AN ASCITES TUMOR DERIVED FROM EARLY SPLENIC LESION OF FRIEND'S DISEASE: A PRELIMINARY REPORT*1

(Plate C)

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Friend's disease occurs in mice only a few days after the inoculation of Friend leukemia virus, and is characterized by the proliferation of polygonal or spindle cells in the red pulp of the spleen. It offers quite a good model for analyzing viral leukemogenesis, since the transformation of the target cells may take place within a day or two under a certain condition.

It is considered quite important for the morphological approaches to murine viral leukemogenesis to recognize the true target cells in the spleen.

As to the histogenesis of Friend's disease, Friend,3) Metcalf, Furth, and Buffet,9) Kasuga and Oota,7) and Siegler and Rich12) have investigated it in detail, and the proliferating cells in the infected spleen have been reduced to reticulum cell tumor. The origin of the proliferating cells, however, has not been clarified yet, and the relation of the cells to the associated erythroblastosis is still obscure.

To solve the above problems, the spleen foci of Friend's disease were produced in male DDD mice by the inoculation of diluted virus solution following the method of Axelrad and Steeves,1) and were examined histologically and by electron microscopy.4,5) The spleen foci were proved to be early sites of Friend's disease, and histologically consisted of large polygonal or spindle cells, consistent rather with a reticulum cell tumor. The electron microscopic observation of the spleen foci in early stages, however, revealed the tumor cells resembling proerythroblasts and basophilic erythroblasts.5)

Although the authors thus succeeded in realizing some characters of the proliferating cells in Friend's disease by analyzing the spleen foci in early stages, it was still questionable whether the minute spleen foci consisting of erythrogenic tumor cells would really be continuous with the final tumorous proliferation, and the isologous transplantation of the macroscopic spleen foci was attempted, and six strains of subcutaneously transplantable solid tumors were established on different occasions.6) After several subcutaneous passages, each strain was converted to an ascites form, and the six ascites strains, originally derived from the early splenic lesion of Friend's disease, have been established.

One of them, SFAT–3 (spleen focus-derived ascites tumor–3), showed a classical cytological pattern of an erythrogenic ascites tumor, and here is a preliminary report on the ascitic strain.

**History of SFAT–3**

Establishment of SFST–3 (spleen focus-derived solid tumor–3): An accessory spleen and three macroscopic spleen foci of a male DDD mouse, 11 days after the inoculation of 0.1 ml of 10^2 dilution of the routinely prepared viral inoculum, were transplanted to the subcutaneous areas around the neck of 5-week-old, male DDD mice, and 18 days after the transplantation, a small, pea-sized tumor was noted at the site where the accessory spleen had been transplanted. The transplantation of the other three spleen foci was unsuccessful. The tumor was 100% transplantable to DDD mice when 1 mm³ of the tumor mass was transplanted in the subcutaneous area around the neck of 4–6-week-old DDD mice. The mean survival days, then, were approximately
31 days. An example of SFST-3 strain is shown in Photo 1. The solid tumor often grew to over 10 g in weight. The tumor mass consisted monotonously of large polygonal cells, resembling reticulum cell sarcoma (Photo 2).

Ascitic conversion to SFAT-3: The solid tumor of SFST-3-3-32 (a male DDD mouse, 32 days after the subcutaneous transplantation of the SFST-3 strain in the 2nd generation, to be the 3rd when transplanted) was dissected in a warm Ringer solution, and the cells in suspension were inoculated intraperitoneally to 10 male, 5-week-old DDD mice. Seven out of the 10 mice showed the increase of ascites within 3 weeks after the inoculation, and the transplantable ascites tumors were established. One of them has been serially passaged through male DDD mice intraperitoneally, designated as SFAT-3 (Photo 3).

