A CASE OF TRANSPLANTABLE PLASMA CELL SARCOMA PRODUCED EXPERIMENTALLY IN A RAT (With Plates XX and XXI)

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INTRODUCTION

With exception of a report presented by Bloom (1947) that a plasma cell tumor occurred in the humerus of a male dog pertaining to English setter, no investigator ever reported on the incidence of this kind of tumor in animal (Engelbreth-Holm, 1942). During a serial study on the glucose-induced sarcoma of rat with primary stress on the role of monoiodoacetic acid in the sarcogenetic mechanism, the author encountered a tumor of the histologic picture of plasma cell sarcoma arising in a rat subjected to repeated injections of monoiodoacetic acid alone. The author successfully transplanted this tumor serially in rats until it died out after 7 generations. That this tumor arose primarily in the subcutaneous tissue and that fairly extensive study was made on the cytological features of this tumor, induced the author to present this paper.

EXPERIMENTAL METHOD AND MATERIAL

This sarcoma arose during the course of the study described in “IInd Report” (preceding communication, Tagashira, 1954). The carcinogens employed in this study were: concentrated glucose solution injected subcutaneously for the purpose of evoking a glucose-induced sarcoma (G); o-aminoazotoluene injected subcutaneously in order to facilitate the sarcogenesis (A); and monoiodoacetic acid, newly established carcinogen by the author (1954), injected subcutaneously in order to inhibit enzyme systems involved in the metabolism of glucose (M). Animals were divided into 8 groups each receiving different combination of carcinogens. Namely, Group 1 received all of the carcinogens (G+M+A); Group 2 (G+M); Group 3 (M+A); Group 4 (G+M+olive oil); Group 5 (G+M); Group 6 (M); Group 7 (G); Group 8 (A). Frequency of the incidence of the induced tumor in each group was discussed elsewhere. Most tumors evoked by the above listed carcinogens, according to the authors experience, are either one of fibroma, fibrosarcoma and polymorphocellular sarcoma. It happened that a plasma cell sarcoma occurred in a rat from Group 6 (No. 69) receiving monoiodoacetic acid alone subcutaneously in dose of 0.5 cc (0.4 g/dl) every other day for the period of one year (180 injections).
Following the cessation of the injections, this group had been placed under the author's observation, and it was around the 540th experimental day that a palpable nodule arose in one of the rats at its right back exactly at the site of the subcutaneous administration of monoiodoacetic acid. Thenceforth this tumor increased its size progressively and reached the size of $3.2 \times 2.4 \times 1.1$ on the 556th day when it was felt elastic hard in the absence of conspicuous vascular dilatation on its surface. This tumor mass softened for most part around the 562nd day, and the animal died on the 564th day when it weighed 185 g, while its pre-treatment weight was 100 g. Sections were prepared from the tumor and studied histologically. Pieces of the tumor were transplanted in 3 rats either by subcutaneous or by intraperitoneal route.

**Findings at the Necropsy (Text-fig. 1)**

The primary tumor arose in the subcutaneous tissue and had the size of $3.6 \times 3 \times 1.6$ cm without any conspicuous vascular dilatation. There was a focus of viscous, bloody fluid together with that of soft, necrotic mass each occupying about one-third portion of the tumor. Tumor was adherent to the underlying tissue, and was encapsulated by a thick layer of the connective tissue. The skin covering the tumor could be peeled off easily. The tumor was found growing through the muscular layer into the abdominal cavity. It invaded the retroperitoneal cavity and grew into the right posterior abdominal wall to extend further along the abdominal aorta and into the entire area of the diaphragm to form a massive tumor in the anterior mediastinum wherein to implicate the heart and medial portions of the lungs.

Left lung was invaded by the tumor by way of the diaphragm and the hilus. Right lung was invaded in a similar way, but somewhat weakly. Distinction was sharp between the tumor invading the lungs and healthy portion of pulmonary parenchyma. Nodular metastases spread in the parenchyma of the lungs. The tumor mass in the pleural and abdominal cavities appeared whitish gray and was felt elastic soft and there was scanty signs of necrosis. Pleural effusion and ascites were absent. One of the peri-aortic lymph nodes was tumorous with histologic evidence of metastasis. In the absence of enlarged axillary or inguinal glands, metastasis was established histologically in the axillary glands. Vertebrae were intact.

The liver weighed 8.7 g, with histologic signs of congestion associated with hypertrophy of the stellate cells. The spleen weighed 1 g and was characterized by congestion and atrophied follicles. Kidneys weighed 1.4 g (right), and 1.2 g (left). Renal capsule was adherent to the tumor, but parenchymatous infiltration was absent. Omentum and mesenterium were intact.

