ON THE LOSS OF CERTAIN ORGAN-SPECIFIC ANTIGENS BY CANCERS OF THE HUMAN STOMACH

G. I. Avdeev and I. S. Bashkaev
From the Laboratory of Virology (Head—Professor V. V. Gorodilova) of the
P. A. Gertsen State Institute of Oncology (Director—Professor A. N. Novikov)
(Presented by Active Member Akad. Med. Nauk SSSR L. A. Zil'ber)
Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 52, No. 12,
pp. 76-79, December, 1961
Original article submitted February 27, 1961

In 1952, Weiler [17] succeeded in first showing that certain antigens present in an organ were absent from the tumor developing in it. Following this, the question of "antigenic reduction" in tumors was studied by a number of investigators, using various immunological methods. They confirmed the basic fact that a tumor loses certain antigens present in the normal tissue. The data of Hughes [12, 13], which subjects certain of Weiler's works to criticism, more readily confirms than disproves the basic facts obtained by the latter.

It was observed that "antigenic reduction" occurs in the precancer period [3, 21]; by growing normal tissues in culture [21], it was shown that organ-specific antigens were lost [19, 1]. One of the organ-specific antigens lost by mouse liver through the process of malignancy was isolated in the pure form [1, 2].

It should be noted that the phenomenon of antigen loss has been studied on a comparatively small number of tumors. Careful studies have been made on liver tumors in mice and rats [1-6, 10, 14, 17, 18, 21] and on a renal tumor in the golden hamster [15, 19, 20, 21]. Human tumors have been studied considerably less effectively; the loss phenomenon was observed in one patient with primary cancer of the liver [7] and in association with leukemia [8, 16].

Considering the comparative dearth of experimental data on the question of antigen reduction in tumors, especially in regard to human tumors, and taking into account the important theoretical conclusions of certain authors [11, 18], linking the indicated phenomenon with the pathogenesis of the cancer, we believe that our data on this question may be of definite interest.

METHODS

The tumors were obtained from operations performed in the P. A. Gertsen Institute, and the gastric mucosa—from the cadavers of individuals who died of trauma or cardiovascular diseases. In a number of cases, we also used macroscopically uninvolved portions of the mucosa from cancerous stomachs. The tissues were frozen, and kept at −15°C. Rabbits were immunized with suspensions of cells from the gastric mucosa (1 part ground tissue and 3 parts physiological saline).

The antigens were simultaneously injected subcutaneously, intramuscularly, and intraperitoneally. The cycle consisted of 5 immunizations, with 2-3 day periods between each one. The dose of the injected antigens increased from 3 ml in the beginning of the cycle to 10 ml at the end.

Several immunization cycles were carried out, with monthly intervals between them. The most active serum was obtained after 3-4 cycles.

All the sera were concentrated by 7-10 times, following the method of Z. A. Avenirova; this involved salting out the globulin fraction with a 50% saturated solution of ammonium sulfate.

In order to elevate the specificity of the concentrated sera, which initially had a rather wide spectrum of activity, we exhausted them by the addition of a mixture of 20-30 human sera, belonging to different blood groups, and then adding aqueous-saline extracts from gastric tumors and certain normal organs. When the exhaustion was completed, small doses of the antigens were added to the sera in divided portions, under the control of the agar precipitation reaction, until the serum stopped reacting with the corresponding antigens.

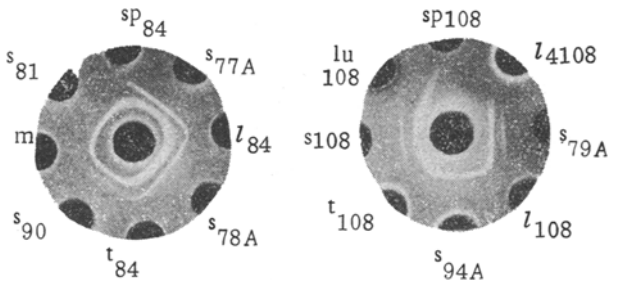
In a number of cases the final exhaustion was carried out by the method of Björklund [9]. The exhausting antigen was poured into a hole in the agar-agar beforehand (usually 24 hours earlier). The remainder of the antigens, not absorbed into the agar, was drawn up from the hole before the reaction was set up. Aqueous-saline extracts of tumors and normal tissues, made up of 1 part tissue to 3 parts physiological saline, served as the antigens for the precipitation and neutralization reactions of the sera.

