Characterization of Myelomonocytic Leukemia Cells Induced by in vitro Infection of Bone Marrow with FLV

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A. Introduction

The development of systems in which pluripotent haemopoietic stem cells and committed progenitors proliferate and differentiate in vitro has allowed the analysis of factors involved in the regulation of normal haemopoiesis (Bradley and Metcalf, 1966; Pluznik and Sachs, 1966; Axelrad et al., 1974; Stephenson et al., 1971; Dexter and Testa, 1976) and of events associated with leukemia, both in experimental systems (reviewed by Metcalf, 1977) and in patients (e.g. Moore, 1974).

A recent report (Dexter et al., 1977) shows that in vitro infection of mouse bone marrow cells with murine leukemia viruses affects the proliferation and differentiation of the stem cells, and in particular, that infection with Friend leukemia virus complex (FLV) induces extensive proliferation and differentiation of pluripotent stem cells and committed granulocytic progenitor cells, which maintain normal characteristics for several weeks. The present work reports the occurrence of leukemic cells of myelomonocytic type 2 weeks after in vitro infection of mouse bone marrow cells with FLV.

B. Materials and Methods

I. Initial Bone Marrow Culture

This technique has been described fully elsewhere (Dexter and Testa, 1976; Dexter et al., 1977). Donor mice were 8 week old ♀ BDF_1. Briefly, the content of a mouse femur was flushed into a culture bottle containing 10 ml of Fischers medium plus 25% horse serum and antibiotics. Replicate cultures were kept at 33 °C in an atmosphere of 5% CO_2 in air for a period of 3 weeks to allow the development of an adherent layer which is essential for the maintenace of haemopoiesis (Dexter et al., 1977), and were fed weekly by replacing one half of the growth medium with fresh medium. After that time, a fresh inoculum of 10^7 syngeneic bone marrow cells was added to each culture, followed by infection with FLV within 2 hours (NB-tropic pool, F-B strain) as described by Dexter et al. (1977). The cultures were maintained by weekly feeding as described above.

II. Isolation of the Leukemia Cell Line

Cells collected 14 days after the infection with FLV were plated in 0,8% methylcellulose in alpha medium plus 30% foetal calf serum enriched with bovine serum albumin, transferrin and sodium selenite (Gilbert and Iscove, 1976) as described previously (Testa and Dexter, 1977). After 10 days of incubation at 37 °C, these cultures contained atypical colonies which were aspirated, pooled and subcultured as a single cell suspension in similar growth medium, minus methylcellulose, in culture bottles gassed with 5% CO_2 in air and also maintained at 37 °C. The cells were subcultured when saturation growth was reached. A summary of the isolation procedure for this cell line, identified as 427E-MC, is shown in Fig. 1.

