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Differential Reactions of Homologous Antiserum to Tumor Cells of Different Rat Ascites Tumors

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(With 1 Plate, 4 Textfigures and 2 Tables)

In a review of the chromosome cytology of ascites tumors in rats, Makino (1957) with some available evidence has expressed the view that the stability of the stem-cells is not always permanent, though the constancy is very pronounced, and that the tumors have undergone numerical and structural changes of chromosomes in correlation with shift of the neoplastic condition. Evidently the genetic constitution of a rat ascites tumor is closely correlated with the chromosome pattern of its stem-cells. Change in the stemline chromosomes induce alteration of the tumor genotype, resulting in the rise of a new tumor. According to Makino and Sasaki (1958), the Yoshida sarcoma subline D was a derivative of subline C produced through the inoculation of tumor cells subjected to freezing; these two ascites tumors differ from one another not only in their chromosome pattern, but also in their property such as transplantability, though they are cellular descendants from the stock tumor. The transplantability of Yoshida sarcoma subline C to rats of Fischer strain was about 0 per cent, though that of subline D was 80 per cent.

In a series of experiments, the author has been engaging in research on the property of disease in the subline derivatives of the Yoshida ascites tumor, with special reference to the antigenicity of tumor. The present paper deals with the specific action of the antiserum obtained from homologous rats which were previously immunized against the tumor cells of Yoshida sarcoma subline C, to other kinds of ascites tumors of rats.

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Material and method

The tumor cells employed in this experiment were obtained from the Yoshida sarcoma sublines C and D, from the MTK-sarcoma II and from the MTK-sarcoma III, all being ascites tumors of rats. The general characters of these tumors have been described

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previously by many investigators (Kanô and Tanaka 1951, Hamazaki *et al.* 1955, Makino and Sasaki 1958).

For immunizing rats of the Fischer strain, the tumor cells of Yoshida sarcoma subline C were employed; 0.05 cc of the ascites in which ca. 80 million tumor cells were contained, was injected intraperitoneally. In these rats the second inoculation was made with a larger dosage than the first after the lapse of about 30 days. Seven days after the second injection, the whole blood was withdrawn by a cardiac puncture, and the serum was separated by the routine technique. The antiserum thus prepared was immediately used for agglutinin- and cytotoxin-reaction. For agglutinin-reaction, the antiserum was inactivated at 56°C for 30 minutes. The control sera were collected similarly from rats of Wistar (W/Ma) strain which showed over 90 per cent susceptibility to subline C, and from Fischer rats (Fischer-344).

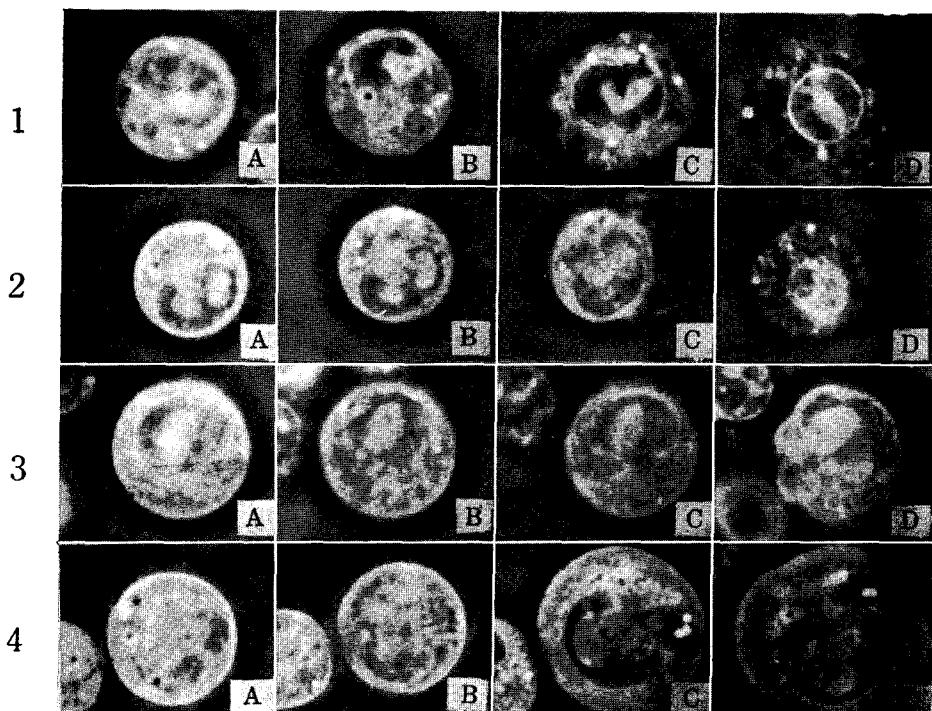
1. *Cytotoxin test.* For microscopic observations of cytotoxic activity of the antiserum *in vitro*, the mixture of 0.01 cc of tumor ascites, 0.02 cc of antiserum and 0.01 cc of fresh serum obtained from Wistar rats was prepared per each tumor. The resultart reaction between living tumor cells and antiserum in the presence of the complement was precisely observed by phase-microscopy according to the method of Makino and Nakahara (1953). The control experiment, for comparison, was made with the mixture of tumor ascites, normal serum from rats of either Wistar or Fischer strain and saline in the same proportion as above.

