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Regulator-Dependent Haemopoiesis and Its Possible Relevance to Leukemogenesis

T. M. Dexter

A. Introduction

Haemopoiesis is regulated by a variety of factors determining proliferation, differentiation, amplification and maturation of stem cells and their committed progeny, the myeloid and lymphoid precursor cells. Investigation of putative regulatory molecules has been facilitated by the development of clonogenic in vitro systems whereby restricted progenitor cells are induced to undergo clonal expansion and maturation in soft gel media, resulting in discrete colonies of mature cells. Using these systems, most committed precursor cells can be grown in culture (Reviewed in Metcalf 1977). Furthermore, haemopoietic stem cells can now be maintained in vitro for many months, continuously generating myeloid and lymphoid restricted cells (Dexter et al. 1977a; Dexter et al. 1978). In all aspects so far studied the haemopoietic cells produced in such long-term culture are apparently normal and possess characteristics in common with their counterparts present in freshly isolated bone marrow (Dexter et al. to be published a). Using these cultures we have heen investigating the regulation of stem cell proliferation and differentiation and the effects of a variety of RNA C-type leukaemia viruses on in vitro haemopoiesis (Dexter et al. 1977b; Teich and Dexter 1979; Teich et al. 1979; Dexter and Teich 1979; Testa et al. 1980).

B. Isolation of Normal and Leukaemic Cell Lines and Their Response to Haemopoietic Regulators

Several cell lines have been isolated from long-term cultures infected with Friend leukaemia virus (FLV):

1. In one experiment, cells isolated from long-term cultures 14 days after FV infection showed GM-CSF independent colony growth in soft agar. When individual colonies were isolated, it was found that the cells grew antonomously in suspension and that injection of the cells in vivo produced a rapidly progressing myelomonocytic leukaemia (Testa et al. 1980). This cell line, designated 427E, was aneuploid with a mean of 78 chromosomes and a constitutive producer of GM-CSF. When plated in soft agar, colony formation occurred in the absence of added stimulatory molecules. However, if the cells were plated in the presence of excess exogenous GM-CSF, it was found that although the initial colony forming efficiency was not altered, the self-renewal ability of the colony forming cells (measured by re-plating ability) was dramatically reduced. These leukaemia cells, therefore, show at least some biological response when cultured in the presence of excess GM-CSF – a proposed regulator of granulopiesis. Similar effects of GM-CSF have been observed in other myelomonocytic leukaemia cell lines (Metcalf et al. 1969; Ichikawa 1969; Fibach et al. 1972).

Infection of marrow culture with FBJ osteosascoma virus has similarly led to the rapid emergence of a malignant myelomonocytic clone of cells (426-C) with characteristics similar to those described above.

2. Long-term cultures treated with Abelson leukaemia virus readily undergo malignant transformation to produce poorly differentiated B-cell leukaemia cell lines (Teich et al. 1979; Teich and Dexter 1978). The cells grow autonomously in suspension and in soft agar and are inducible for intracytoplasmic IgM production by various reagents.

3. The infection of marrow cultures with FLV can also lead to the production of apparently normal, non-leukaemic cell lines which possess characteristics of either stem cells or committed granulocyte progenitor cells (Dexter et al. 1979; Dexter and Teich 1979; Dexter et al. 19780b). These cell lines are characteristically isolated only from long-term cultures which have been maintained for several months. 416B cells were isolated from a culture more than 5 months after infection with FLV and were established as a continuous cell line, growing in suspension independently of added stimulatory molecules. Upon isolation the cells were initially bipotential (Dexter et al. 1979) when injected into irradiated mice and formed spleen colonies containing granulocytes and megakayocytes, although they grew in suspension (in vitro) as an undifferentiated cell population. The cells had a normal diploid karyotype and were non-leukaemic. Colony formation in soft agar was only seen in the presence of exogenous GM-CSF (Dexter et al. 1978a). Eventually, karyotype instability was seen, the cells became restricted to erythroid development when injected in vivo and colony formation in soft agar occurred in the absence of added GM-CSF. However, the cells were still non-leukaemic.

Another cell line, 458C, was isolated more than 5 months after FLV infection (Dexter et al. 1980b), also grew autonomously in suspension culture, maintained a diploid karyotype and was non-leukaemic. Colony formation in soft agar initially occurred only in the presence of added GM-CSF, and the colonies produced consisted of neutrophil granulocytes. Presently, this cell line also has aquired the ability to undergo clonal expansion in soft agar in the absence of GM-CSF, and a karyotype investigation is in progress.

