Studies on the Specific Antigens of Human Gastric Cancer. III

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Kelling¹⁾ and Hecht²⁾ have maintained that a saline extract of human cancer presents an antigenicity specific to that cancer and a protein is responsible for it. Though this was confirmed by Matsuura³⁾, Furusawa⁴⁾, and Yamamoto⁵⁾ in our country, Oka⁶⁾ has quite recently reported that rabbits subjected to injection of a saline extract of human gastric cancer failed to produce specific precipitin. Further, by referring to the results reported by Dochez, Heidelberger and others⁷⁾⁸⁾, Sunada and Komiya⁹⁾ isolated from human cancer a polysaccharide-like substance showing a specific antigenicity; Murata¹⁰⁾ also has maintained that a water-soluble substance of human cancer, mainly consisting of polysaccharide, presents a specific antigenicity and a close antigenic relationship with the placental tissue.

With the recent progress in immunology and biochemistry modern techniques have been applied to this line of study. Thus Kidd, Mann and others¹¹⁾¹²⁾ isolated by means of an ultracentrifuge a protein showing a cancer-specific antigenicity, and Macula¹³⁾ found that the nucleoprotein of a transplantable tumor is clearly different from that of the normal tissue.

Thus, opinion is much divided on the nature of specific antigens of human cancer. A view that the substance responsible for the specific antigenicity is a protein or a polysaccharide can not be accepted blindly so long as the substance was not chemically pure.

In view of such a situation of this field of study attempts were made to clarify the nature of the specific antigenicity by more precise procedures, with the results to be reported in the following.

Essentially, the experiments consisted of (1) producing antisera in rabbits by injection of saline extracts of human gastric cancer, normal gastric mucosa, and placenta, (2) preparing antigens for tests from highly purified fractions of saline and aqueous extracts of the above three kinds

of tissue, and (3) performing reciprocal precipitin tests aided by reciprocal absorption tests. Especially in carrying out the serological test, I received generous guidance of Prof. Murakami (director) and members of the legomedical Institute of our university.

EXPERIMENTAL

Preparation of Antigens for Immunization

The specimens of gastric cancer and normal gastric mucosa were taken from the fresh excised stomachs of patients with gastric cancer and with gastric or duodenal ulcer, respectively, operated on in our clinic. They were cut into fine pieces and washed in water to remove blood. Excess water being fully removed with filter paper. 10 g. of the material was weighed out, to which was added 90 cc. of physiological saline. The mixture was shaken on a shaking apparatus for 48 hours to extract the material and centrifuged. Phenol was added to the supernatant to give a final concentration of 0.5 per cent and the solution was stored in a stoppered brown bottle in an ice chamber until used.

The specimens of placenta were taken immediately after birth in the gynecological clinic. The antigenic preparation was made and stored in the same manner as above. In order to avoid the appearance of an antibody specific to group A, tissues belonging to group O or B were used exclusively, as described in the first report.

Immunization

Healthy rabbits, weighing about 2.5 kg., of serum group O' or β' and saliva group A (+) were used for immunization as described in the first report. Immunization was successful with 11 out of 50 rabbits. Each rabbit received 9 injections (into the ear vein) of increasing amounts of antigen, 2,3,4, . . . 9 and 10 cc., at intervals of twice a week. A preliminary titration was made 7 days after the last injection. If the titer was found high enough, total bleeding was made from the carotid artery. The serum separated from the blood was inactivated at 56°C. for 30 minutes, to which was added phenol to give a final concentration of 0.5 per cent, and stored in a stoppered brown bottle in an ice chamber.

Precipitation Tests

Precipitin reaction was examined by ring tests, in which the amount of antiserum was held constant and antigen was varied in doubling dilution series. The results were read after 2 hours' incubation as follows: +, positive; -, negative; $\pm$, obscure, but possibly positive. As antigenic controls, physiological saline was superposed instead of antigen; as

TABLE I
Tests Using Immune Antigens

Antigen: Doubling dilutions of the saline extract of 3 tissues.

[illegible]

antiserum controls inactivated normal serum was used.