2. *Agglutinin test.* Prior to the test, the antiserum was serially diluted in two-fold steps with a test-tube of ca. 10 mm diameter. After repeated washing and centrifuging of tumor cells with saline, the packed tumor cells were adjusted to keep constant per unit quantity (0.5 per cent tumor cell suspension) with appropriate saline dilution. Thereafter 0.2 cc of this suspension was added to each antiserum dilution tube, and the titer reading of the agglutinin-reaction was taken after 2 hours' incubation at 37°C. In this manner, five readings were made for each individual test, and the most reliable value was adoptd to denote the titer. As a control, 0.5 cc saline was mixed with 0.2 cc of the cell suspension, according to the above procedures.

In order to supplement the results of cytotoxin- and agglutinin-reaction, the absorption test was carried out; viable packed cells of various types of tumors were mixed with antiserum at the rate of 1:4, and then incubated at 37°C for 2 hours and further kept at 5°C overnight. Then the cells were removed from the solution by repeating centrifugation, and the supernatant was again absorbed by means of the same tumor cells. In this manner, the absorption of the antiserum was repeated three times, and finally the supernatant was exposed to the cells of Yoshida sarcoma subline C, according to the same procedures as employed for agglutinin and cytotoxin tests respectively.

Results of experiments

1. *Cytotoxin-reaction* Prior to the experiments with antiserum, examination was made of possible effects of normal sera obtained from rats of Fischer and



Figs. 1 to 4. Photographs of tumor cells, under the influence of anti-Yoshida sarcoma subline C cell antiserum, taken by phase microscopy. Showing successive series of degenerative changes. $\times 1200$.

Figs. 1-A,B,C,D. Showing degenerative changes in a cell of the Yoshida sarcoma subline C under the effect of the antiserum diluted with physiological saline in 1 : 2. Cytolytic degeneration occurring in a cell is shown in series. A ; 10 minutes after exposure. B ; 20 minutes. C ; 30 minutes. D ; 60 minutes.

Figs. 2-A,B,C,D. Showing degenerative changes of a cell of the MTK-sarcoma II. Sixty minutes after exposure (2-D) to the antiserum, the cell underwent cytolysis showing coarse granules in the cytoplasm.

Figs. 3-A,B,C,D. Showing a cell of the Yoshida sarcoma subline D under the influence of the powerful antiserum. At 30 to 40 minutes exposure, the cell remains normal (3-C), while 60 minutes (3-D), the cell shows a degenerative change and became damaged by the serum 5 hours after the treatment.

