Nucleoside Deaminase Activity in Viral Leukemia

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INTRODUCTION

Inoculation of mice with Friend leukemia virus induces a disease characterized by fulminant proliferation of reticulum cells and erythroblasts in the spleen (4, 10, 11, 22). It is generally believed that the reticulum cells represent neoplastic cells and have the potential for autonomous growth (2, 3, 6, 11, 13). The nature of the erythroblastic response, however, and its relationship to the proliferating reticulum cells remain uncertain. The erythroblasts have been considered by some investigators to be neoplastic cells (7, 8, 10, 21). Alternatively, the erythroblastic activity could represent a separate virus-induced hyperplastic process that is distinct from the neoplastic manifestations of the disease (1, 11, 13).

Previous investigations in this laboratory have demonstrated a marked increase in the activity of nucleoside deaminase in the spleens of mice inoculated with Friend virus (23). High levels of this enzyme were also present in rapidly proliferating erythroid tissue of uninfected mice (18). In this study, nucleoside deaminase activity was measured in transplantable tumors and tissue culture lines derived from Friend virus-induced leukemia (6, 7). The results offer further data compatible with the erythroid nature of the neoplastic cell in this murine leukemia.

SUMMARY

Nucleoside deaminase activity was determined following inoculation of mice with several leukemia viruses. The erythroblastic disorders induced by Friend virus and Rauscher virus were associated with markedly elevated levels of spleen nucleoside deaminase. High enzyme activity was also found in transplantable solid tumors and tissue culture lines derived from Friend virus-induced leukemia. The lymphocytic leukemia resulting from Moloney virus infection did not lead to alterations in the level of nucleoside deaminase. It is suggested that the appearance of nucleoside deaminase activity during Friend virus and Rauscher virus leukemogenesis is a reflection of the erythroid nature of these disorders.

MATERIALS AND METHODS

Cytidine-2-\(^{14}\)C with a specific activity of 23 mCi/mmole was obtained from Schwarz BioResearch, Inc., Orangeburg, N. Y. Unlabeled nucleosides were purchased from Calbiochem, Los Angeles, Calif. Mice were obtained from Blue Spruce Farms, Altamont, N. Y.

Friend virus inoculation of female Swiss Webster mice weighing 20 to 25 g was performed by i.p. injection of a cell-free homogenate of leukemic spleen, as previously described (24). Under these conditions, a 10-fold increase in spleen weight was observed 5 days after virus inoculation. The spleen was enlarged to approximately 20 times normal on the 10th day postinoculation. The virus stock was originally obtained from Dr. C. Friend and has been maintained in this laboratory for several years by serial passage in female DBA/2 mice.

Rauscher virus (Lot No. R5-S200) and Moloney virus (Lot No. M8-S200) were generously provided by the National Cancer Institute, Bethesda, Md., in sealed ampuls containing frozen, cell-free spleen extracts. Female BALB/c mice weighing 20 g were inoculated i.p. with 0.1 ml of the Rauscher virus preparation. Subsequent passage of Rauscher virus in BALB/c mice was performed by the same method that was used for Friend virus passage (24). Moloney virus infection was produced by the i.p. injection of 0.15 ml of the virus preparation into 4-day-old BALB/c mice.

Erythropoiesis was stimulated in uninfected mice by phenylhydrazine-induced hemolysis. Female Swiss Webster mice weighing 25 g received an i.p. injection of 3 mg of phenylhydrazine, which was prepared immediately before use by dissolution of phenylhydrazine HCl in 0.15 M NaCl and adjustment of the pH to 7.0.

Friend virus tumor variants were made available through the kindness of Dr. C. Friend, Mount Sinai School of Medicine, New York, N. Y. SQ-4B and SQ-1A are transplantable s.c. tumors that were originally derived from s.c. implantation of tissue fragments from DBA/2 mice in advanced stages of virus-induced leukemia (6). These tumors had been in serial passage for 58 and 187 generations, respectively. Tissue culture lines C-1A, 300, and 502 were established from these solid Friend tumors (7) and had been transferred in vitro over 350 times. Dr. Friend also supplied the 58-11A s.c. carcinoma, derived from a hydrocarbon-induced mammary carcinoma, and the 5825 ascites lymphoma. The EL4 ascites lymphoma was obtained through the courtesy of Dr. L. J. Old, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Tissues (with the exception of the tissue culture cells) were...
homogenized with a Teflon Potter-Elvehjem homogenizer in 0.01 M Tris (pH 8.0), 2.0 ml/g of tissue. The homogenate was centrifuged at 11,000 X g for 20 min at 0 to 5°C, and the supernatant fluid was used for the determination of enzyme activity. Extracts of tissue culture cells were prepared by freezing and thawing of the harvested cells in 0.01 M Tris (pH 8.0).