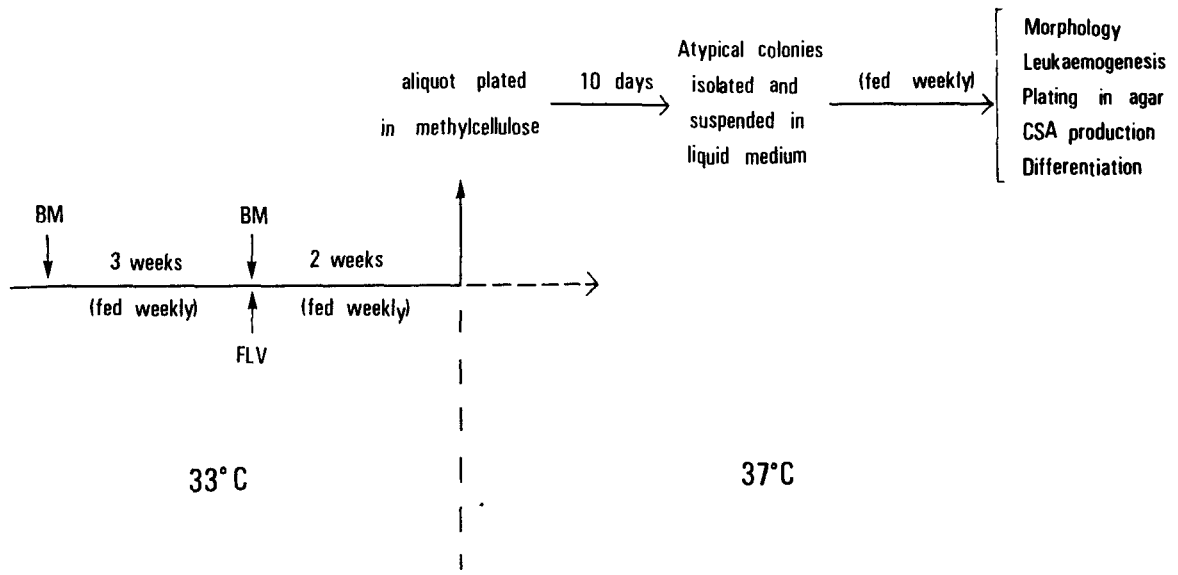

Fig. 1. Isolation of 427 E-MC cells

III. Cloning Studies

The cells to be assayed were plated in 0,3% agar in Fischers' medium plus 25% horse serum, with or without mouse heart conditioned medium as an exogenous source of colony stimulating activity (CSA, which is essential for the growth of granulocytic colonies derived from normal progenitor cells). Cultures were incubated in a humidified atmosphere of 5% CO_2 in air and colonies of more than 50 cells were scored after 7 days.

IV. Induction of Leukemia by Cultured Cells

Cells harvested from the suspension cultures (B.II.) were injected into syngeneic neonatal or adult mice, at doses between 10^6–5×10^6 cells per mouse. The mice were killed within a week of the observation of the growth of a local tumour at the site of subcutaneous inoculation, or, in the case of i. v. injection, when they appeared ill. Leukemia was confirmed by histological examination of the haemopoietic tissue.

C. Results

I. Characteristics in Suspension Culture

These are summarized in Table 1. The 427E-MC cells grow in suspension in the absence of an adherent layer (see B.I.) with a doubling time of 12–16 hours to a density of $1–2 \times 10^6$ cells per ml, and have a high plating efficiency in agar, even in the absence of exogenous CSA. They produce CSA which stimulates the growth of granulocytic colonies from normal bone marrow cells. Morphologically, 20–40% are classified as blasts, a similar proportion as promyelocytes and the rest as myelocytes and metamyelocytes, with some monocytic characteristics.

Only the lymphatic leukemia helper virus (LLV) of the FLV complex replicates in this culture.

Table 1. Characteristic of 427 E-MC cell line

Maximum number of cells/ml	$1–2 \times 10^6$
Morphology	Blasts, promyelocytes, myelocytes
Differentiation to segmented granulocytes	Not observed
Virus production:	
LLV (lymphatic leukemia helper virus)	Yes
SFFV (spleen focus-forming virus)	Not observed
CSA production	Yes
Plating efficiency in agar	10–30%
Induction of leukemia	Yes

II. Characteristics in Agar

These are summarized in Table 2. Colonies derived from 427E-MC cells are spherical, compact, and contain up to 5×10^3 cells after one week of growth. Both the morphology of the cells (similar to that found in suspension cultures) and the plating efficiency are similar in the presence or absence of exogenous CSA. The colony cells have high replating efficiency in agar and induce leukemia when injected into mice.

Table 2. Characteristics of in vitro colonies derived from 427 E-MC cells

Appearance of colonies	Compact
Morphology of colony cells	Blasts, promyelocytes, myelocytes
Differentiation to segmented granulocytes	Not observed
Dependance on exogenous CSA	No
Replating efficiency	10–15%
Induction of leukemia	Yes

III. Leukemia Inducing Ability

Injection of 427E-MC cells into neonatal and adult mice results in the induction of leukemia which reaches an advanced state at the time shown in Table 3.

Table 3. Leukemia-inducing ability of 427 E-MC cells

Mice	*No. of leukemic mice* No. inoculated	Inoculation	No. of cells	Time of observation (days)
Neonatal	8/8	s.c. or i.p.	10^{-6}–2×10^{6}	19–41
Adult	3/4	s.c. or i.v.	5×10^{6}	35–96

D. Discussion

Stimulation of granulopoiesis showing normal characteristics with regard to growth and differentiation has been reported after injection of FLV into mice (Golde et al., 1976) and for several weeks after in vitro infection of bone marrow cultures (Dexter et al., 1977). In the latter report, myeloid leukemic transformation was observed in one instance 12 weeks after the infection, although in contrast with the present work, normal differentiation and CSA dependance were observed when the cells were cloned in agar.

Our results confirm that cells other than erythroid precursors may be the target of FLV, and indicate that a long latency period is not an absolute requirement for malignant transformation to lake place. It is of interest that in spite of the capacity of the bone marrow culture system (B.I.) to support the growth of early erythroid precursor cells (Testa and Dexter, 1977), no erythropoietin-independent erythropoiesis was observed at the time at which the 427E-MC line was isolated.

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