In Vitro and Preliminary in Vivo Studies of Compounds which Induce the Differentiation of Friend Leukemia Cells

H. D. Preisler, M. D.

Roswell Park Memorial Institute
Department of Medicine A
Buffalo, New York 14263

The progeny of normal hematopoietic stem cells ultimately become mature functional cells through a process involving both cell replication and differentiation. The proliferative aspect is limited and in fact as full maturation is approached the cells lose their proliferative potential. Built into the process of differentiation is an inherent limitation of the life span of the maturing cells. In essence, acute myelogenous leukemia (AML) results from a disruption of the normal maturation process. Leukemic stem cells proliferate without normal constraints and their progeny for the most part remain at the stem cell level. This latter phenomenon results in the cells retaining their proliferative potential and endows the cells with a life span which is longer than that of normal cells. The resulting increasing mass of leukemic cells, through an as yet undefined process, interferes with the proliferation and maturation of normal myeloid elements ultimately causing the pancytopenia which is directly responsible for the morbidity and mortality which accompanies AML.

Current approaches to the therapy of AML are directed towards the destruction of the leukemic cells by the administration of cytotoxic chemotherapy. Unfortunately the chemotherapeutic agents are also toxic for normal hematopoietic stem cells and one of the major side effects of this form of therapy is pancytopenia – with attendant morbidity and mortality.

A variety of studies have demonstrated that malignant cells possess the potential to differentiate and that differentiation is frequently associated with a decrease in or a loss of malignant potential (1–4). These observations suggest that it may be possible to treat cancer (including AML) by inducing the differentiation of the malignant cells. Such an approach, even if only moderately effective, might result in the production of mature granulocytes which could alleviate the granulocytopenia associated with the disease.

Friend Leukemia as a Model System

The Friend leukemia is a viral induced murine leukemia (5). The disease is characterized by the proliferation of blast cells with the spleen being the major site of leukemic cell proliferation. Some of the progeny of leukemic cells prolif-

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rating in the spleen undergo erythroid differentiation while the same leukemic cells
growing subcutaneously produce tumors which are devoid of differentiating eryth­
roid cells (6). These tumors have been classified as “reticulum cell sarcomas” (7).

Several investigators have established long-term suspension culture cell lines
from the leukemic mice (8-10). These leukemic cells grow in suspension culture as
morphologically undifferentiated blast cells. A small proportion of the tissue cul­
ture cells (usually \(< 1\%\)) spontaneously differentiate along the erythroid pathway.
It was found that the addition of either dimethylsulfoxide (DMSO) (11) or
dimethylformamide (DMF) (12) to the culture media of these cells induced a
substantial proportion of the leukemic cells to differentiate along the erythroid
pathway. These observations led to the conclusion that the Friend leukemia cells
growing in suspension culture represented a class of committed erythroid precursor
cells whose normal maturation had been prevented by their neoplastic trans­
formation.

Recent studies in our laboratory have made the question of the nature (multi­
potential stem cell vs. erythroid progenitor) of the Friend cells growing in culture
more complex than had previously been appreciated. We have found that when
these tissue culture cells (line 745A) are inoculated subcutaneously into mice,
the majority of tumor cells growing at the site of inoculation contain chloroacetate
esterase (CAE) and some of the cells are peroxidase positive as well (13). Both
enzymes are felt to be characteristic of granulocytic cells and have not been re­
ported to be present in cells of the erythroid series nor in reticulum cell sarcomas.
Some of the tumor cells appear morphologically to be undergoing abortive
granulocytic maturation. The tissue culture cells themselves are all chloroacetate
esterase positive (14). It should be noted that Friend virus infection of mice is
associated with a significant increase in granulocyte colony forming units (15). It is
not known if this increase is due to neoplastic transformation of granulocytic
progenitor cells or if it is merely reactive proliferation of normal progenitor cells.

Thus the question as to the nature of the Friend leukemia cell is more compli­
cated than had hitherto been realized. Are they erythroid progenitors whose
maturation has been arrested by the leukemic state and whose genomic regulation
has been so distorted by malignant change that it programs for the synthesis of
enzymes which are normally not present in erythroid cells? Or are they granulo­
cytic (CAE positive) stem cells which in the in vitro environment have a greater
potential for erythroid rather than granuloid differentiation? One interesting
aspect of this problem is the fact that while DMSO-induced erythroid differen­
tiation in vitro appears to be associated with a decrease in the degree of CAE posi­
tivity of the cells, the majority of cells remain CAE positive and in fact we have
found cells which are simultaneously strongly positive for both CAE and heme
(benzidine stain). At present we do not know if the CAE present in cells which are
synthesizing heme is being actively synthesized or if CAE synthesis ceases with the
onset of erythroid differentiation and we are detecting residual enzyme. In any
event, it is clear that the cells which are induced to differentiate along the eryth­
roid pathway are the cells which had previously synthesized an enzyme believed
to be characteristic of granulocytic differentiation. Hence if CAE positivity de­
notes granulocytic differentiation then either the cell can simultaneously dif­
ferentiate along two different pathways or alternatively the cell must dediffer­