DETECTION OF THE ANTIGENIC PROPERTIES OF EHRLICH'S MOUSE CARCINOMA CELLS AFTER TREATMENT WITH LONIN-3

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Research into the question of vaccination against tumors with cancer cells treated with x-rays has shown that after their rate of growth has been slowed by the action of ionizing radiation, the tumor cells become more antigenic [8, 11, 13, 14], and their antigenic properties are modified [1, 2]. Immune sera obtained by immunization with irradiated cells differ from those obtained by immunization with ordinary tumor cells [6]. After the injection of irradiated tumor cells into a recipient, the resistance of the latter to growth of tumor tissue is increased [10, 12]. It has also been reported that chemotherapeutic agents which depress tumor cells increase their immunogenicity [4] and increase the resistance of the recipient to growth of malignant tumors [3, 7, 9, 16].

The object of the present investigation was to study the immunogenicity of Ehrlich's mouse carcinoma cells after treatment with the preparation Lonin-3 [p-(di-2-chloroethylamino)-phenylacetamide] in vitro.

METHOD

The gel precipitation reaction of Ouchterlony [15] was used in the investigation. Saline extracts of ordinary cells of the ascites form of Ehrlich's mouse carcinoma and extracts of Ehrlich's carcinoma cells treated in vitro for 1 h with the preparation Lonin-3 (doses of 2,000, 4,000, and 8,000 mg/kg tumor tissue) were used as antigens. The ascites carcinoma cells were obtained by centrifugation of the ascites fluid of mice with an ascites form of Ehrlich's carcinoma for 10 min at 2500 rev/min, and after being rinsed twice with physiological saline, they were again centrifuged in the same conditions. Extracts of the liver and spleen of healthy mice and extracts of mouse liver treated in vitro with 20,000 mg Lonin-3 per kg tissue for 1 h were also used as antigens. The controls were saline suspensions of the same preparation of Lonin-3 in the doses used for treating the cells.

Antisera obtained after immunization of rabbits (weight about 2 kg) with ordinary Ehrlich's ascites carcinoma cells, and with the same cells treated in vitro before immunization for 1 h with the preparation of Lonin-3 (in doses of 2,000, 4,000, and 8,000 mg/kg tumor tissue), were used as antibodies. Sera could not be obtained after treatment of the cells with the preparation in doses of 10,000 and 20,000 mg/kg tumor tissue because all six experimental rabbits died from irritation of the peritoneum with the preparation and foreign protein after intraperitoneal immunization. Other antisera were obtained by immunization of rabbits with the liver and spleen of healthy mice. "Antilonin" sera also were obtained by immunization of rabbits with a pure preparation of Lonin-3. For one injection a dose of 10 mg of the preparation in 1 ml physiological saline was given (the same dose of the preparation was injected into each rabbit during immunization with cells treated with a dose of 20,000 mg/kg tissue).

The rabbits were immunized in two or three cycles. Each cycle lasted 3 weeks, in the course of which the rabbits were immunized six times, and at each injection 500 mg of the corresponding material was given [5]. The first injection of this material was given subcutaneously together with Freund's adjuvant, and all the other injections were given intraperitoneally. The intervals between the separate cycles were 1.5-2 months. The presence of antibodies in the serum was verified 7, 14, and 21 days after the end of the immunization cycle. Altogether 24 rabbit sera were investigated in the gel precipitation reaction.

