MURINE HEMATOPOIETIC CELLS WITH PRE-B OR PRE-B/MYELOID CHARACTERISTICS ARE GENERATED BY IN VITRO TRANSFORMATION WITH RETROVIRUSES CONTAINING *fes*, *ras*, *abl*, and *src* ONCOGENES

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The development of in vitro hematopoietic assay systems has permitted the identification of specific cell subpopulations that are susceptible to transformation induced by retroviruses containing different oncogenes. In particular, studies using a colony-forming assay that is dependent upon the presence of 2-ME have revealed that pre-B cells were transformed when cultures were infected with viruses containing *abl* or *fes*, oncogenes that code for tyrosine-specific protein kinases (1, 2). By comparison, cultures infected with viruses containing *bas*, H-*ras*, or K-*ras*, oncogenes that code for proteins with guanidine diphosphate (GDP)[1]-binding activity (3), produced transformed hematopoietic cell lines that lacked characteristics of cells in the B, T, or erythroid cell lineages (4). Although one continuous line was shown to have myeloid characteristics (5), most of the cell lines transformed by *ras*-containing retroviruses expressed high levels of terminal deoxynucleotidyl transferase (TdT) activity, suggesting that they were derived from cells at an early stage within the lymphoid differentiation pathway. Transformed lines with characteristics similar to those of the *ras*-induced neoplasms were also obtained from hematopoietic cell cultures infected with a murine recombinant retrovirus containing the *src* oncogene that, like *abl* and *fes*, codes for a tyrosine-specific kinase (6).

To more clearly define the characteristics of the cell populations transformed by the *bas*, H-*ras*, K-*ras*, and *src* oncogenes, continuous clonal cell lines produced from infected bone marrow and fetal liver cultures were examined for expression of lineage-specific cell-surface antigens, expression of cytoplasmic μ chain (cμ), and the organization of Ig heavy and light chain genes. The results show that cell lines with characteristics of pro-B and pre-B cells were obtained from cells infected with viruses containing *bas*, H-*ras*, K-*ras*, and *src*, as well as *abl* and *fes*. In addition, two cell lines transformed by *ras*-containing viruses were found to

[1] *Abbreviations used in this paper:* ABL, Abelson MuLV; BMSV, Balb murine sarcoma virus; cμ, cytoplasmic μ chain; FeSV, Snyder Theilen feline sarcoma virus; FMF, flow microfluorometry; FU, fluorescence units; GDP, guanidine diphosphate; HMSV, Harvey murine sarcoma virus; KMSV, Kirsten sarcoma virus; MRSV, murine Rous sarcoma virus; MuLV, murine leukemia virus; NSE, nonspecific esterase; sIg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase.

TABLE I
Antibodies Used in This Study

Antigen	Clone	Nominal specificity	Reference
Thy-1.2	NEI-005*	T cells	9
Ly-1	NEI-017*	T cells, B cell subpopulation	9
Ly-2	53-6.7‡	Some T cells	9
Lyb-2.1	10.1-D2	Pre-B, B cells	10
Ly-5	30-F11	Pan leukocyte	9
Ly-5 (B220)	RA3-6B2	Pre-B, B cells	11
Lyb-8.2	CY-34-1.1	Pre-B, B cells	12
Ly-17	2.4G2	Pre-B, B cells, myeloid cells	13, 14
Ly-17.1	Alloantisera	Pre-B, B cells myeloid cells	14, 15
Ia^k	10.3-6	B cells, macrophages	16
ThB	53-9.2	Pre-B, B cells, thymocytes	9
MAC-1	M1/70	Monocytes, macrophages	17
MAC-2	M3/38	Thioglycollate-elicited PEM§	18
MAC-3	M3/84	PEM, Thioglycollate-elicited PEM	19
"6C3"	6C3	B Lineage neoplasms	20

* Purchased from New England Nuclear (Boston, MA).
‡ Purchased from Becton Dickinson and Co. (Mountain View, CA).
§ PEM, Peritoneal exudate macrophage.

coexpress antigens usually restricted to the B cell or myeloid pathways of differentiation. Detailed analyses of these two lines suggest that the initial transforming event giving rise to these lines occurred in a precursor common to the B cell and myeloid lineages.

Materials and Methods

Cell Lines. The cell lines examined were obtained from cultures of fetal liver cells or bone marrow cells from 2–3-wk-old NFS/N mice that were infected as described (4) with amphotropic murine leukemia virus (MuLV) or Moloney MuLV pseudotypes of replication-defective retroviruses containing the Rous sarcoma virus (MRSV) *src* oncogene (6), the Harvey sarcoma virus (HMSV) H-*ras* oncogene (4), the BALB sarcoma virus (BMSV) *bas* oncogene (4), the Kirsten sarcoma virus (KMSV) K-*ras* oncogene, the Snyder-Theilen feline sarcoma virus (FeSV) *fes* oncogene (2), or the Abelson murine leukemia virus (ABL) *abl* oncogene. Individual colonies appearing in the soft agar cultures were harvested, cultured in suspension in microtiter wells, and grown to bulk cultures, usually with the use of adherent feeder layers. Cell lines expanded in the presence of feeder layers were recloned in soft agar before further characterization. All the cell lines were shown to contain and express the oncogenes used in their induction (2, 4–6).