Cytological Observation of SFAT-3
The smears of SFAT-3, about 2 weeks after the inoculation of 10^7 purely cultured SFAT-3 cells to male DDD mice, were air-dried, fixed in methanol, and stained with Wright-Giemsa solution. The SFAT-3's examined were in their 7th, 8th, and 9th generations. The tumor cells of SFAT-3 varied in size. The cytoplasm of the larger cell was stained pale blue, and the chromatin in their nuclei were finely aggregated. They remarkably resembled proerythroblasts (Photos 4 and 5). The smaller tumor cells, about half as large as the larger tumor cells, had markedly basophilic cytoplasm and their nuclei often showed coarse chromatin aggregation, resembling basophilic erythroblasts (Photos 4, 5). Both kinds of cells showed frequent mitosis (Photo 6), and occasional intracytoplasmic vacuoles (Photos 4~6). Imprints of an SFAT-3 solid tumor presented similar cytologic patterns (Photo 7).

Subcutaneous Transplantation of SFAT-3 Cells
To find the behavior of SFAT-3 cells in a mass, subcutaneous transplantation of SFAT-3 cells was performed and it produced the same histological pattern as seen in Photo 2.

**Discussion and Conclusion**
In the present investigation, an ascites tumor, which had originally been derived from an early splenic lesion of Friend's disease, was cytologically proved to be erythrogenic, providing characteristic chromatin aggregation of the nuclei. This fact strongly supports the hypothesis that the proerythroblastic tumor cells, which had been electron microscopically observed in the spleen foci of Friend's disease in early stages, are continuous to the final neoplastic proliferation.

The cells in SFAT-3 mostly resembled proerythroblasts or basophilic erythroblasts. They proliferate rapidly in the peritoneal cavity and kill the hosts by leukemic infiltration to the liver and hemorrhagic tendency without causing marked splenomegaly. Slight to moderate splenomegaly was present in most of the cases, which had survived over 5 weeks after routine transplantation of the ascites strain. Mesenterial, retroperitoneal, and paratracheal lymph-node involvements were often observed at autopsy.

Transplantable Friend ascites tumors have so far been established by Oboshi et al.,10) Kobayashi,8) and by Fieldsteel et al.,2) but there have been no such erythroblast-like tumors as SFAT-3.

The facts that the subcutaneous transplantation of SFAT-3 cells formed a solid tumor with the same histological pattern as that of SFST-3 (cf. Photo 2), and the imprints of SFAT-3 solid tumors showed the same cytological pattern as that of SFAT-3 strongly suggest that the cells in SFAT-3 resembling younger erythroblasts are the tumor cells themselves.

The mean survival days of DDD mice, inoculated with 10^7 SFAT-3 cells in their 9th generation were 38 days. A Friend ascites tumor, which had been converted by Oboshi et al.,10) from the Friend solid tumor, established by Kasuga et al.,7) showed 8~12 survival days in its 180th generation in ddOM mice.11)

If the red blood cells could be induced from the SFAT-3 strain, the rôle of the Friend virus...
in tumor cell, or, further, a mechanism of 'dedifferentiation' in Friend cells might be suggested.

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EXPLANATION OF PLATES C

Photo 1. A male DDD mouse, 4 weeks after the subcutaneous transplantation of SFST-3 solid strain in its 3rd generation.

Photo 2. Histology of the tumor of the mouse in Photo 1. Formalin-fixed, Hematoxylin-Eosin stain. ×480.

Photo 3. A male DDD mouse, 5 weeks after the intraperitoneal transplantation of the SFAT-3 ascites tumor in its 8th generation. Mesenterial and paratracheal involvement observed. No marked splenomegaly present. The fluid in the dish underneath is the ascites, turbid, slightly reddish.

Photos 4–6. Smears of the SFAT-3 ascites strain. Tumor cells resemble immature erythroblasts. Wright-Giemsa stain (Photos 4 and 6, ×960; Photo 5 ×800).

Photo 7. Imprint of SFST-3 solid tumor. ×960.