106. *Origin of the Yoshida Sarcoma.*

I. *Experimental Production of "Ascites Hepatoma" in the Rat.*

By Tomizo YOSHIDA, Haruo SATO, and Tomohiko ARUJI.

Dept. of Pathology, Tohoku University Medical School, Sendai.

(Comm. by T. SASAKI, M.J.A., Oct. 12, 1951.)

The manner of origin of the Yoshida Sarcoma is remaining obscure, although we have postulated that the mother cell of the tumor might be the "monocyte".¹⁾

The present strain of the Yoshida Sarcoma originated in 1943 in one of 20 rats which had been subjected to investigations to determine whether slight changes in the liver cells to be initiated by feeding carcinogenic amino azo dye for a short time could be succeeded to by successive application of other carcinogenic compounds, so as the collective effect of the both carcinogens realize an acutal carcinoma development in the liver. This was confirmed by the experiments of successive superpositions of carcinogenic hydrocarbon or arsenite through the skin to preceding amino azo dye feeding (Y. Muta).²⁾ But, the yield of the Yoshida Sarcoma was an accidental case in the series of these experiments, although just at that time we had been earnestly working on the experimental production of ascites tumor.

The animal presented the Yoshida Sarcoma was treated as follows: Immediately after cessation of feeding "o-Amidoazotoluol" (4'-Amino-2,3'-dimethylazobenzene) for just three months the animal received to its skin on the back paintings of diluted potassium arsenite solution (prepared by adding of equal amount of absolute alcohol to Fowler's solution) three times a week. Nearly at the end of the third month of the painting, a tumor was marked in the scrotum of the animal, which gradually increased in size, the painting being continued. In about one month after that the tumor arrived at a state ready to ulceration. The animal was sacrificed and by transplantation of materials from both the white massive tumor in the scrotum and milky ascites containing tumor cells the strain of "ascites sarcoma", the Yoshida Sarcoma at present, was established. The liver of the animal appeared some cirrhotic and presented multiple small hepatoma nodules.

Lacking in the knowledge of consistent and reproducible means of experimental production of this peculiar tumor, series of experiments were made repeating the same procedure described, but in

vain. Hereupon, another strain of the same ascites sarcoma has originated, once more by chance, in a rat which Tanaka had been feeding with o-Amidoazotoluol for some 300 days for the purpose of inducing hepatoma.³⁾ The animal fed at the end of the treatment 4-dimethylaminoazobenzene (DAB) for about a month, but was never painted with potassium arsenite solution. The autopsy of the animal was performed in our Institute with the purpose of transplantation of hepatoma which had been expected to be in an advanced stage because of enlarged abdomen of the animal. Contrary to the expectation, it was found there a milky ascites similar to that of the Yoshida Sarcoma, which was at once transferred and "took". The nature of the new strain cannot be distinguished from the original Yoshida Sarcoma in any point throughout its consecutive transplantations. The liver of the second animal was in gross and histological appearances analogous to that of the first case.

The basis for the assumption that origin of the Yoshida Sarcoma might be closely related with the development of induced hepatoma through azo dye, and azo dye alone, became all the more confirmed. Consequently, we have discussed from various points of view the histogenesis, histological structure, and histopathological phenomenon to be observed in hepatoma including histological behavior of the tumor tissue in the case of transplantation. Then, we obtained in this relation following points:

(1) Hepatoma has a peculiar histological architecture, without being provided with any stroma in usual meaning.⁴⁾ As the stroma in this case is demonstrable nothing else than irregularly shaped blood space ("Blutraum" Yamagiwa's) (Fig. 1 and 2). The coast of this blood sea is margined by the endothelial layers which tightly cover up the surface of masses of the hepatocellular tumor cells, which vary in size and shape. The endothelium in this case does not seem to perform enclosed tubes as usually to keep in the streaming blood, but appears, morphologically considered, to belong unseparably to the parenchymal masses. It remains thus between the masses of islands of hepatic tumor cells only irregularly shaped rooms filled up with the blood. The endothelial layers are undoubtedly those derived from the wall of the sinusoids within the liver lobules.

(2) It has been emphasized by Yoshida that, at the transition of benignant adenomatous hyperplasia of liver cells into malignant hepatoma, elongation of the wall of the sinusoid through cell proliferation plays an essential role as to bring about the typical hepatoma structure.⁴⁾ At the earliest stage of malignant change of adenoma into hepatoma the increase in the cellular elements in the sinusoidal wall becomes manifest. It follows this that the

sinusoidal spaces which have been very narrow in the adenoma tissue becomes extended and irregularly shaped. At the same time the increasing hepatic cells appear to be divided into groups of various size and shape being covered over their surface by concurrently increasing endothelial layers. This observation implies that the endothelial cells appear to have acquired a neoplastic or malignant character hand in hand with the hepatic cells.