Absorption Tests

For tracing the specific antigenicity of human cancer, the absorption test with the 3 antisera was an important means. The necessary and sufficient amount of a fraction of each tissue to be used for absorption was determined by preliminary tests. A given amount of a fraction was added to 1 cc. of antiserum; the mixture was well rubbed up with a glass rod and placed in an incubator at 37°C., being shaken every 15 minutes for a full mixing. After 2 hours the mixture was centrifuged and the supernatant was separated for use as "absorbed" antiserum.

Esperimental Results

1) Tests using immune antigens

The results of precipitin tests of immune antigens with the 3 antisera are summarized in Table I (A, B, C), where it will be seen that there were no definite differences in their reactions with each antiserum among the 3 antigens. This may be explained as follows: A tissue has species specificity as well as organ specificity, and the former is generally more influential, if blood or body fluids in mixed with the tissue extract, the species specificity becomes further influential. Thus, normal serum (having the predominant species specificity over all other human tissues) was added to each antiserum with a capillary pipette at a rate of 4 drops per 1 cc. of the antiserum in order to absorb species-specific antibodies completely (i.e., in order to specialize the antiserum). The results of tests in which each antiserum thus specialized was used instead of the untreated antiserum are shown in the left corner of the main part of Table I (A, B, C), (surrounded by heavy lines). It will be seen that each antigen reacted most strongly with its homologous antiserum. Therefore, the next step was to trace the specificity and similarity of each antigen by chemically separating it into various fractions and making precipitin tests with each of these fractions.

2) Tests using crude fractions as antigen

The 3 tissues that had been stored in 95 per cent alcohol were extracted in distilled water, and (1) the precipitates produced from each extract by barium hydroxide at pH 9.0, (2) the precipitates produced by 30 per cent acetic acid at pH 4.5, and (3) the supernatant after the above treatment with acetic acid, were purified as shown in Table II and used as antigens for precipitin tests. The 3 antisera to be used had previously been absorbed on saturation with normal human serum. The results of tests with each fraction of pH 9.0 precipitates are shown in Table III (A, B, C). It

TABLE II

will be seen (1) that gastric cancer and normal gastric mucosa did not react with the rabbit serum against the saline extract of human placenta (hereafter to be referred to as anti-placenta serum), (2) that placenta did not react with the rabbit serum against the saline extract of normal human gastric mucosa (hereafter to be referred to as anti-normal gastric mucosa serum) and the rabbit serum against the saline extract of human gastric cancer (hereafter to be referred to as anti-gastric cancer serum), and (3) that accordingly the fraction of pH 9.0 precipitates was highly organ-specific. After absorption with 15 mg. of the pH 9.0 precipitate fraction of normal gastric mucosa, the anti-gastric cancer serum reacted only with gastric cancer as shown in the upper left corner (surrounded by heavy lines) of Table III A, whereas the anti-normal gastric mucosa serum absorbed with 20 mg. of the pH 9.0 precipitate fraction of gastric cancer did not react with any of the 3 antigens, which results are suggestive of the presence of antigens specific to human gastric cancer.

Precipitin tests with the pH 4.5 precipitate fraction of each tissue as antigen yielded the results shown in Table IV (A, B, C), where it will be

TABLE IV
Tests Using Crude Fractions as Antigen
Antigen: The pH 4.5 precipitates of 3 Tissues $\times 50 \times 8000$.

[illegible]

found that the relations among the 3 antigens were quite similar to those in the case of the pH 9.0 precipitate fraction.

Precipitin tests with the pH 4.5 supernatant fraction of each tissue as antigen yielded the results shown in Table V (A, B, C). Comparing the results of tests using the anti-gastric cancer serum with those using the antiserum absorbed with 20 mg. of the pH 4.5 supernatant fraction of normal human gastric mucosa (shown in the left corners of Table V A, surrounded by broken lines), it will be understood that gastric cancer had some antigens in common with placenta. The tests using the anti-normal gastric mucosa serum absorbed with the pH 4.5 supernatant fraction of placenta yielded the results shown in the area surrounded by heavy lines (Table V B), while the tests using the same antiserum but absorbed with the pH 4.5 supernatant of gastric cancer gave the results shown in the area surrounded by broken lines. The differences between both results indicate that gastric cancer and normal human gastric mucosa had some different antigens. As for tests with the anti-placenta serum (Table V C), the differences between the results before and after being absorbed with the pH 4.5 supernatant of normal gastric mucosa indicate that gastric cancer had some antigens in common with placenta. (Table V C, surrounded area by heavy lines)

