Table 1 Generation of lymphoblastoid colonies by cocultivation of murine splenic B cells with virus-producing BALB/3T3 cells

Splenic B-cell target population	Virus (helper)	Positive wells	Estimated efficiency
TNP-binding $(C3H \times DBA/2)F_1$	KiSV (ampho-MuLV)	43/250	0.86×10^{-5}
TNP-binding $(C3H \times DBA/2)F_1$	KiSV (Moloney-MuLV)	5/50	0.5×10^{-5}
TNP-binding $(C3H \times DBA/2)F_1$	Ampho-MuLV	0/50	0
TNP-binding $(C3H \times DBA/2)F_1$	None	0/200	0

TNP-binding spleen cells were purified from $(C3H \times DBA/2)F_1$ ($C3D2F_1$) mice by binding to and elution from TNP-gelatin plates. These cells were mixed with γ-irradiated BALB/3T3 cells (10,000 rad) producing Kirsten sarcoma virus pseudotyped with either amphotropic or ecotropic (Moloney) murine leukaemia virus in RPMI 1640 supplemented with 20% fetal bovine serum, 2.0×10^{-5} M 2-mercaptoethanol and 5 $\mu g\ ml^{-1}$ polybrene (Sigma). B cells (2×10^{-4}) were plated with 5×10^{-4} BALB/3T3 cells in 200 μl flat bottom microculture wells. Cultures were fed with fresh medium every 2-4 days. Wells were microscopically assayed for the presence of growing cells. The number of microwells with growing lymphoblastoid clusters appearing within 1-6 weeks (positive wells) and the fraction of plated B cells induced to grow out into colonies, assuming a clonal origin of these colonies (estimated efficiency), are given. Control cultures were plated with either no BALB/3T3 cells, or BALB/3T3 cells producing amphotropic MuLV alone. The data are pooled from three separate experiments.

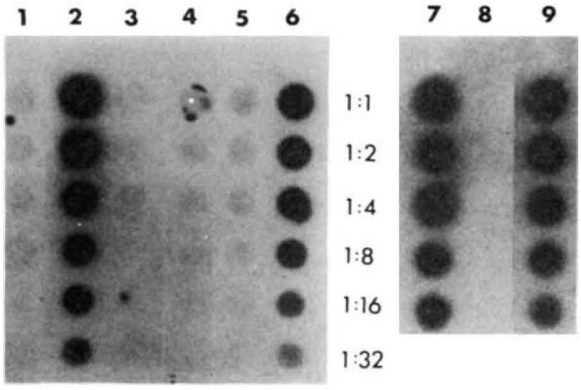

Fig. 3 RNA dot blot analysis of B-cell lines for Ki-*ras* specific RNA. Ki-*ras* homologous cytoplasmic RNA was analysed in: lanes 1 and 8, Abelson-MuLV-immortalized B-cell lines 1D12 and 12E11 (respectively); lanes 2 and 7, KiSV-immortalized B-cell lines 2F9 and 6H3 (respectively); lane 3, normal murine splenic B cells; lane 4, the murine B-cell lymphoma A20.2J; lane 5, Abelson-MuLV (amphotropic MuLV)-producing BALB/3T3 cells; lanes 6 and 9, KiSV (amphotropic-MuLV)-producing BALB/3T3 cells. Cytoplasmic RNA was prepared from 4×10^6 cells of each type by NP-40 lysis and denatured in 5% formaldehyde at 55 °C for 15 min. Samples were serially 2-fold diluted and blotted onto nitrocellulose with a vacuum manifold (Schleicher & Schuell). Blots were baked at 80 °C for 1 h under vacuum and hybridized to a ^{32}P-labelled Ki-*ras* specific probe (the 600 base-pair *Sst*II/*Hin*dII fragment from KiSV) (ref. 7) in 10% dextran, 5 × SSC, at 42 °C, for 18 h. The final washes of the blots were in 0.2 × SSC at 50 °C. Autoradiography was carried out at −80 °C for 18 h using intensifying screens[7,16].

a poly(A) template and trichloroacetic acid precipitable radioactivity is measured[17]. Supernatant from cultures of 2F9 (10 ml) yielded 8,110 counts per minute (c.p.m.) in this assay, compared to 677 c.p.m. from an ininfected BALB/3T3 culture supernatant, and 128,761 c.p.m. from the culture supernatant of a Mo-MuLV-producing BALB/3T3 line. Thus 2F9 produces low levels of viral particles.

We have shown here that introduction of the v-*ras* oncogene into mature B lymphocytes by retroviral infection can immortalize them. This is the first demonstration of *ras*-induced immortalization of mature lymphocytes *in vitro* and it is likely that the use of an amphotropic helper virus was important to our success. KiSV pseudotyped with an ecotropic helper (Moloney-MuLV) generated a few lymphoblastoid colonies none of which could be established as permanent lines (Table 1). The amphotropic envelope allows infection of cells that do not express membrane receptors for ecotropic viral envelope glycoproteins[9,10]. The same approach has been used to immortalize differentiated human umbilical vein endothelial cells[7], and to infect mature helper T-cell lines *in vitro*[11]. Mature B cells may have few or no functional receptors for ecotropic retroviruses, but do have amphotropic retroviral receptors.

The cell lines described here retain their mature phenotype, and preliminary functional studies indicate that they can be further mitotically stimulated by reagents which stimulate normal B cells, particularly the helper T-cell-derived lymphokine, B-cell stimulatory factor 1 (data not shown). These cells do not secrete immunoglobulin constitutively; we are attempting to induce increases in immunoglobulin gene transcription, biosynthesis and/or secretion with activating stimuli, including lymphokines. The technique of infecting purified, functionally well-defined lymphocyte populations with acute transforming retroviruses will be useful for the development of model systems to study lymphocyte physiology as well as the role of oncogene expression in lymphocyte growth deregulation.

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Erratum

Energy conversion catalysis using semiconducting transition metal cluster compounds

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In this letter, the metal atoms in the Fig. 1 legend were incorrectly defined. They should read: ●, Mo; ○, Ru; ⊜ Se.