Microscopically, bone marrow (vertebrae, femur) was intact in its cellular constitution. The kidneys did not contain such protein crystals and giant cells as
are elicited from the kidneys of human subject bearing plasma cell myeloma. There were occasional renal tubules containing hyaline cylinders (Fig. 4).

**Histological and Cytological Findings of the Tumor**

Data of the supravital observation are not available as to the tumor earlier than the 5th transplant generation. Since there is no essential difference in the histological appearance of the tumor cells from a generation to another so far as smear and sections are concerned, histological findings of this tumor are summarized in the following paragraphs.

The induced tumor grew into the subcutaneous muscle layers on one hand (Fig. 2), and extended through the retroperitoneum and into the mediastinum per continuitatem on the other. The appearance of this growth bears a perfect resemblance to a sarcoma. Owing to the fact that repeated injections of monoiodoacetic acid resulted in the necrosis associated with induration of the local tissue, it could hardly be determined whether the primary location of the scar tissue was intramuscular or exactly subcutaneous. In view of the fact that tumor carried a capsule superficial to it, however, it appears rather likely that this tumor arose from a scar tissue deep in the muscle layer, and grew deeper into the body cavity. The area invaded by the growth was extensive, but the pathway of the infiltration is characteristically interstitial, while parenchymatous organs remain fairly intact. Massive tumors in the retroperi-toneum or the mediastinum did not grow into the kidneys or the heart beyond their capsules. Either lung escaped a deep infiltration save its hilar region. Tumor invaded no lymph glands other than those draining the area involved in the primary tumor. Generally it appeared that the growth was vigorous in the interstitial tissue, but slow in the parenchymatous organs.

Histologically, rich network of silver fiber was present in tumor tissue, probably because the tumor grew into the interstitial tissue which is abundant in

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![Text-fig. 1](Image)

Invasion of the tumor
connective tissue. However, exact relationship between tumor cells and silver fiber was indistinct (Fig. 3). Appearance of the growth through the muscle layers of the body trunk resembled human chloroma.

Cytologically, tumor cells presented a picture compatible with a plasma cell with characteristically scanty cytoplasm as is usually elicited from plasma cells migrating in the inflammatory focus. There is a clear zone adjacent to the nucleus which is invariably cart-wheel like with nucleoli somewhat smaller than those elicited from myeloma cells in man. There are occasional cells simulating giant cells with relatively narrow cytoplasm. Inclusion body was absent (Fig. 1).

Giemsa-stained smear presented microscopically identical pictures with sections (Fig. 7). The cells contained somewhat small, basophilic cytoplasm, and cart-wheel like nuclei. Nucleoli were hardly detectable. Perinuclear clear zone was generally small. Inclusion bodies were absent. Tumor cells cotained cart-wheel like nuclei, but none of them exhibited fine nuclear net similar to our "lymphogonia." Dividing cells were encountered abundantly in sections secured from vigorous-tumor-bearing animals, while scanty cell division occurred in sections secured from regressing-tumor-bearing animals. Tumor cells took methyl-green pyronine stains in a way compatible with normal plasma cells. Peroxidase reaction was negative.

Supravital observation revealed the following pictures (Text-fig. 2): mitochondria stained by janus-green were distributed fairly extensively throughout the cytoplasm, in a lower frequency, they clustered around the nucleus. A minority of neutral-red vacuoles occured in the perinuclear clear zone either sparsely or in groups. Carbon particle phagocytic test (employing film-method) was negative.

A; B, C—Tumor cells; two of them binucleated. D, E—Degenerate tumor cells. Mitochondria: rods, Neutral red vacuoles: black and rounded, vacuole degeneration: rings.
Phase contrast microscopy made a detailed observation of mitochondria possible (Fig. 8-13). The predominating numbers of mitochondria were rod-shaped, somewhat smaller and narrower in shape than those contained in normal plasma cells. It is reported that, at the time of mitosis, mitochondria increase in number and become somewhat slender in shape (Dalton, 1951). This kind of change was noted in an exaggerated degree in this tumor cells. The author studied the morphologies of cells at different stage of mitotic division. As was reported by Amano, Unno, Hanaoka previously (1951), the nucleus of plasma cell normally contains chromatin mass which hangs from its nuclear membrane like stalactite, and this obscures the morphologies of the nucleus at prophase. It was difficult, therefore to observe the distinct morphologies of chromosomes at the stages equivalent to the 1st and 2nd stage of usual tumor cells under mitotic process. The 3rd stage of contraction of the chromosomes immediately before metaphase was the first sign of the prophase noted by the author. This fact may be partly attributable to the fact that the nucleus of a plasma cell is physiologically in a state of fused prochromosomes (Amano, 1954) and it completes the prophasic process very rapidly. On the other hand, phase contrast microscope elicited detailed figures of the cell division at telophase where the loosening of chromosomes occurs. Supravital observation depicted occasional tumor-cell-nuclei taking neutral-red stains at an early staining suggestive of degeneration. Such nuclei, phase-contrast-microscopically, lost chromatin mass rapidly and turned into transparent bodies containing a small nucleolus. This probably means a disentangle of chromatine blocks through the only loss of tonicity of the chromosomal structure. The like of such pictures had not been encountered in the mitotic division of plasma cells appearing in the inflammatory focus. These facts depict an aspect of the degeneration of the neoplastic plasma cells and presents an interesting problem with regard to the cytological natures of the plasmacytic nucleus.