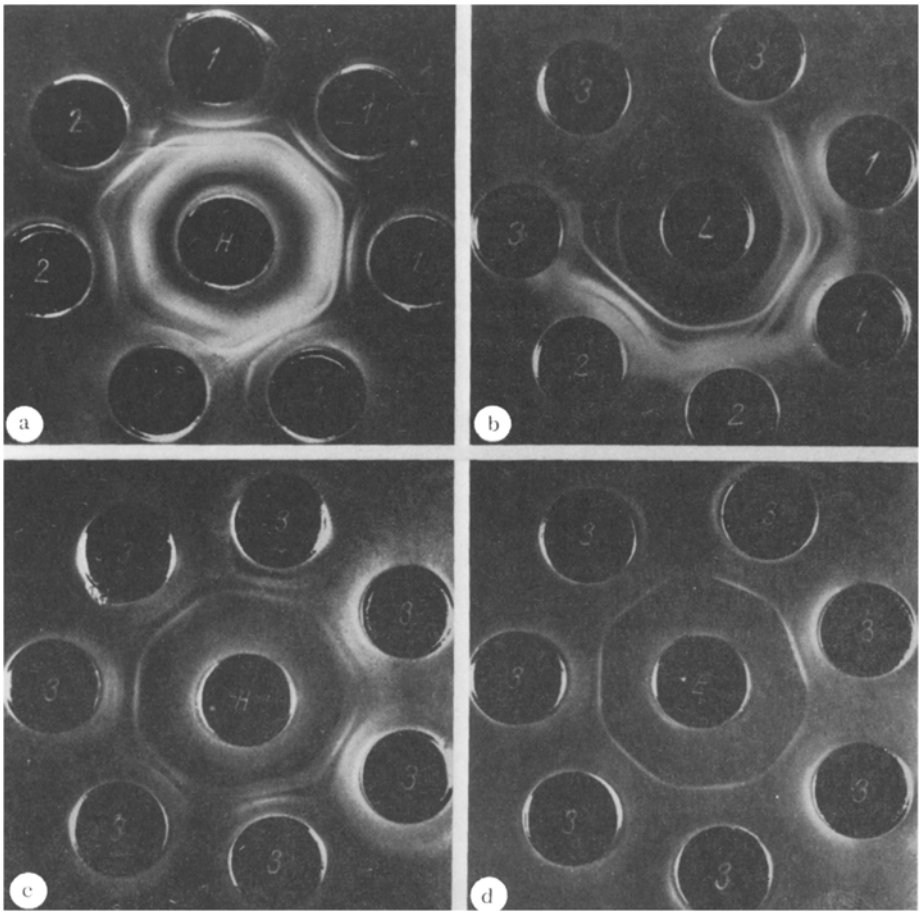

Fig. 1. Gel precipitation reaction of extracts of mouse liver (H), spleen (L), and Ehrlich's mouse carcinoma cells (E) with mouse antiliver (1) and antispleen (2) sera and with antisera to ordinary Ehrlich's carcinoma cells (3). a, b, c, d) different variants of the reaction.

RESULTS

Investigations of the antigenic properties of the pure preparation of Lonin-3 showed that neither the saline suspensions of the Lonin-3 preparation itself nor saline extracts of the Ehrlich's tumor treated in vitro with different doses of Lonin-3 gave precipitation lines in the precipitation reaction with the "antilonin" sera obtained by immunization of rabbits with Lonin-3. These results showed that pure Lonin-3 does not possess antigenic properties.

A saline extract of mouse liver gave a series of precipitation lines with antiliver mouse sera, so many in fact that they merged together and could not be counted. The same saline extracts of liver gave a series of precipitation lines in the gel with the antispleen sera (Fig. 1a). The saline extracts of mouse spleen also gave numerous precipitation lines with the mouse antispleen sera. Many precipitation lines also appeared during the reaction of the same saline extracts of spleen with mouse antiliver sera (see Fib. 1b and Fig. 2c). Different results were obtained in experiments with ordinary antitumor sera. In the precipitation reaction between the same saline extracts of mouse liver and the sera mentioned above, only one or two lines, and not groups, were formed (see Fig. 1c). The same small number of lines developed in the case of precipitation of the saline extracts of mouse spleen with the same antitumor mouse sera (Fig. 1b and Fig. 2c). In the reactions of the extracts of Ehrlich's mouse carcinoma with the corresponding antisera, only one or two precipitation lines developed (Fig. 1d).

Antisera obtained by immunization of rabbits with ascites cells of an Ehrlich's carcinoma treated in vitro with the Lonin-3 preparation for 1 h were studied next. In the gel precipitation reactions of saline extracts of mouse liver with antisera obtained after immunization of rabbits with Ehrlich's carcinoma cells treated in vitro

