Heme Synthesis in Friend Ascites Tumor Cells* (32732)

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The Friend virus causes a leukemia characterized by proliferation of reticulum cells in the spleen associated with erythroblastosis (9). Foci of proliferating reticulum cells surrounded by an accumulation of erythroblasts is evident on histologic examination of the spleen (9). Relationship of these erythroid cells to the reticulum cells, however, is not clear (1).

In 1966, Ikawa *et al.* (7) established several lines of ascitic Friend tumors. They reported that the ascitic Friend tumor cells, although closely resembling proerythroblasts morphologically, showed little tendency to mature along the erythrocytic line (8). Since the main characteristic of erythroblasts is the ability to synthesize hemoglobin, it was considered important to study this capacity in these ascitic Friend tumor cells.

Materials and Methods. Weanling mice of DDD strain weighing 18–20 gm, inbred at the Institute for Infectious Diseases, University of Tokyo, were used.

The Friend ascites tumor cells were serially transplanted through DDD mice at the Cancer Institute, Tokyo. Of the 6 strains of Friend ascites tumor, 2 strains were used in the present experiment [spleen-focus-derived ascites tumor type-3 (SFAT-3) and type-6 (SFAT-6)]. For SFAT-3, 10 mice were inoculated with 1.5×10^7 cells. For SFAT-6, 10 mice were inoculated with 10^7 cells. The origin and the morphology of SFAT cells were described in the previous report of Ikawa *et al.* (7). Controls consisted of mice inoculated with 10^6 cells of Ehrlich ascites tumor cells (serially transplanted at the Division of Clinical Pathology, Central Hospital of Japanese National Railways in Tokyo), and mice inoculated with about 10^6 of SN-36 ascitic lymphoma cells (11) in the six hundred-fifteen transplant generation (kept at the Sasaki Institute in Tokyo).

About 2 weeks after inoculation of the tumor cells, the mice showing a marked ascites accumulation were injected intraperitoneally with 0.33 μC of radioiron ($^{59}FeCl_3$, specific activity 19.6 mC/mg of Fe, Dainabot Radioisotope Lab., Tokyo) in 0.5 ml of saline solution. Four hours later, they were sacrificed and the ascitic peritoneal fluid was withdrawn in heparinized saline solution (heparin sodium 50 units/ml). The volume of packed cells was measured to the nearest 0.01 ml by microhematocrit tubes.

The collected cells were washed 3 or 4 times until the washings were free of radioactivity. One ml of hemoglobin solution (45 mg/ml) was added to the packed cells as a carrier to facilitate heme extraction. Hemin chloride was extracted and crystallized according to the method of Chu and Chu (2).

Extracted hemin was dissolved in 3 ml of 1 *N*-NaOH, and the radioactivity was counted in a low background gamma-ray spectrometer (Atomic Instr. Co., Model 988A). The amount of recovered hemin was determined spectrophotometrically and the radioactivity was corrected as follows:

$$\text{Expected cpm} = \text{Observed cpm} \times \frac{\text{Amount of carrier Hb (mg)} \times 0.04^{1}}{\text{Yield of hemin (mg)}}.$$

[1] 0.04 = amount of hemin in hemoglobin molecule.

Results. The volume of packed ascites cells ranged from 0.32 ml to 0.78 ml per mouse. Some cells showed a slight contamination of red cells, which occurred equally in the SFAT cells and controls. Hemoglobin from contaminating red cells, however, was negligible when compared to the amount of added carrier hemoglobin.

The yield of hemin ranged 0.56–1.38 mg (31–76% yield), which was a fairly good recovery with this method.

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TABLE I. Heme Synthesis in Control Ascites Tumor Cells and Friend Ascites Tumor Cells.

Group	Type of ascites tumor	No. of mice	Packed volume of ascites cells (ml)	^{59}Fe incorporation[c] into heme (cpm/ml packed cells)	
Control	Ehrlich	2	0.600 ± 0.180[b]	77 ± 13[b]	
	SN-36	8	0.441 ± 0.055	1018 ± 259	(ns)[d]
Friend	SFAT-3[a]	10	0.400 ± 0.018	9485 ± 2500	($p<0.01$)
	SFAT-6[a]	9	0.427 ± 0.018	8273 ± 1680	($p<0.01$)

[a] Abbreviations: SFAT-3, spleen focus-derived ascites tumor type-3.
SFAT-6, spleen focus-derived ascites tumor type-6.

[b] Mean ± SD.

[c] $^{59}FeCl_3$ (0.33 μC) was injected intraperitoneally into mice 4 hours prior to sacrifice. Ascites tumor cells were collected and washed 4 times with cold saline. Hemin chloride was extracted and crystallized by the method of Chu and Chu (2).

[d] t test.