The agar precipitation reactions were set up according to the micromodification developed in the L. A. Zil'ber laboratory [5].

RESULTS

Two organ-specific sera against the gastric mucosa were obtained.

Serum No. 22: exhausted by the addition of a mixture of human sera and antigen from gastric cancer. In the reaction of precipitation in gel (Fig. 1a), it yielded from one to three lines with antigens from the gastric mucosa, and did not react with the mixture of sera, nor with antigens from normal organs, taken from the same cadaver (gastric mucosa from this cadaver yielded a clear reaction with this serum).



a b

Fig. 1. Reactions with sera No. 22 and 23. The sera were poured into the central holes: a—No. 22; b—No. 23. The antigens were poured into the peripheral holes: s—stomach; c—cancer of the stomach; sp—spleen; k—kidney; l—liver; t—thyroid gland; lu—lung; m—mixture of human sera; S_{11a}—uninvolved mucosa from stomach with cancer C_{77}; C_{77b}—metastasis from cancer C_{77} of the liver. Numerals indicate the antigen number.

Serum No. 23: exhausted by the addition of a mixture of human sera, as well as antigens from the spleen and from a stomach cancer. In the reaction of precipitation in gel (Fig. 1b), it did not react with antigens from the spleen, kidneys, liver, thyroid gland or lung of the cadaver from which the gastric mucosa, yielding a clear reaction, was taken.

In tests with antigens from the stomach tumors, the sera, as a rule, did not react. Fig. 2 shows the reaction of serum No. 22 (additionally exhausted with cancer antigen) with antigens from cancerous and normal gastric mucosa. The reaction was set up with holes, alternately filled with normal and cancer antigens, arranged around a central hole containing the serum; in separate set-ups, the same arrangement was made, using only cancer antigens and using only normal antigens. It is apparent from Fig. 2 that in all variations the precipitation lines formed only against holes with the normal antigens, and were absent against holes containing the cancer antigens, with the one exception of cancer No. 69, which produced a weak reaction.

The reaction with serum No. 23 was set up in the same manner (Fig. 3). In this case also we observed a selective reaction with normal mucosa, and its absence with tissues involved in the stomach cancer.

A total of 12 samples of gastric mucosa were studied (4 of them were uninvolved portions of mucosa from a cancerous stomach). Every one of them yielded from one to three precipitation lines with the organ-specific sera against normal gastric mucosa.

We studied 12 carcinomas from various parts of the stomach, with different histological structure and a differing degree of cell differentiation. As a rule, the antigens from these cancers did not react with the organ-specific sera, and in those cases (3 antigens) where a reaction was observed, it was very weak and doubtful. These weak reactions could not be coordinated with the characteristics of histological structure in the studied cancers. Apparently, they are explained by the fact that in certain cases with preparation of the tumor antigens from uninvolved tissues, the latter were not sufficiently pure.

Thus, the precipitation reactions set up by us showed from one to three organ-specific antigens in the human gastric mucosa (including normal gastric mucosa from cancerous stomachs in that group), which were absent from human gastric carcinoma tissue.

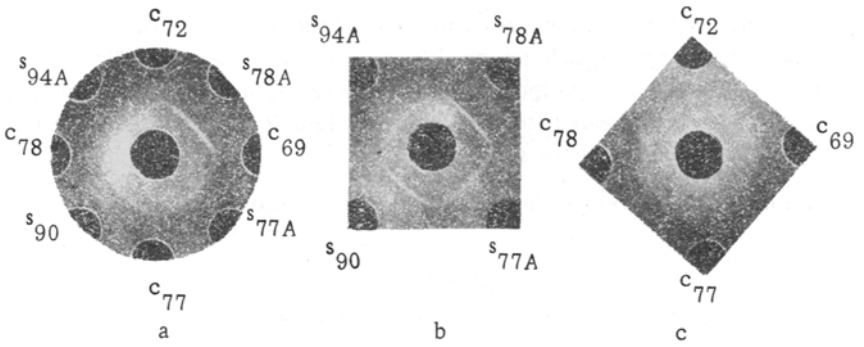


Fig. 2. Reaction with serum No. 22. Symbols are the same as in Fig. 1.