**Transplantation of the Tumor**

Since pieces of the primary tumor were transplanted from rat No. 69 after its death, resulting tumor was superimposed by mixed infection. Probably because of this infection, tumor regressed in the 2nd generation. Removal of the infection was attempted by the administration of penicillin, streptomycin in the 3rd generation, with the result that tumor regained its vigor in the 4th generation. Intravenous transplantation of the tumor from regressing-tumor-bearing animals in this period failed to take in hosts. On the other hand, pieces of the tumor transplanted intraperitoneally from vigorous-tumor-bearing animal of the 4th generation produced a marked anemia in hosts on the 15th post-transplantation day associated with extramedullary hematopoiesis in the spleen.
Bone marrow (Fig. 6) and other hematopoietic organs were free from neoplastic invasion. This fact is interesting as compared to the fact that plasma cell myeloma of man induces a marked anemia of the patient.

The results of serial transplantation are given in Text-fig. 3. Analysis of this table suggests that successful takes occurred only in transplantation by subcutaneous and intraperitoneal route, whereas the liver, testis and blood rejected a take. It may be of interest to discuss briefly the amount of tumor tissue to be implanted to secure a successful take. Namely, pieces of tumor implanted in an amount smaller than several trocars failed to take in hosts. This fact suggests that a successful take requires a fairly large amount of the tumor tissue. The rats used in this study, including both 1st and the subsequent serial generations, were all hybrids. The author feels that neoplasm derived from a highly differentiated tissue like blood-cell should have been produced and transplanted serially in an inbred strain of rats.

**SUMMARY AND DISCUSSION**

That the histological appearance of the cells predominating in this tumor...
are compatible with plasma cells has ample evidences. Another characteristic feature of this tumor is its slow proliferation as was shown in the result of the transplantation experiment. In view of these two features this tumor may be concluded to be a neoplasm derived from a blood cell type with proliferation not leukemic, but simulating sarcoma of fairly mild malignancy. For these reasons the author designates this tumor as "plasma cell sarcoma" or "extramedullar plasmoma."

Supravital appearance of the mitochondria in the plasma cell myeloma of man was reported by Fukase (1949). In comparison with the reported appearance, the author's tumor appeared more sarcomatous in that it contained smaller, but more numerous mitochondria. Since Yoshida sarcoma is now accepted as a monocyte-sarcoma, so is the author's tumor compatible with a plasma cell sarcoma. It is regretted that this tumor died out after 7 generations. There must have been some means to preserve the strain of this tumor, since Furth successfully transplanted leukemia of mouse serially for many generations of inbred strain.

It deserves attention that this tumor arose primarily in the subcutaneous tissue exposed to the repeated doses of monoiodoacetic acid and in the absence of bone marrow involvement. This fact sharply contrasts to the fact that most plasma cell tumors in man are derived from the bone marrow in the form of a myeloma with the exception of a plasma cell sarcoma reported by Maresch (1909) to have invaded lymph glands systematically, and a non-myelogenic plasma cell sarcoma arising primarily in the connective tissue of the neck (Kagamiyama, unpublished data).

Since plasma cell derives its origin from the adventitia cells around the blood vessel (Amano, et al, 1944), plasma cell sarcoma arising primarily from tissues other than bone marrow is no surprising matter. It is rather strange that plasma cell sarcoma like this is considered to be so rare.

