Infection of Mouse Bone Marrow Cells with Abelson Murine Leukemia Virus and Establishment of Producer Cell Lines

Teich, N., Boss, M., Dexter, T. M.

Imperial Cancer Research Fund Laboratories. Lincoln's Inn Fields. London. WC2A 3PX. Great Britain
and Paterson Laboratories. Wilmslow Road. Manchester. M20 9BX. Great Britain

We have recently described a technique for the establishment of long-term cultures of mouse bone marrow cells (Dexter et al., 1977). In that communication we reported the successful infection of the cells in vitro with the Friend leukemia virus complex and the subsequent development of producer cultures of the Friend spleen focus-forming virus. Here we describe the infection of the mouse bone marrow cultures with Abelson murine leukemia virus (MuLV-A) (Abelson and Rabstein, 1970); establishment of lines of primitive "blast" cells which chronically produce MuLV-A in vitro has been accomplished and a proportion of these cells contain intracytoplasmic immunoglobulin M (IgM). Thus these transformed cells can be classified as early B lymphocytes.

MuLV-A causes a thymus-independent rapidly progressing lymphosarcoma in adult mice (Abelson and Rabstein, 1970). Viral infection in vitro and in vivo leads to the production of tumours which can be classified as null (non-B, non-T) cell tumours. If mice are pre-treated with the mineral oil pristane, there appears to be a high incidence of immunoglobulin-producing (B cell) plasmacytomas (Potter et al., 1973; Sklar et al., 1974; Rosenberg et al., 1975). Previous reports of in vitro transformation of splenocytes (Sklar et al., 1974), foetal liver (Rosenberg et al., 1975) or bone marrow (Rosenberg and Baltimore, 1976), documented a requirement for a reducing agent such as 2-mercaptoethanol for the establishment of virus-producing cell lines; in our system, this requirement is obviated.

Bone marrow cultures from 6–8 week old female BALB/c mice were established from femoral marrow aspirates suspended in Fischer's medium with 20% horse serum. After 3 weeks in culture, with weekly withdrawal of and replacement of one-half of the medium, a second aspirate of syngeneic cells was added to each culture. Approximately 2 hours later, 7×10^4 plaque forming units (pfu) of the Moloney MuLV-M helper virus and 2×10^3 focus forming units (ffu) of the MuLV-A were added to each experimental culture. All cultures were then incubated at 37° with weekly demi-depopulation and addition of fresh medium. The cells and media removed each week were assayed by several techniques to ascertain the virus status and the nature of the cell population. Two assays were used to determine virus production: 1. the XC syncytial plaque assay (Rowe et al., 1970) for quantitation of the MuLV-M helper virus population and 2. the focus assay (Hartley and Rowe, 1966) on NIH/3T3 cells for measurement of the transforming capacity of the defective

Abelson virus component (Scher and Siegler, 1975). After the first week in culture, the titre rose considerably (to $> 10^5$ pfu/ml) and thereafter remained constant. As expected, the MuLV-M was consistently found to be in excess (3–400 fold) of the MuLV-A.

The numbers and types of cells maintained in the suspension fluids were analysed by several criteria (Table 1). The cultures showed an initial decrease in total cell number after one week post-infection. In uninfected cultures, the cell number remained relatively constant throughout the course of the experiment. On the other hand, the MuLV-A infected cultures showed a dramatic increase in total cell number at 5 weeks. At about the same time, the cell population in the infected cultures showed a predominant shift toward undifferentiated blast cells and a decline in the proportion of granulocytes (of all stages) and mononuclear macrophages. This is in marked contrast to the control cells in which the predominant population was composed of mononuclear cells by week 7.

Table 1. Differential analysis of bone marrow cell cultures

Weeks in culture	Culture	Morphology (%)		
		Blast	Granulocyte	Mononuclear
3	Control	36	34	30
	Infected	48	12	37
5	Control	41	5	54
	Infected	96	0	4
7	Control	0	0	100
	Infected	99	0	1
9	Control	0	0	100
	Infected	100	0	0

Two assays for analysis of stem cell differentiation were also utilized. The agar colony assay for granulocytic cell precursors (CFU-C) (Bradley and Metcalf, 1966) was performed according to the technique of Dexter and Testa (1976). By 4 weeks, neither control nor infected cells formed CFU-C. However, by the time that the infected cells demonstrated feeder-independent autonomy (see below) at week 15, several thousand cells per culture were capable of growing as agar colonies. These differed from normal CFU-C in forming tightly clustered colonies composed of undifferentiated blasts with no evidence of granulopoiesis. In addition, there was little or no decrease in the number of "CFU-C" in the absence of granulocyte colony stimulating factor (CSF). Furthermore, such CSF-independent colony forming cells could be found in infected cell cultures by week 6 if mercaptoethanol (5×10^{-5} M) was present in the agar medium. These data indicate that the cells are not true CFU-C and represent blast cells which have been "transformed" by viral infection, thus acquiring the ability to replicate in an agar medium.

Pluripotent haematopoietic stem cells (CFU-S) (Till and McCulloch, 1969) were lost early during the culture period.

At the earliest period checked (one week post-infection), the MuLV-A infected cells were capable of inducing leukemia within 2–4 weeks in adult syngeneic BALB/c mice and in heterogeneic BDF_1 mice as well. The leukemias were characterised by massive enlargement of the spleen and of most lymph nodes (cervical, inguinal, brachial, axillary and mesenteric). The leukemic cells were identified morphologically as large, undifferentiated blast cells. Of particular interest was the finding that these cells lacked both θ and surface Ig antigens by immunofluorescent staining techniques.

At 15 weeks, the cultured bone marrow cells appeared to be autonomous and were capable of forming agar colonies in the absence of 2-mercaptoethanol. Thereafter the cells exhibited an increased ability to grow in suspension without feeder cells. The cell lines thus established were uniform in morphology and appeared to be large vacuolated, undifferentiated blast cells.

Although the cells appear to be of the lymphoid series, they do not show the typical identifying markers of T (θ antigen) or B (surface Ig) cells and are perhaps best classified as null cells. As cells infected with Abelson are thought to be pre-B lymphocytes, we attempted to characterise the nature of the established cell line. It was found that 2–5% of the cells contained intracytoplasmic IgM as identified by immunofluorescence techniques. We attempted to induce lymphoblastoid differentiation by a variety of compounds. As shown in Table 2, the proportion of cells with intracytoplasmic IgM was greatly stimulated by treatment with dimethyl sulphoxide (DMSO) or lipopolysaccharide (LPS). No treatment has yet been found to stimulate the production of surface Ig.

Table 2. Stimulation of intracytoplasmic IgM production

Treatment	% Cells staining for	
	Intracytoplasmic IgM	Surface Ig
None	1–4	0
Lipopolysaccharide		
5 μg/ml	23	0
20 μg/ml	39	0
Dimethyl sulphoxide, 1%	23	0
Dextran sulphate, 20 μg/ml	4	0
Tuberculin PPD, 10 μg/ml	2	0

We thus conclude that Abelson virus infection of bone marrow cells has led to the transformation of cells in the early B-lymphocyte lineage. In the uncloned population, there are two cell populations: One is IgM-negative and another which constitutively produces IgM. The experiments using DMSO and LPS suggest that such treatment can either cause preferential proliferation of the IgM-positive cells or induce some of the IgM-negative cells to synthesize IgM. Attempts are now being made to select clones from each of

these cell types. However, this provides the first proof that Abelson virus can transform cells of the B-lymphocyte series in vitro and suggests that such cells may be the target for leukemogenesis in vivo.

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