Figs. 4-A,B,C,D. Showing the MTK-sarcoma III cell under the effect of the powerful antiserum. A; 10 minutes exposure. B; 20 minutes. C; 30 minutes. D; 60 minutes. At 30 minutes (4-C), the cell shows an expansion into a round outline. The enlarged cell then is lobulated during 3 to 4 hours after exposure.

Wistar strains upon tumor cells of different tumors.

The results of this experiment showed that there was no remarkable cytotoxin-reaction in the serum from Wistar rats nor in that from Fischer rats, though Fischer serum showed a slight value of agglutinin titer (Matsumoto 1958 unpublished).

The changes *in vitro* of tumor cells upon exposure to antiserum were successively traced under a phase microscope in single tumor cell (Figs. 1-4). As shown in Figures 1A to 1D, the antiserum exerts a remarkable cytotoxic effect on the cells of Yoshida sarcoma subline C (antigen-cell); all the tumor cells were damaged by the antiserum within 30 minutes of the reaction, even though diluted antiserum (1:2) was used. The antiserum seems to exert its first effect on the cytoplasm; the cell is damaged by a breaking down of the cell body following a severe blebbing of the cell surface (Figs. 1C-1D). The appearance of cytoplasmic granules, rather coarse in outline, is also one of the typical reactions. The disintegration of the naked nuclei occurs subsequently (Fig. 5).

The reaction of the antiserum to cells of Yoshida sarcoma subline D is quite different; it is weak in effect upon the tumor cells in this case (Figs. 3A-3D). As shown in Figures 3A to 3D, no remarkable change is observed in cells during 30-minutes exposure of the antiserum. Sixty minutes after exposure, the cell becomes rather irregular in outline showing a striking movement of the cytoplasmic particles. Most of the cells, however, have remained viable even after 5-hours exposure, showing an active movement of mitochondria in the cytoplasm (Fig. 7).

The cells of the MTK-sarcoma II, however, showed a somewhat intense reaction to the antiserum, including a cytotoxic influence of the tumor cells. The reaction in this case seems to be more efficient than in the case of the Yoshida sarcoma subline D (Figs. 2A-2D and 6). Thirty minutes after exposure the cell becomes smaller in size. The next step in degeneration is a gradual damage of the cytoplasm with active movement of the cytoplasmic particles (Fig. 2D). There are certain numbers of resistant cells persisting unchanged by the antiserum even in this case.

In the experiment with the MTK-sarcoma III, no remarkable cytotoxic reaction of the antiserum is seen, although an amoeboid movement of the cytoplasm with a blebbing of the cell surface is common in affected cells (Fig. 8). Figures 4A to 4D show a series of the above picture. The cells become larger and larger in size with time. A very few of them were damaged after displaying an amoeboid movement or blebbing of the cytoplasm, while many others survived for five hours, though there was a slight vibration of the cytoplasmic particles (Fig. 8).

2. *Agglutinin-reaction.* The data regarding the agglutinin titer is given in Table 1. It is evident from this table that the cells of Yoshida sarcoma subline C (antigen-cell) show the highest titer value (1:512) amongst those obtained from the other four types of tumors, though there is a slight difference in titer value between cells of the MTK-sarcoma III (1:2) and those of either the MTK-

sarcoma II (1 : 16) or the Yoshida sarcoma subline D (1 : 16). The result implies that the agglutinin-reaction of the tumor cells is somewhat correlated with the cytotoxin-reaction. In reference to the above evidence, the agglutinin titers of the normal sera from rats of Fischer and Wistar strains were measured against the cells of different types of tumors. The normal serum from Wistar rats shows no agglutinin-reaction to any of the tumors. The normal serum from Fischer rats, however, shows a weak reaction to the Yoshida sarcoma subline C and to the MTK-sarcoma II, but not to the Yoshida sarcoma subline D and the MTK-sarcoma III. The evidence presented here may be supplemented by the results of transfer experiments of Sasaki (1958) showing that the transplantability to the Fischer rats of subline C and MTK-sarcoma II is almost negative.