(3) The structure of transplanted hepatoma is consistent with that of spontaneous one, whose development is just above discussed. But in the case of transplantation of hepatoma, it may need some consideration on the formation of histological structure, because in the transplanted tumor in general the "stroma" is supplied from the normal tissue of the host animal.

At the transplantation of tumors it is repeatedly confirmed that inoculated grafts are subjected to necrosis, with exception of few, usually very few, surviving cells in the peripheral parts of the grafts. The survivors are the parenchymal cells, being epithelial ones in the case of carcinoma. The stroma of the grafts fades away entirely. When the survived parenchymal cells set into proliferation there is nothing for the stroma but to be supplied from the host tissue.

How could, then, the implanted hepatoma reproduce the original architecture of the spontaneous hepatoma in which the epitheliums and endotheliums represent an intimate combination? Satomi¹³⁾ has earlier traced at Yoshida's suggestion the histological development of the inoculated hepatoma, starting from its very beginning stage and come to the conclusion that in the case of hepatoma transplantation the endothelial part also "takes", and proliferates in the host hand in hand with the epithelial (hepatocellular) tumor cells. If this is true, the hepatoma represents an exceptional case of cancer consisting of two "parenchymas" of both epithelial and endothelial origin, and having nothing of tissular "stroma".

(4) At histological observations of induced hepatoma in rats it occurs often to meet with sporadic gatherings of large mononuclear cells filling up the blood room of the tumor (Fig. 3). They have been regarded by many investigators as infiltrations of myeloid cells, and caused to arise an assumption that myeloid leukemia could occasionally accompany the induced hepatoma. But any change in white blood cell count has not yet been demonstrated.

We looked through a great deal of histological preparations of o-Amidoazotoluol hepatoma in rats arranged according to its developing stages, and found that in advanced stages of hepatoma the mononuclear infiltrates are almost always met with, varying in

their numbers and extensions in fairly wide range. Further, it was histologically confirmed that the mononuclears are endothelial origin in loco, because it happens to the endothelial cells of the hepatoma that they begin to proliferate locally, presenting at first a rosary-like figures and then a dense aggregation of rounded free cells (Fig. 4). The discussed assumption that the endothelial elements of hepatoma are of neoplastic nature seems to have been supported by this finding with a firm basis.

In connection with origin of the Yoshida Sarcoma in question, we observed these mononuclears with particular respects, because it is apparently impossible in usual sectioned preparations to differentiate them from Yoshida Sarcoma cells infiltrated into the liver tissue, which often occurs following intraperitoneal inoculation of the latter.

Thus, we have naturally come to postulate a tentative working hypothesis that the mononuclears in the hepatoma tissue might be identical with Yoshida Sarcoma cells. In other words, if they could be made alone separated from the epithelial cells, and unloosed into a spacious place like peritoneal cavity they would disclose their intrinsic potency of neoplastic proliferation to present a fluid sarcoma of mononuclear origin, so-called Yoshida Sarcoma.

Our subsequent experiments have been planned simply as follows: Hepatoma induced in the rat by amino azo dye feeding is minced and transplanted into the peritoneal cavities of normal rats. When it takes, the tumor is transplanted further under the same procedure, and so on. The smear of the peritoneal fluid of every inoculated animal is subjected to careful microscopical controls. It is expected that during repeating animal passages the endothelial elements will sooner or later get chance of separation from their partner, the hepatic tumor cells, and eventually overwhelm in the tendency of proliferation the latter. The peritoneal cavity is supposed to provide a more suitable living condition for the endothelial cells than for the tumor cells of epithelial origin in separated state.

To date, 19 hepatomas developed in rats fed DAB for various periods ranging from 145 to 215 days have been successfully transplanted. Several of them are being continued more than seven generations.

To our interest, we have, as a first step, gained an ascites form of hepatoma which we are provisionally calling "Ascites Hepatoma". In many animals received coarsely minced hepatoma mush intraperitoneally, it happened to be found that there arose an ascites in which numerous number of small "hepatoma-islands"

were increasing (Fig. 5). Hepatoma-islands, as we call, are those small bodies or dense aggregates of hepatic tumor cells of various sizes ranging from microscopically small globes to gross clumps visible with naked eyes. It is to be noted that all the islands or clumps, smaller or larger, are tightly covered over their surface by endothelial layers (Fig. 6). Plenty of mitotic figures are present in the cells of the covering surface layers, and they are ready to be detached from there becoming rounded free mononuclears (Fig. 7-8). A great deal of these emancipated cells are found floating in the fluid between the islands (see Fig. 5). They are not seldom in mitosis.

A droplet of the ascites freshly mixed with a small amount of aceto-gentianviolet or aceto-carmin solution and covered slightly with the cover glass presents very fine view of the islands informing almost intact of their living condition in the peritoneal cavity (Fig. 5-12).