4. It has recently been reported by Greenberger et al. (1979) that infection of susceptible long-term bone marrow cultures with FLV is followed consistently by the generation of promyelocytic leukaemia cell lines. For their continued growth such cells must be sub-cultured in medium conditioned by the growth of WEHI-3CM. Since one component of WEHI-3CM is GM-CSF, it was assumed that this moiety acted as the growth promoter for the proliferation of these cells (Greenberger et al. 1979). According to this report, WEHI-3CM dependent cell lines could not be obtained from control (non-infected) cultures. However in recent work we have shown that cells from uninfected long-term marrow cultures will consistently generate cell lines in the presence of either WEHI-3CM or pokeweed-mitogen spleen cell conditioned medium (Dexter et al. 1980c). Such cell lines are non-leukaemic, maintain a diploid karyotype and from colonies containing . granulocytes when plated in soft agar. Growth in suspension culture or in soft agar is absolutely dependent upon the continued presence of WEHI-3CM or SCM. Other GM-CSF containing conditioned media or highly purified GM-CSF preparation did not support the growth of these cells (Dexter et al. 1980c). This suggests that GM-CSF is not the regulatory molecule involved in the maintenance of proliferation. We have further suggested that such cells represent a population of committed granulocyte progenitor cells which are capable of extensive self renewal and which are responding to a hitherto unrecognised regulator. Thus far cell lines have been produced from marrow cultures of strain DBA/2, C57BL/6, BDF_1 and Swiss mice. No evidence of viral replication can be found in these cell lines, which are designated Factor-Dependent Continuous cell lines, Paterson Labs (FDC-P).

A summary of these cell lines is given in Table 1. FDC-P lines produced from untreated cultures demonstrate factor dependency for growth in suspension and in soft agar. Initial isolates of 416B and 458C showed independent growth in suspension but dependent growth in soft agar. These cell lines are characterised by being diploid and non-leukaemic and apparently undergo normal differentiation. Karyotype changes occurring in 416B are associated with a restriction in development potential and acquisition of factor-independent growth in soft agar.

Other cell lines produced from virus infected long-term cultures show a restricted developmental potential, are aneuploid, leukaemic and are independent of the addition of exogenous factors for growth in suspension or in soft agar. The Ml cells represent a line derived from cultures treated with the carcinogen methylnitrosourea (MNU). These cells are also factor independent, aneuploid and leukaemic with restricted differentiation ability.

Table 1. Production of factor dependent and independent cell lines from long-term marrow cultures[a]

Cell Line	Treatment	Factor dependence		Karyotype	Differentiation	Leukaemogenic
		Suspension	Agar			
FDC-P (Several)	–	+	+	Diploid	Mature grans	No
458-C	FLV	–	+	Diploid	Mature grans	No
416-B	FLV	–[b]	+	Diploid	E+G+Meg	No
416-B	FLV	–[b]	–	Aneuploid (41 chromosomes)	Erythroid	No
AB-1	Abelson	–	–	?	IgM production	Yes
426-C	FBJ virus	–[c]	–[c]	Aneuploid	Metamyelocytes	Yes
427-E	FLV	–[c]	–[c]	Aneuploid	Metamyelocytes	Yes
M1	MNU	–	–	Aneuploid	TdT production	Yes

[a] MNU = methylnitrosourea
[b] No response to Fraction IV (CFU inhibitor) (Lord et al. 1976)
[c] Constitutive producers of GM-CSF

C. Do the Cell Lines Represent Different Stages in Leukaemic Transformation?

Treatment with a leukaemogen may have diverse effects one or more of which is important in leukaemogenesis. In one case there may be a direct transformation of a haempoietic "target cell", leading as a result to regulator-independent growth or to an altered response to the regulator. Such a leukaemogen may be expected to produce a rapid disease such as that seen with Friend Leukaemia virus infection, where the growth of erythroid progenitor cells becomes independent of the requirement for erythropoietin, Abelson disease may also fall into this category.

Alternatively, tratment with leukaemogens may result in alterations in the level of the various regulatory molecules, such as the factors specifically controlling stem cell proliferation (Lord et al. 1977) or GM-CSF or BPA production, or in the levels of factor required for the sustained proliferation of FDC-P cells. In this case there would be population changes in the factor dependent but normal cells – presumably leading to hyperplasia or aplasia of one or more cell lineages. The next stage in leukaemogenesis may be represented by a mutation event leading to the generation of cells which have acquired proliferative autonomy (i.e. grow independently of growth factors) but which none the less still respond to differentiation stimuli. Cells in this category would include 416B and 458C cell lines, which may be regarded as "pre-leukaemic" cells. The final stage would be the generation of clones which do not, or only partially, respond to differentiation signals – such as the ABl, 426C, 427E and Ml cells. These are characteristically aneuploid cells. The generation of such cell lines having a multi-step process, would take a relatively long time as is seen after treatment with most viruses, X-rays and chemicals.

Using the long-term cultures, the hypothesis presented can be tested in some detail. Not only can the levels of regulators be monitored in the cultures, but the cell lines produced (particularly FDC-P cells) represent a model for transformation studies including a variety of leukaemogens and analysis of the subsequent response of the cells to the various regulators. Such studies are now in progress.

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