Table 1. Agglutinin titers of unabsorbed antiserum and those of normal serum from untreated Fischer rats, calculated against tumor cells from four ascites tumors. The antiserum was prepared from the Fischer rats against tumor cells of subline C.

Antigen (agglutinogen) cell				
	Yoshida subline C	Yoshida subline D	MTK-II	MTK-III
Titer of normal serum	1:4	—	(1:1)	—
Titer of antiserum	1:512	1:16	1:16	1:2

3. *Absorption test.* The results of agglutinin-reaction and observation of the cell behavior following the antiserum exposure are presented in Table 2 and Figures 9 to 12. As already mentioned, both agglutinin- and cytotoxin-reactions of the antiserum are powerful to the cells of Yoshida sarcoma subline C (antigen-cell). When the antiserum is absorbed 3 times by the antigen cells of subline C, the titer value falls to 1 : 16, and no remarkable cytotoxic change of the cell is longer observed within about 2-hours exposure (Table 2 and Figs. 9-10). Two to three hours later, however, a slight cytotoxic reaction is observed in some tumor cells as evidenced by an irregular blebbing of the cell surface. The above results seem to show that most of the cytotoxins contained in the antiserum are removed by the absorption by the cells of Yoshida sarcoma subline C. In the case of absorption with cells of either the Yoshida sarcoma subline D or the MTK-sarcoma II, the agglutinin titer of the antiserum which was determined against the cells of subline C is 1 : 128, and the absorbed antisera in this case are strikingly cytotoxic to the cells of subline C (Table 2 and Figures 11 to 14). Figures 11 and 13 show the typical course of cell damage after the exposure of the antigen cells of subline C to the antisera absorbed either by subline D or MTK-sarcoma II. The degenerative change of these tumor cells becomes more intensive with time (Figs. 12 and 14).

The antiserum absorbed by the cells of MTK-sarcoma III follows a similar pattern of events to the above two cases, showing a rather intense cytotoxic reaction; the damage of the cell body becomes complete within 30 minutes after the exposure, showing a high agglutinin titer at 1:256. It is then apparent that the cytotoxin of the antiserum is not removed by the MTK-sarcoma III in this case.

Table 2. Agglutinin titers of antisera absorbed by tumor cells of various types of ascites tumors to the cells of Yoshida sarcoma subline C.

Titer of absorbed sera to Yoshida subline C	Tumor cells for absorption			
	Yoshida subline C	Yoshida subline D	MTK-II	MTK-III
	1:16	1:128	1:128	1:256

On the basis of the data obtained for cytotoxin-reaction and judging from the detectable difference of agglutinin titer value, the statement may be possibly made that the reactivity of the four different types of tumors to the antiserum are arranged in the following order as far as the scope of the present observations is concerned:

Yoshida sarcoma subline C \gg MTK-sarcoma II = Yoshida sarcoma subline D $>$ MTK-sarcoma III.

Discussion

One of the main purposes of this study was to learn the specific action of the antiserum on living tumor cells. The action of anti-tumor antibody in homologous antiserum prepared against the tumor cells of the Yoshida sarcoma subline C was investigated in respect to tumor cells of different types of rat ascites tumors: the Yoshida sarcoma sublines C and D, the MTK-sarcoma II and the MTK-sarcoma III. The data at hand indicate that the anti-tumor antibody contained in the antiserum reacts differentially upon the tumor cells of the different tumors. The cytotoxic effect of the antiserum here under study was most powerful against the cells of Yoshida sarcoma subline C (antigen-cell), though the antiserum reacts not only on the antigen cells from which the antiserum is originated, but also to some extent on the cells of the other tumors here employed. Such a cross-reaction was also found in the case of agglutinin-reaction.