After we had obtained ascites hepatoma by the inoculation of minced hepatoma tissue, we examined the peritoneal fluid of 20 animals bearing hepatoma induced by feeding DAB for 6 months, and found in 13 of them free hepatoma islands floating and increasing in the peritoneal fluid. With these ascites one can readily transfer the tumor. Therefore, it is evident that in advanced cases of hepatoma, the islands are released into the peritoneal cavity without any experimental treatment, just like as the dissemination of tumor cells in other carcinoma cases. The time of this occurrence is assumed to be about 200 days after the beginning of azo dye feeding. There are also some animals bearing hepatoma, which do not show such a change even after 200 days.

We keep at present three strains of ascites hepatoma in consecutive transplantation respectively. One of them was derived from a hepatoma of 145 days, in which the condition could not be yielded till the fourth generation of grafting of minced tumor tissue.

With the hepatoma ascites effective transplantation is done with ease using small glass pipette just in the same way as in the case of the Yoshida Sarcoma. In the peritoneal cavities of the inoculated animals there arises ascites hepatoma as well as multiple nodular tumors of various sizes and locations invading the omentum, pancreas, mesenterium, abdominal wall, and many others. The histological pattern of these coincide with that of spontaneous hepatoma.

Now, it remains a little doubt in identifying the hepatoma islands as elemental constituents of hepatoma. This will offer an evidence for our histological acception on the principle of hepatoma architecture discussed above. Injection of India-ink solution into

the peritoneal cavity of the animal bearing ascites hepatoma has demonstrated an appreciable phagocytic activity of the cells covering the islands as well as detached mononuclears, indicating their origin from the reticuloendothelial system of the liver, i.e. Kupffer's stellate cells (Fig. 12).

In the ascites, microscopically examined, there are scattered between the large islands many of such tiny ones as consisting of a few hepatic tumor cells enclosed by endothelial layers containing only one or two nuclei (Figs. 6 and 9). They, decreasing much more, reach at last at the extremity represented by pairs of two cells, the one being hepatic and the other endothelial in origin (Fig. 10). Sometimes the cells in the pairs are found in mitotic division (Fig. 11). It may be, therefore, safe to interpret these symbiotic, paired cells as ultimately analysed elements of hepatoma. The macroscopical islands may be increased states of the pairs. The hepatoma tissue is principally a conglomerate mass of the islands, although in sectioned pictures of the tumor some inversed arrangement of epithelial and endothelial elements is occasionally found, which requires as to its formation further discussions.

The investigations are in progress. Although the ultimate end of our work to separate from each other the symbiotic neoplastic cells of two different sources has not yet been achieved, it may be worthy to note to have obtained new strains of ascites hepatomas.

We are also taking into consideration possible responses in the reticuloendothelial system throughout the body of the animals of prolonged amino azo dye treatment. On the other hand, experiments in the mice treating in the same way as in the rats are concurrently going on in our laboratory. Ascites hepatoma in mice is also being observed. At the same time conditions similar to the leukaemia are often found in the treated mice, although their transplantations are not yet succeeded.

References.

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Fig. 1. Sectioned figure of a rat hepatoma tissue induced by o-Amidoazotoluol (4'-Amino-2, 3'-dimethylazobenzene). Irregularly shaped blood spaces as the stroma.

Fig. 2. Do. Extended blood spaces. Island-shaped hepatoma cell masses covered by endothelial layers. (Holes within the masses most probably derived from the bile capillaries.)

Fig. 3. Infiltration of large mononuclear cells in rat hepatoma tissue.

Fig. 4. Local increasing of the endothelial cells of rat hepatoma tissue. (Occasionally mitosis among the increased, enlarged, rounded endothelial cells.)

Fig. 5. "Ascites Hepatoma". Many "Hepatoma-islands" floating in increased peritoneal fluid. Many of the large, free mononuclears between the hepatoma-islands are detached endothelial cells. (Aceto-gentianviolet preparation of the ascites)

Fig. 6. A rather small hepatoma-island. Covering endothelial layer is clear. On the right side lower a very small one consisting of one endothelial and two (probably) hepatic cells. (Do.)

Fig. 7. Mitosis in the covering endothelium. (Do.)

Fig. 8. A dividing endothelial cell appears to be ready to detach. (Do.)

Fig. 9. A very small hepatoma-island consisting of one hepatic and two endothelial cells. (Do.)

Fig. 10. The smallest hepatoma-island, a pair of hepatic and endothelial cells. (Do.)

Fig. 11. One of paired cells in mitosis. (Do.)

Fig. 12. Covering endothelial cells of the hepatoma-island phagocytize India-ink particles introduced into the peritoneal cavity. (Aceto-carmin preparation of the ascites.)