The results of heme synthesis by each tumor cell line are shown in Table I. In the Ehrlich ascites tumor cells, no significant heme synthesis was observed. In SN-36, no significant heme synthesis was recognized; however, there was a considerable amount of heme synthesis in SFAT-3 and SFAT-6 cells ($p<0.01$).

Discussion. There has been much controversy over the origin of erythroblasts in Friend leukemia (1,7,9,10,13,14). Metcalf *et al.* (10), from their pathologic studies in Swiss mice, and Brodsky *et al.* (1), from their studies on ferrokinetics in Swiss Webster female mice, concluded that the altered erythropoiesis in Friend leukemia was benign and not a form of leukemia. However, Ludwig *et al.* (9), by their pathologic studies in BALB/c and RF mice, suggested that the Friend virus caused a type of erythroleukemia. Zajdela (14) came to the conclusion, from histologic and autoradiographic studies, that the Friend tumor cells, although resembling undifferentiated reticulum cells, were primitive erythroblasts. Sassa *et al.* (13) have recognized, on the basis of hypertransfusion studies in ddO mice, that the erythroblastosis in Friend leukemia was not regulated by a normal physiological mechanism which was mediated through erythropoietin (6).

In the present investigation, ascites tumor cells of SFAT-3 and SFAT-6, originally derived from an early splenic lesion of Friend leukemia (7), were used. Several transplantable Friend ascites tumors were established by Oboshi *et al.* (12), Kobayashi (8), and Fieldsteel *et al.* (3,4). However, appearance of tumor cells morphologically resembling erythroblasts as seen in SFAT-3 and SFAT-6 has never been reported. Because subcutaneous transplant of SFAT cells formed a solid tumor with the same histological pattern as the spleen-focus-derived solid tumor, and because the imprint specimen of SFAT-derived solid tumor showed the same cytological pattern as that of ascitic Friend tumor cells, Ikawa *et al.* (7) suggested that the SFAT cells are the tumor cells.

In this study we have demonstrated an apparent heme synthesis in these SFAT cells, while the other tumor cells showed none or a slight amount of heme synthesis which might be due to heme formation unrelated to hemoglobin, for example, catalase or cytochrome. Because the amounts of hemin isolated were about the same in controls and in SFAT, it is unlikely that nonhemin contamination of the crystals was responsible for the observed counts.

Recently, Friend *et al.* (5) reported that the reticulum cells infected by Friend virus, when cultivated *in vitro,* incorporated iron and contained benzidine-positive material. They concluded that these cells appeared to carry the genetic information which permits expression of a normal function; i.e., hemoglobin synthesis.

Whether the heme synthesized in these SFAT cells is due to hemoglobin synthesis awaits further clarification. However, our data

on the Friend ascites tumor cells are considered to substantiate these *in vitro* findings of Friend *et al.* The analysis of hemoglobin synthesis in pure tumor cell culture should provide the evidence and such studies are in progress in our laboratory.

Summary. Significant heme synthesis was observed in 2 Friend ascites tumor cell lines, when radioiron incorporation was measured and compared to Ehrlich ascites tumor cells and SN-36 ascites lymphoma cells.

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Effect of Lysosomal Labilizers and Pro-Inflammatory Substances on Connective Tissue Repair as Measured by Tensile Strength (32733)

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Many types of substances have been shown to influence wound healing. These include anti-inflammatory compounds, histamine depletors, nutritional additives, thyroxin, DL-penicillamine, zinc sulfate, deuterium oxide, cartilage powder, etc. (1–11). Few of these substances accelerate wound healing; most inhibit. Prudden *et al.* (12,13) have shown that the local application of bovine cartilage powder moderately increases wound tensile strength (WTS) on days 7 and 11 after wounding. This effect appears to be relatively specific, as other substances which promote growth of granulation tissue (e.g., talcum powder) do not accelerate healing and carrageenin actually reduces WTS (14). The increased strength of cartilage-treated wounds appears to be effected by some mechanism other than the laying down of increased numbers of collagen fibers (15) or by the sulfhydryl content of the cartilage (16).

The wound healing process is conveniently divided into (a) substrate phase (1–5 days); (b) collagen phase (5–15 days) and (c) maturation of scar phase (15 days) (17). The substrate phase is characterized by an acute inflammatory process whereas the collagen phase is associated with an increased tensile strength. The increased WTS is dependent upon both the amount and quality of collagen laid down (18, 19).

Glucocorticoids have been reported to inhibit both the inflammatory process and wound healing (20). Since a lysosomal mechanism may be involved in both of these processes, it was of interest to evaluate the influence on wound healing of substances which affect biological membranes. It was also of interest to determine if substances which augment the inflammatory reaction would likewise accelerate wound healing.

Materials and Methods. Male Wistar rats weighing 170 ± 10 gm were used in these studies. On day 1, the rats were anesthetized