From the results stated above it may be assumed that human gastric cancer has some specific antigens and is related to placenta more closely than to normal gastric mucosa. Judging from the chemical procedures through which the 3 fractions of each tissue (9 fractions in all) were obtained, however, each fraction may possibly be a mixture of several substances. Therefore, from the results of precipitin tests using such fractions as antigen, no decisive conclusions can not be drawn concerning the nature of antigens specific to human gastric cancer and of antigens common to this cancer and placenta. Thus, the next step was to isolate certain chemically purer substances from the 3 tissues stored in alcohol and make precipitin tests using these substances as antigen.

3) Tests using a nucleic acid fraction as antigen

A nucleic acid fraction was prepared from each of the 3 tissues stored in 95 per cent alcohol by the method as shown in Table VI. The results of qualitative tests of this preparation are as follows: phosphorus test, +; Molish's test, $\pm$; biuret test, $\pm$; Bial's test, +. The results of precipitin tests using as antigen the nucleic acid fraction of each tissue are summarized in Table VII (A, B, C), where it will be seen that definite difference in their reaction with the anti-gastric cancer serum was presented between gastric cancer and normal gastric mucosa (Table VII A), while gastric cancer and placenta still reacted with the antiserum absorbed with normal gastric mucosa (shown in the left corners of A, surrounded by heavy lines),

TABLE VI

thus indicating that the gastric cancer nucleic acid has some antigens in common with placenta as well as highly specific antigens.

The anti-normal gastric mucosa serum reacted positively with all the 3 tissues, though differently in intensity (Table VII B), but did not react with any of the 3 antigens after being absorbed with the gastric cancer nucleic acid.

The anti-placenta serum reacted with gastric cancer more strongly

TABLE VII
Tests Using a Nucleic Acid Fraction as Antigen
Antigen: The nucleic acids of 3 Tissues $\times 50\text{--}7000$.

[illegible]

than with normal gastric mucosa, and after absorption with normal gastric mucosa nucleic acid it reacted with both placenta and gastric cancer, which results demonstrate some common antigenicity between the nucleic acids of placenta and gastric cancer (Table VII C, surrounded area by heavy lines).

In summary, it may be concluded that the gastric cancer nucleic acid has some specific antigenicity, since it is incapable of being absorbed on full saturation with the normal gastric mucosa nucleic acid, and is related with placenta more closely than with normal gastric mucosa.

4) Tests using a carbohydrate fraction as antigen

A carbohydrate fraction was prepared from each tissue stored in 95 per cent alcohol by the method shown in Table VIII, and gave the following qualitative reactions: Molisch's test, ++; biuret, phosphorus, and Bial's test, —. The results of precipitin tests using as antigen this carbohydrate fraction of each tissue are summarized in Table IX (A, B, C), where it will be seen that the anti-gastric cancer serum reacted with normal gastric mucosa and gastric cancer strongly and almost equally, but, after absorption with the normal gastric mucosa carbohydrate, only slightly with gastric cancer and placenta (Table IX A surrounded area by heavy lines).

Now, when absorbed with the gastric cancer carbohydrate, the anti-normal gastric mucosa serum reacted only with normal gastric mucosa (Table IX B). The reactions of the anti-placenta serum with gastric cancer and normal gastric mucosa were the same in intensity (Table IX C). After absorption with the normal gastric mucosa, the antiplacenta serum reacted only with placenta slightly (surrounded area by heavy lines), after further absorption with the gastric cancer carbohydrate, this antiserum reacted only with placenta as before and a little more slightly (surrounded area by broken lines).

Considering these results, the antigenic specificity of the gastric cancer carbohydrate seems to be low, antigenic differences of carbohydrate between gastric cancer and normal gastric mucosa being indistinct, though there is something in common between the carbohydrates of gastric cancer and placenta.