Evidence presented by the absorption tests is that the cells of Yoshida sarcoma subline C have a capacity to neutralize the cytotoxic antibody contained in the antiserum prepared, and that the MTK-sarcoma II can reduce to some extent both the cytotoxic nature and the agglutinin titer of the antiserum which were determined against the Yoshida sarcoma subline C cells. On the contrary, the Yoshida sarcoma subline D and the MTK-sarcoma III have failed almost completely to remove the antibody from the antiserum of subline C.

From the above findings, it is apparent that the antiserum has an antibody, specific to the tumor type, which reacts almost selectively to cells from different types of tumors, and that a cross-reaction is also found in cytotoxin-reaction. The author interprets the above cross-reaction as indicating that the antiserum contains at least two sorts of antibodies; the tumor-specific antibody and the so-called "strain-specific" antibody, and that if the above relation be possible, cross-reaction may be caused by the strain-specific antibody in the antiserum. Probably the strain-specific antibody would be associated with two factors; one is the tissue contamination of donors at the time of immunization, and the other is the presence of a strain-specific antigen in tumor cells. The presence of a strain-specific antigen in tumor cells is rendered possible by the fact that the antigen-cells concerned here are derived from an original tumor-rat of mixed strain, and therefore they may contain a wide antigenic system common to the other three tumors here examined. Since the tissue contamination is negligible in the case of ascites tumors, there is a possibility that the strain-specific antigen occurring in the tumor cells induces the formation of the antibody which is responsible for the cross-reaction of the antiserum.

It is of much interest to learn that the antiserum which is derived from the cells of Yoshida sarcoma subline C has little effect upon the cells of Yoshida sarcoma subline D, though both tumors are cellular descendants of the stock tumor, and subline D is a derivative of subline C. This fact indicates that subline D and subline C possess different immune response which seem to be associated with the changes in chromosome pattern and antigenicity of the tumor. Similar evidence has been presented by some investigators on the basis of cytological and immunological investigations in several mouse tumors (Hauschka *et al.* 1953, Amos 1956). It may be suggested, therefore, that the difference in antigenicity between the two sublines of the Yoshida sarcoma may be attributable to the difference between them in the chromosome constitution (or change in genic constitution) of the stem-cells.

Summary

Investigation was made of the action of anti-tumor antibody in homologous antiserum prepared against the tumor cells of Yoshida sarcoma subline C in relation to tumor cells of different rat ascites tumors, such as the Yoshida sarcoma sublines C and D, the MTK-sarcoma II and the MTK-sarcoma III.

The antiserum gave an agglutinin titer value of 1:512 for the Yoshida sarcoma subline C cells, 1:16 for subline D and MTK-sarcoma II cells, and less than 1:2 for the MTK-sarcoma III cells.

The antiserum most effectively damaged tumor cells of Yoshida sarcoma subline C, and showed to some extent, a cross-reaction to tumor cells of different tumors. The anti-tumor antibody of the antiserum was intensively absorbed by viable Yoshida sarcoma subline C cells, but not by those of the other three types of tumors.

The above findings indicate that the antiserum possesses an antibody specific to the tumor type, that the antibody reacts selectively to the various types of tumors, and that sublines C and D differ in their immune response. The cross-