Nucleoside deaminase was assayed by measurement of the amount of labeled uridine formed by the deamination of 14C-labeled cytidine. The assay mixture contained 20 μmoles of Tris-HCl buffer, pH 8.0; 0.05 μCi of cytidine-2-14C (23 mCi/mmole); and the enzyme preparation in a total volume of 40 μl. This was incubated at 37°C for 10 to 60 min, and the reaction was terminated by the addition of 20 μl of a mixture of unlabeled cytidine, uridine, and uracil (each 5 mM) in 3 N HCl. Samples to which the acid and reference compounds were added prior to the enzyme served as blanks. The precipitate was removed by centrifugation, and aliquots of the supernatant fluid were spotted on Whatman No. 1 paper and subjected to ascending chromatography for 16 hr. The solvent system consisted of 1-butanol:water:formic acid (77:13:10). The positions of marker compounds were identified under LW, 40 μl. This was incubated at 37°C for 10 to 60 min, and the reaction was terminated by the addition of 20 μl of a mixture of unlabeled cytidine, uridine, and uracil (each 5 mM) in 3 N HCl. Samples to which the acid and reference compounds were added prior to the enzyme served as blanks. The precipitate was removed by centrifugation, and aliquots of the supernatant fluid were spotted on Whatman No. 1 paper and subjected to ascending chromatography for 16 hr. The solvent system consisted of 1-butanol:water:formic acid (77:13:10). The positions of marker compounds were identified under LW.

RESULTS

Nucleoside deaminase activity was determined in the spleen following various stimuli that lead to the development of splenomegaly (Table 1). As previously reported (23), inoculation with Friend virus produced a massively enlarged spleen in which the specific activity of nucleoside deaminase had increased to 400 times the normal value. Rauscher virus infection, which produces an erythroblastic disorder that is indistinguishable from the Friend virus-induced disease (4, 22), also resulted in marked splenomegaly and an elevation of spleen enzyme activity. The increase in nucleoside deaminase activity following Rauscher virus inoculation is less than that observed after Friend virus inoculation. This difference may be attributable to the longer latent period of Rauscher virus disease. Moloney virus induces a widely disseminated lymphocytic leukemia of thymic origin (15). There was no alteration in the level of nucleoside deaminase in the mildly enlarged spleen or in the greatly enlarged thymus (3.8 milliunits/g), which are found in this disorder. The stimulation of erythropoiesis in normal mice by the administration of phenylhydrazine produced an 80-fold increase in spleen enzyme activity, confirming earlier observations (18).

Table 2 shows the levels of nucleoside deaminase in various neoplastic tissues. Two solid s.c. tumors derived from Friend virus leukemic tissues (SQ-4B, which had been serially transplanted 58 times and still synthesized infectious Friend virus, and SQ-1A, which was in its 187th transplant generation and had little leukemogenic activity) possessed high nucleoside deaminase activity. Three tissue culture lines established from Friend virus tumors (C-1A, 300, and 502) had moderate levels of nucleoside deaminase. The other tumors listed in Table 2 are not related to Friend virus disease. The ascites lymphomas EL4 and 5825 did not contain appreciable levels of nucleoside deaminase. Moderate enzyme activity was found in 58-11A, a transplantable s.c. carcinoma.

DISCUSSION

The erythroblastosis in Friend leukemia appears to be independent of the normal physiological control mechanism, which is mediated through the action of erythropoietin (12, 14, 19). The question of whether or not the erythroblasts are neoplastic has been a subject of considerable discussion (1, 4,
The repeated observation that transplantable solid tumors (reticulum cell sarcomas) established from Friend leukemic tissue are devoid of erythropoietic activity (2, 3, 6, 7) has suggested that the tumorigenic cells are not erythroid. However, Friend et al. (7), Patuleia and Friend (16), and Rossi and Friend (17) have demonstrated that tissue culture lines derived from these solid reticulum cell tumors have, to a limited extent, the capacity to differentiate along the erythroid series and appear to synthesize hemoglobin. In addition, Ikawa and Sugano (8) reported that transplantable Friend ascites tumor cells resemble proerythroblasts morphologically, and Sassa et al. (20) have shown that appreciable heme synthesis occurs in these cells. These data strongly support the concept that the neoplastic reticulum cells in Friend leukemia are actually undifferentiated erythroid precursors. The maturation of these cells into erythroblasts could account for the altered erythropoiesis observed during Friend virus-induced leukemogenesis.

Erythropoietic stimulation in uninfected mice results in the appearance of high levels of nucleoside deaminase in the spleen and blood (18). This enzyme activity is located in the erythrocytes that are produced during the period of accelerated erythropoiesis. These findings suggest that the marked elevation of spleen nucleoside deaminase activity that occurs following inoculation with Friend virus or Rauscher virus is a consequence of the intense erythroblastic response induced by these agents. The present observation that Friend virus-induced transplantable solid tumors and tissue culture lines contain considerable nucleoside deaminase activity is in keeping with the concept that the undifferentiated cells of these neoplastic tissues are erythroid in nature. However, the finding of a high enzyme level in the 58-11A carcinoma indicates that this response is not limited to erythroid neoplasms.

Ellem (5) screened a number of tissue culture lines and found detectable levels of nucleoside deaminase only in transformed cell cultures (e.g., an SV40 transformed culture contained nucleoside deaminase activity, whereas the diploid strain from which it was derived lacked this activity). This would suggest that the appearance of nucleoside deaminase during Friend virus-induced leukemogenesis might be the result of in vivo viral transformation. From the data presented here, however, another interpretation can be offered. The failure to find elevated enzyme activity in Moloney virus-induced lymphocytic leukemia appears to indicate that viral transformation is not always associated with high nucleoside deaminase levels. The increase in enzyme activity following erythropoietic stimulation in uninfected animals demonstrates that viral transformation is not the sole mechanism that could account for the alteration in enzyme levels. It is, therefore, possible that the elevated enzyme levels in Friend virus leukemia are a reflection of the erythropoietic response rather than of viral transformation per se.

REFERENCES


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