Plasma cell myeloma of man is classified into Ɣ-globulin type and β-globulin type. The author performed assays of globulin fraction, both qualitative and quantitative, on this tumor. With a cooperation of Dr. Hanaoka the author examined blood samples obtained from tumor-bearing animals of the 2nd generation on the 15th post-plantation day for their protein fractions electrophoretically. There was no evidence of increasing globulin fraction. The author does not intend to generalize this fact on the entire strains of the induced-tumor, since the tumor-bearing-animals used in this assay had received repeated doses of antibiotics in order to subdue the superimposing mixed infection and the tumor itself was regressing at the time of this assay. After the 4th generation tumor regained its vigor, but no electrophoretical analysis of the protein fractions was made either on blood or the tumor extracts.
That hybrid strains were used in this study constituted the primary reason for the author's failure to transplant this tumor for many generations. Another experiment is being contemplated employing inbred strain of animal. During the course of this study occasional cysticercus sarcoma (reticulosarcoma) of the liver occurred. This tumor could be transplanted from many generations without any particular difficulty, contrasting sharply to the failure of the plasma cell sarcoma to be carried beyond 7 generations despite the best caution employed by the author. In this failure the highly differentiated character of the tumor cell—a blood cell type—probably plays a part. With this failure, this study still merits a publication, since occurrence of plasma cell sarcoma has not reported on.

**CONCLUSION**

(1) Monoiodoacetic acid, a carcinogen, was injected repeatedly for a long period of time to a group of rats from hybrid strain. One of the rats developed a plasma cell sarcoma at the site of the injection.

(2) This tumor was consisted of predominant cells which bear a striking cytological and histological resemblance to the tumor cells of the plasma cell myeloma of man, except that the former cells contained smaller nucleoli. This tumor neither arose from the bone marrow nor metastasized in the bone marrow, but occurred as a diffuse, continuous infiltration in the connective tissues.

(3) Abundance of mitochondria in the tumor cell suggested a higher malignancy of this tumor in comparison with the known plasma cell myeloma of man. Sections prepared from this tumor contained abundant cells under mitotic division. Such features of the tumor sharply responded to the vigor of the growth.

(4) Blood samples obtained from regressing-tumor-bearing animals were assayed for protein fractions. No marked change was present. It is conjectural, however, whether this is attributable to a decreased vigor of the growth or to a high degree of the anaplasia.

(5) Pieces of the tumor were transplanted subcutaneously, intraperitoneally or into the testis, liver, bone marrow or blood of hosts. Successful takes occurred following the implantation by subcutaneous and intraperitoneal routes, whereas other routes rejected a successful take. Transplantation to the 7th generation was successful only in small percentage of the animal, and the tumor died out after the 7th transplant generation.

**REFERENCES**

1) Amano, S.: Fundamental problems on hematology. 1948, Japan (Monograph)


**LEGEND FOR PLATES XX AND XXI**

Fig. 1: Microscopic picture of tumor cells in section (hematoxyline-eosin staining); typical cart-wheel like nuclei.

Fig. 2: Tumor-cell-infiltration into the subcutaneous muscle layer. (1st generation).

Fig. 3: Silver staining of the tumor tissue (Akazaki's modification).

Fig. 4: Kidney, renal tubulus containing hyaline cylinders (1st generation).

Fig. 5: Spleen, extra-medullary hematopoiesis (4th generation, 15 days after the intraperitoneal transplantation).

Fig. 6: Bone marrow (same as above).

Fig. 7: Giemsa-stained smear of the tumor cells.

Fig. 8—Fig. 13: Observation by phase contrast microscope. Fig. 8, 9—mitotic cells, Fig. 10—binucleated cell, Fig. 13—a pair of asymmetric cell division. (4th generation)
要 旨
実験的に発生させるラッケの可移植性形質細胞肉腫

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動物の形質細胞腫は Bloom が犬の大脛骨髄に自然発生したものを報告しているにとどまり、その他に記載をみない。われわれは葡萄糖肉腫の発生過程において、一酸度酸が如何に作用するかという点を実験的に検索中、単に一酸度酸のみを注射した一定のラッケから悪性形質細胞腫と称すべきものの発生を認めた。

この動物の実験条件をのべると、一酸度酸 0.4 gr/dl 0.5 cc を隔日に皮下注射 180 回 1年と及ぶ。観察中、実験日数 540 日頃に右背皮下の注射部位に相当して、腫瘍を触知した。その後急速に大きさを増し、556 実験日には 3.2×2.4×1.1 cm となり、硬度は弾力性で皮膚面の著しい血管拡張は認め得なかった。562 実験日に腫瘍は一部を除き軟化し始め、564 実験日において発死した。初期体重 100 g、末期体重 185 g である。腫瘍組織は直に組織学的検索に供するとともに、他方三正のラッケに皮下乃至腹腔内移植移植を試みた。