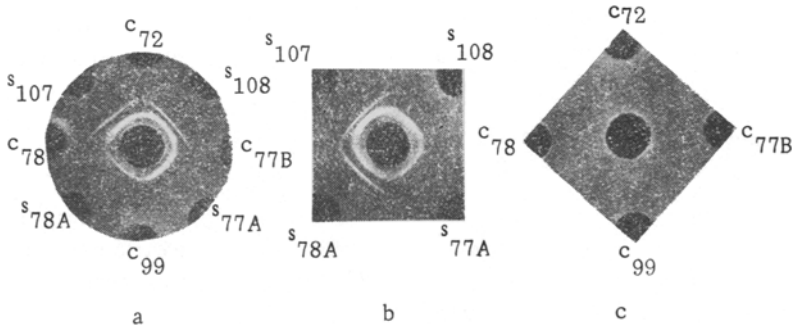


Fig. 3. Reaction with serum No. 23. Symbols are the same as in Fig. 1.

SUMMARY

Two concentrated sera against the tissue of human gastric mucosa were obtained. These sera were exhausted by extracts from normal organs and cancer tumors of the stomach and tested against 12 samples of gastric cancer tissue in the precipitation reaction in agar. This reaction has demonstrated that from one to three organ-specific antigens present in the tissues of the gastric mucosa were absent in the cancer tissues of the stomach studied.

LITERATURE CITED

1. G. I. Abelev, Z. A. Avenirova, N. V. Éngel'gardt et al., Doklady Akad. Nauk SSSR, vol. 124, No. 6 (1959) p. 1328.
2. G. I. Abelev, Z. A. Avenirova and V. S. Tsvetkov, Vopr. Onkol., No. 7 (1960) p. 43.
3. V. I. Gel'shtein, in the book: Works of the 2nd All-Union Conference on Oncology [in Russian], (Leningrad, 1959) p. 233.
4. A. I. Gusev and V. S. Tsvetkov, Labor. Delo, No. 2 (1961) p. 43.
5. L. A. Zil'ber, G. I. Abelev, Z. A. Avenirova et al., Doklady Akad. Nauk SSSR, vol. 124, No. 4 (1959) p. 937.
6. T. A. Korosteleva, Vopr. Onkol., No. 1 (1959) p. 22.
7. P. N. Kosyakov and N. I. Kuznetsova, Byull. Éksper. Biol. i Med., No. 6 (1957) p. 49.
8. V. A. Parnes, The Immunology of Leukemia [in Russian] (Moscow, 1960).
9. B. Björklund, Proc. Soc. Exp. Biol. (N. Y., 1952) v. 79, p. 319.
10. P. De Gregorio, Arch. Sci. Med. (1959) v. 108, p. 6.
11. H. N. Green, Ann. N. Y. Acad. Sci. (1957) v. 68, p. 268.
12. P. Hughes, C. Louis, J. Dineen et al., Nature (1957) v. 180, p. 289.
13. P. E. Hughes, Cancer Res. (1958) v. 18, p. 426.
14. F. Schmidt, E. Liss and R. Coutelle, Z. Krebsforsch (1959) Bd. 62, S. 658.
15. U. Schneeweiss, E. Liss, P. Landen et al., Ibid. (1960) Bd. 63, S. 345.
16. M. Seligman, P. Grabar and J. Bernard, Sang. (1955) v. 26, p. 52.
17. E. Weiler, Z. Naturforsch., (1952) Bd. 7, S. 324.
18. Idem, Strahlenterapie (1955) Bd. 96, S. 269.
19. Idem, Brit. J. Cancer. (1956) v. 10, p. 560.
20. Idem, Ibid., p. 553.
21. Idem, In: Ciba Foundation Symposium on Carcinogenesis, Mechanisms of Action (London, 1959) p. 165.