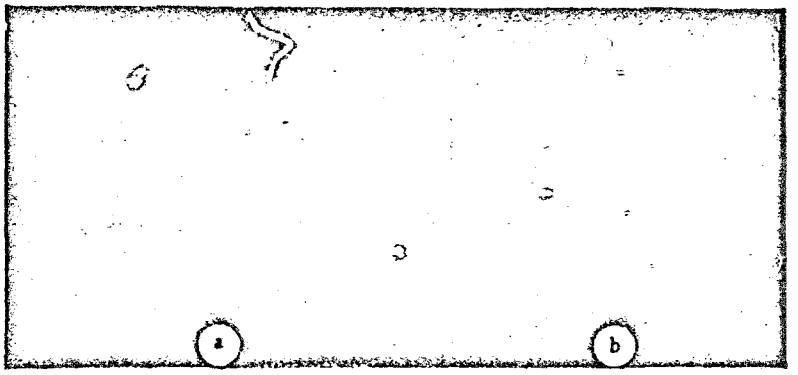

Fig. 2. Gel-precipitation reaction of "anti-lonin-3" (rabbits immunized with lonin-3 in a dose of 10 mg) (L^1) and anti-γ-globulin (γ) serum and of anti-γ-globulin serum obtained by immunization of rabbits with γ-globulin treated before immunization with lonin-3 (γ^1) with 5-10% extracts of mouse spleen (3), mouse liver (5), mouse kidney (6), and γ-globulin (10). a, b) Variants of the reaction.

A suspension of the preparation lonin-3 in physiological saline in various concentrations gave no precipitation reaction with sera of rabbits immunized with compounds lonin-3 and K-4.

When the preparations were immersed in 10% NaCl solution (as described by A. I. Gusev), the haloes around the serum wells disappeared in 2-6 h but the precipitation zones remained for the next 24 h or longer – until the end of observation.

From the results of these investigations and their comparison with data in the literature [1, 3, 5-10] it can be concluded that during immunization with a chemical anticancer preparation not itself possessing antigenic properties, the preparation combines with the body proteins and modifies their antigenic properties. As a result, autoimmunization takes place and autoantibodies appear. The existence of a second (direct) mechanism of appearance of precipitation zones may also be postulated. The diffuse precipitate appears as a result of binding of the preparations by serum proteins and modification of the physicochemical properties of these proteins.*

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