剖検時皮下原発腫瘍の大きさは 3.6×3×1.6 cm で粘稠性液状の液状物及び軟い壊死状物をそれぞれ腫瘍の 1/3 程度に含み、皮下の底部に固着している。腫瘍自体は厚い結締繊維性の被膜を被り皮膚との境界はなが、底部において筋層を破って腹腔内に浸潤している。後腹膜腔内に入った腫瘍は右側後腹壁に浸潤し、さらに背部大動脈に沿って上行、横隔膜を全面において腫瘍化せしめてこれを突破し、前縦隔状に大腫瘍を形成し、この中に心臓を嵌し、さらに両肺内側を完全に侵している。肺は横隔膜面及び肺門から実質内に腫瘍浸潤を受けており、肺実質と銳利に壊されており、その他に実質内の転移結節の散布もみられる。胸水、腹水はないと膣大動脈淋巴腺の腫脹するものの 1 個があり、腫瘍転移を認めた。腋窩、鼠蹊淋巴腺の腫大は認めなかったが、腋窩淋巴腺には組織学的に腫瘍転移を認めた。その他の臓器、例えば心、肝、脾、腎、骨髄等にはいずれも転移形成はなかった。

腎では人の形質細胞腫例で注意されているような蛋白結晶、巨細胞などは認めがないが、その細尿管には硝子円柱が散見された。

固定切片標本の所見では腫瘍細胞は炎症組織においてと同様の比較的細胞の狭い形質細胞の型をとり、核周明膚を有し、核はいずれも定型的車輌状核で、人の形質細胞腫の如き大型の仁は認め難い。この間に巨細胞化せるものが混在するが、その場合には概ね胞体が狭小となって
いる。核分裂像もしばしば認められる。細胞内縁入体はない。鰐側細胞は一見多いように見え
るが、腫瘍が間質内を発育しつつある関係で残存結締繊に割込むことと関係あるらしく、個々
の細胞との関係は明かでない。

塗抹ギムザ染色所見も切片所見とほぼ同様であるが、マーキンクイダーゼ反応は陰性で、メ
チール緑、ピロニン染色の態度は正常形質細胞と異なるところはなく、

超生体染色所見では、ヤーヌス細染色系粒体は比較的広く胞体内に分布するが、時に核周に
集るものもある。中性赤血球はその核周明庭部に数個集合性乃至分散して認められるものがあ
るが、これは一部である。墨粒貪食能は陰性である（墨微法による）。

位相差観察鏡によると系粒体の状態はさらに明瞭で、正常形質細胞に比して細い短桁状の系
粒体が極めて多数認められる。元来系粒体は分裂細胞では細短桁状化し数を増すが、この場合
にはその傾向が著しい。なおこの方法で核分裂の各期を観察した。元来形質細胞の核膜には色
素塊が細乳状の如く垂れ下っているが、この性質のために分裂前期の態度を明確に把握することは
困難で、一般腫瘍細胞核に見られる染色体の第一乃至第二収縮期の染色像を把握することは困難で
あった。そして第三収縮状態（中期への移行期）から初めて前期として確認し得る。これは天
野が述べている如く形質細胞核が融合性前染色体状態にあり、容易に前期を通じて中期に至
るためと考えられる。これに対して末期の染色体のほぐれてゆく像は確認し得た。

腫瘍細胞の移植は主として膿胞、皮下において成功し第七代まで移植可能であった。その間
睾丸、肝、骨髄、血液内等の移植も試みたがすべて失敗に終った。また移植動物の血液並びに
腫瘍組織の蛋白像を電気泳動法により検査したが、グロブリン分層における著明な変化を認め
得なかった。唯一、第4代の腹腔内移植例において移植後15日で強度の貧血を来し、脾におい
ても観察血像を認めたことは人の形質細胞腫高度の貧血を伴う事実と比較して興味深い。

以上の細胞学的性状から本腫瘍細胞が形質細胞の性状と一致することはほとんど疑入れな
いところであるが、その増生の性格が比較的緩徐であることから、一血球腫の腫瘍性増生では
あるが白血性ではなく、むしろ比較的悪性性の強い肉腫、従って形質細胞肉腫と呼ぶことが
出来よう。また天野の述べる如く形質細胞が血管外膜細胞から発生するものである以上、そ
れが骨髄腫以外の形で発生することのあらたのは当然で、むしろその頻度の少きに疑問のある程
である。累代移植が細胞の注意にかかわらず第7代までしか行われず、ために腫瘍蛋白等につ
いて詳細な研究を行い得なかったことは返す返すも残念であるが、これは本腫瘍の母細胞が血
球という高度に分化した細胞であったということも大きな理由である。従ってかかる腫瘍は純
系において発生せしめ、かつ同系動物に移植することが必要で、この点失敗に鑑み今後考慮す
べきことであろう。

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