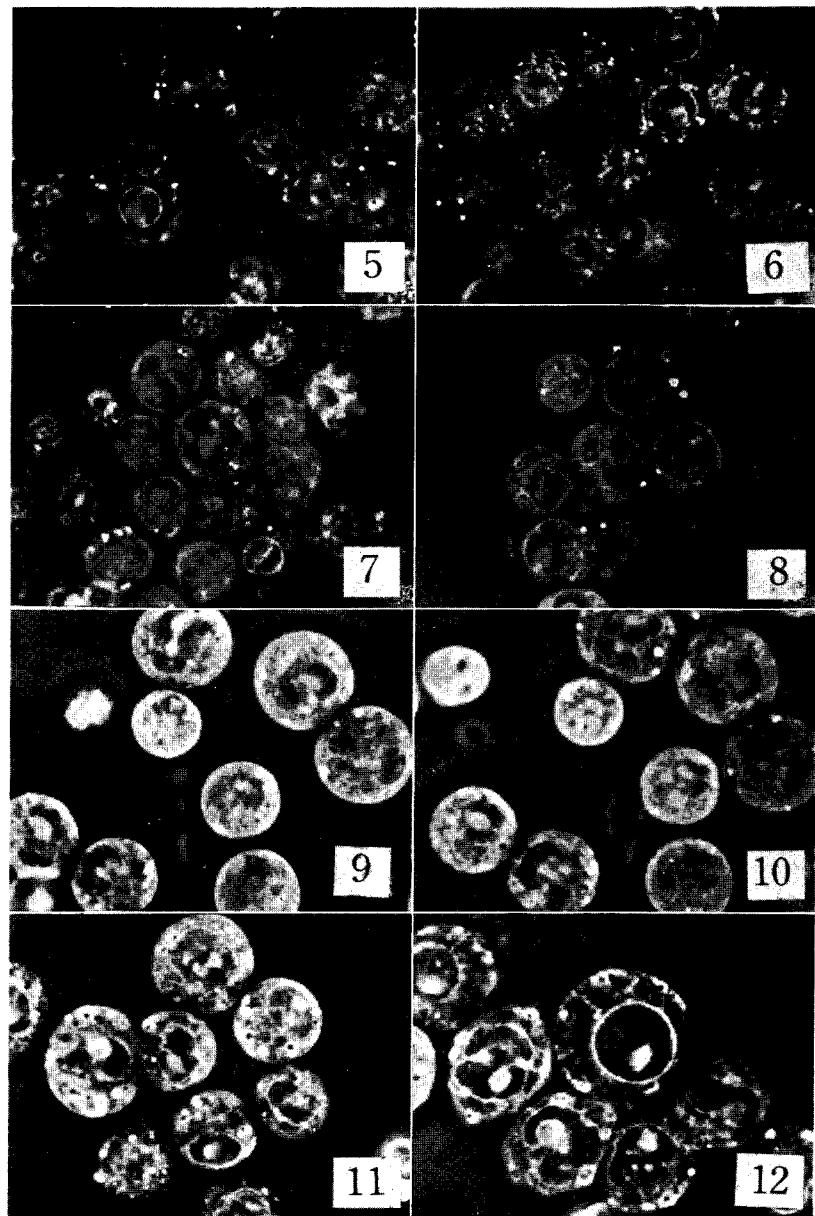
reaction of the antiserum here observed may be connected with the action of strain-specific antibody derived from antigens of tumor cells.

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Explanation of Plate II

- Fig. 5. Damage of tumor cells of the Yoshida sarcoma subline C under the influence of the antiserum diluted 1:2 with saline, observed 90 minutes after exposure. $\times 400$.
- Fig. 6. Damaged tumor cells of the MTK-sarcoma III in the powerful antiserum, 90 minutes after treatment. A few survived for 5 hours. $\times 400$.
- Fig. 7. Tumor cells of the Yoshida sarcoma subline D in the antiserum, 90 minutes after treatment. Some cells are unaffected and remained still viable for 5 hours. $\times 400$.
- Fig. 8. Tumor cells of the MTK-sarcoma III in the antiserum, showing lobulation 90 minutes after exposure. A greater part of cells survived for over 5 hours after exposure. $\times 400$.
- Figs. 9 to 12. Tumor cells of Yoshida sarcoma subline C under the influence of absorbed sera with two types of tumor cells. Figs. 9 and 11; 5 minutes after exposure. Figs. 10 and 12; 60 minutes after the treatment with each absorbed serum. $\times 900$. 9 and 10 : No remarkable change in the serum absorbed with viable Yoshida sarcoma subline C cells. 11 and 12 : Damaged tumor cells in the serum absorbed with cells of Yoshida sarcoma subline D. Toxic antibody of the antiserum has remained still effective even after repeated absorptions with subline D cells.



T. Matsumoto : Differential Reactions of Homologous Antiserum to Tumor Cells