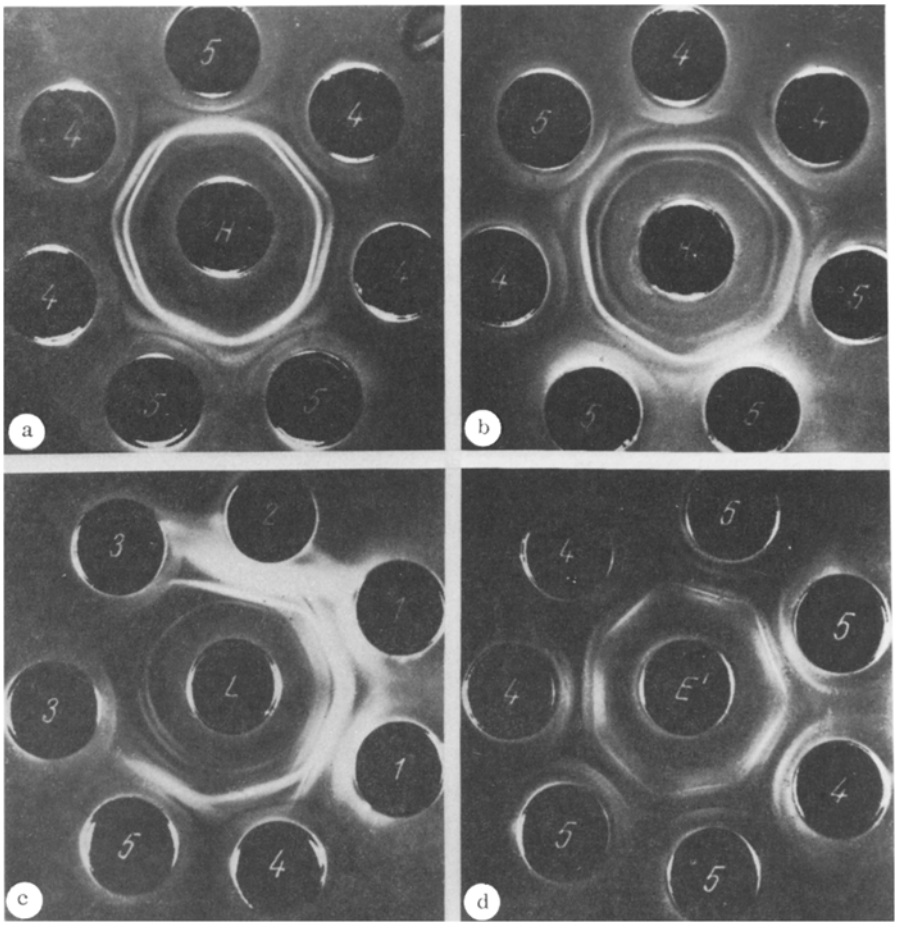

Fig. 2. Gel precipitation reaction of extracts of normal mouse liver (H), mouse liver treated with 20,000 mg of a Lonin-3 preparation per kg tissue for 1 h (H'), spleen (L), and cells of an Ehrlich's mouse carcinoma (E') treated with Lonin-3 in a dose of 8,000 mg/kg tissue for 1 h, with antisera to ordinary Ehrlich's carcinoma cells (3), and with Ehrlich's carcinoma cells treated before immunization with Lonin-3 in doses of 2,000 mg (4) and 8,000 mg (5) per kg tumor tissue for 1 h. a, b, c, d) Different variants of the reaction. Remainder of legend as in Fig. 1.

with Lonin-3 in a dose of 2,000 mg/kg tissue three lines were observed, while in the gel precipitation reactions of the same saline extracts of liver with antisera obtained after immunization of rabbits with Ehrlich's carcinoma cells treated in vitro with Lonin-3 in a dose of 8,000 mg/kg tumor tissue there were four precipitation lines (Fig. 2a and b). Much better results were obtained in the reaction of saline extracts of mouse spleen with these same antisera: the precipitation lines could not be counted for a whole complex of antigens was revealed (Fig. 2c). The antigenic properties of Ehrlich's mouse carcinoma were also revealed better in the reaction between Ehrlich's carcinoma cells, both treated with Lonin-3 and not so treated, and antisera to the same cells treated with the Lonin-3 preparation before immunization (Fig. 2d).

In the control variants of all the experiments, not one of the tumor extracts mentioned above and not one of the extracts of normal mouse tissues reacted with the four sera of the unimmunized control rabbits. The results show that during immunization of rabbits with Ehrlich's carcinoma cells preliminarily treated in vitro with 2,000-8,000 mg of Lonin-3 per kg tissue, more antibodies are detected in the sera than during immunization with ordinary cells. It is difficult at present to interpret this increase in the number of components of the precipitation complexes. Either autolysis of the cells or the influence of the chemical preparation on them may be postulated.*

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LITERATURE CITED

1. I. N. Maiskii and G. V. Suvorova, Byull. éksper. biol., 9, 94 (1957).
2. I. N. Maiskii, G. V. Suvorova, and P. P. Filatov, Byull. éksper. biol., 7, 72 (1959).
3. E. V. Montsevichyute-Éringene, Abstracts of Proceedings of the Fifth Oncological Conference of the Lithuanian SSR [in Russian], Vil'nius (1960), p. 35.
4. E. V. Montsevichyute-Éringene, In the book: Proceedings of the Seventh Oncological Conference [in Russian], Vil'nius (1964, p. 19.
5. R. Ardry and A. Courtin, Ann. Biol. clin., 9 (1961), p. 49.
6. F. Bonmassar and P. Mandruzzato, Arch. ital. Patol., 5 (1962), p. 299.
7. Idem, Ibid, p. 407.
8. D. M. Donaldson and R. J. Michell, Proc. Soc. exp. Biol. (New York) 101 (1959), p. 204.
9. D. M. Donaldson and J. A. North, Fed. Proc., 19 (1960), p. 392.
10. A. Goldfeder, Radiology, 39 (1942), p. 426.
11. J. M. Finey, E. H. Byers, and R. H. Wilson, Cancer Res., 20 (1960), p. 351.
12. C. Mazurek and J. F. Duplan, Bull. Ass. franc. Cancer, 46 (1959), p. 824.
13. R. McKee et al., Acta Un. int. Cancrum, 15 (1959), p. 955.
14. R. McKee, E. Garcia, and M. Troen et al., C. Proc. Soc. exp. Biol. (New York), 102 (1959), p. 591.
15. O. Ouchterlony, Ark. Kemi., 26 (1948), p. 14.
16. F. Sorm and J. Vesely, Neoplasma, 10 (1963), p. 217.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