5) Tests using a glucidamin fraction as antigen

A glucidamin (a protein) fraction was prepared from each of the 2 tissues, gastric cancer and normal gastric mucosa, the purification from placenta being unable on account of shortage of material (Table X). Each fraction gave the following qualitative reactions: biuret and Molisch's tests, ++; Bial's and phosphorus tests, —. The results of precipitin tests using as antigen this glucidamin fraction of each tissue are shown in Table XI (A, B, C). In A it will be seen that the anti-gastric cancer serum reacted with both antigens almost equally, but only with gastric cancer after ab-

TABLE VIII

sorption with the normal gastric mucosa glucidamin, results being suggestive of the specific antigenicity of gastric cancer glucidamin (surrounded area

TABLE X

by heavy lines). On the other hand, the anti-normal gastric mucosa serum reacted with both antigens, but with neither of them after absorption with gastric cancer glucidamin (Table XIB). The anti-placenta serum reacted with gastric cancer more strongly than with normal gastric mucosa.

In summary, it may be concluded from these results that gastric cancer

glucidamin has some specific antigens, part of which are in common with placenta glucidamin.

DISCUSSION AND CONCLUSIONS

A general survey of the results obtained from the precipitin tests using nucleic acid, carbohydrate, and glucidamin as antigen will reveal us that the reaction of each Fraction of human gastric cancer with an antiserum was always different from the reaction of the corresponding fraction of normal human gastric mucosa, and especially in the case of the nucleic acid fraction human gastric cancer showed the most remarkable specific antigenicity and a remarkably close relationship with human placenta (Table VII). Considering the process of fractionation shown in Table VI, the nucleic acid preparation seems to have mainly consisted of ribonucleic acid, which is an interesting thing in view of the fact that ribonucleic acid is closely related with cellular protein metabolism.

The specific antigenicity of human gastric cancer and a close relationship of the cancer with placenta may be taken to have appeared also in the glucidamin fraction (Table XI).

The carbohydrate fraction differed from the other two, in that no marked specific antigenicity of human gastric cancer was presented, there being found only something in common between gastric cancer and placenta (Table IX).

Thus, the specific antigenicity of human gastric cancer does not seem to be restricted to a special fraction, but is presented by several fractions to varying degrees. Thus, serologically, the specificity is highest in the nucleic acid, moderate in the glucidamin, and lowest in the carbohydrate fraction.

SUMMARY

With a view to tracing the specific antigenicity of human gastric cancer a series of experiments were carried out, which essentially consisted of (1) producing antisera in rabbits by injection of saline extracts of human gastric cancer, normal gastric mucosa, and placenta, (2) preparing several purified fractions of the above three kinds of tissue, which were used as antigens for precipitin tests, and (3) performing reciprocal precipitin tests aided by reciprocal absorption tests. It has been demonstrated that the nucleic acid fraction of human gastric cancer presented the highest antigenic specificity, the glucidamin fraction a moderate, and the carbohydrate fraction least specificity.

References

- 1) Kelling, Münch. med. Wschr., 1904, **43**, 1901.
- 2) Hecht, Schweiz. med. Wschr., 1952, **41**, 1058.

- 3) Matsuura, Nihon Fujinka Gakkai Zasshi (Jap.), 1929, **24**, 25.
 - 4) Furusawa, Tokyo Iji Shinshi (Jap.), 1936, **50**, 115.
 - 5) Yamamoto, Nihon Geka Gakkai Zasshi (Jap.), 1918, **17**, 840.
 - 6) Oka, Igaku Kenkyu (Jap.), 1952, **22**, 26.
 - 7) Dochez & Avery, J. Exper. Med., 1917, **26**, 120.
 - 8) Heidelberger *et al.*, *ibid.*, 1925, **42**, 727.
 - 9) Sunada & Komiyama, Geka (Jap.), 1948, **10**, 527.
 - 10) Murata, Nihon Shokakibyō Gakkai Zasshi (Jap.), 1952, **50**, 1.
 - 11) Kidd & Friedewald, J. Exper. Med., 1942, **76**, 557.
 - 12) Mann & Welker, Amer. J. Cancer, 1940, **39**, 360.
 - 13) Maculla, Yale J. Biol. Med., 1948, **20**, 299.
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