Flow Microfluorometry (FMF). FMF analyses were performed on a FACS II (Becton Dickinson and Co., Sunnyvale, CA) using established techniques (7) in which viable cells were electronically gated by narrow forward-angle light scatter and uptake of propidium iodide (8). Cells were reacted with the panel of monoclonal and alloantibodies shown in Table I and with FITC-labeled goat anti-mouse κ antibodies (Southern Biotechnology Assoc., Birmingham, AL) to detect surface Ig (sIg). Binding of antibodies to Lyb-8.2, Ia, and Ly-17.1 was detected using a FITC-labeled goat anti-mouse IgG (Southern Biotechnology Assoc.). Binding of antibodies to Mac-2 and Mac-3 was detected using a FITC-labeled rabbit anti-rat IgG (Zymed Laboratories, San Francisco, CA). All other reagents used were directly labeled with FITC.

Two-color FMF was performed using FITC-labeled anti-Mac-1 and biotin-labeled anti-Lyb-2.1 or anti-Ly-5(B220) (prepared in our laboratory) and Texas Red–labeled avidin (Cappel Laboratories, Malvern, PA). To prevent binding of antibodies via their Fc

portions, 10^6 cells were incubated with 10 μg of unlabeled anti-Fc γ receptor (clone 2.4G2, reference 13) before incubation with labeled antibodies.

Cell Sorting. Cells from the HMSV-infected line, HAFTL3, and the BMSV-infected line, BAMC1, were sorted into Mac-1^+ (Mac-1^+-sorted) or Mac-1^- (Mac-1^--sorted) populations using the FACS II cell sorter and/or were single cell-cloned by sorting Mac-1^+ (Mac-1^+-cloned) or Mac-1^- (Mac-1^--cloned) cells into individual wells of 96-well round bottomed microtiter plates using an Epics V (Coulter Electronics, Hialeah, FL) flow cytometry system.

Measurement of Ig μ and κ Chains. The amount of mouse Ig μ and κ chains present in cells was determined by competition RIA, as described previously (2).

Molecular Assays. High molecular weight DNA was prepared, digested with restriction enzymes, separated on 0.8% agarose gels, and transferred to nitrocellulose using established techniques. Southern blots were hybridized with ^{32}P-labeled DNA probes at 65°C in a buffer containing 3× SSC, 10% dextran sulphate, 10× Denhardt's solution, 0.1% SDS, 5 mM EDTA, and 50 μg/ml salmon sperm DNA. Blots were washed successively in 3× SSC at 37°C for 20 min, 1× SSC at 65°C for 10 min and 0.2× SSC at 65°C for 30 min, and analyzed by autoradiography. Two probes were used, J_H, a 1.96-kb Bam HI/Eco RI fragment of the BALB/c germline J_H region (21) and J_κ, a 2.8-kb Hind III/Hind III fragment of pEC_κ (22).

Assays for Macrophage Function. The assay for nonspecific phagocytosis was carried out in chamber slides (4008; Miles Laboratories Inc., Naperville, IL). Cells were inoculated with 100 μl of 1-μm latex beads (15711; Polysciences, Inc., Warrington, PA) and incubated at 37°C for 3 h. Slides were washed in PBS, stained with Wright's-Giemsa and cells were scored for bead ingestion at × 100 magnification. Lysozyme was assayed by the lysoplate assay using egg white lysozyme as a standard (5). Levels of lysozyme were expressed in μg per 10^6 cells per 24 h. The presence of nonspecific esterase was determined by cytochemical staining using a Sigma Chemical Co. (St. Louis, MO) research kit (5).

Results

Phenotypic Analyses. Cell lines were analyzed by FMF for expression of cell surface antigens characteristic of cells in the B, T, and myeloid lineages (Table I). The results showed that all of the transformed cell lines, irrespective of the oncogene-containing retrovirus used in their induction, had characteristics of early cells in the B lymphocyte lineage (Table II). The phenotypes exhibited by the different lines correspond to several stages of maturation in a recently proposed scheme of B cell development (23, 24; Fig. 1). In this scheme, it was postulated that Ly-17^+,Lyb-2^+ cells, provisionally termed pro-B cells, are the progenitors of Ly-5(B220)$^+$ large pre-B cells defined in other studies (11). Large pre-B cells differentiate into ThB$^+$ small pre-B cells, which become mature B cells with the acquisition of sIg (11). Expression of Ia antigens by B cells occurs either simultaneously with or subsequent to sIg expression (25, 26).

In the present study, two cell lines (BASC6 and HRC3) were Ly-17^+, Lyb-2^+, but Ly-5(B220)$^-$, ThB$^-$, Ia$^-$, and sIg$^-$, a phenotype that corresponds to the pro-B cell stage of differentiation (Table II and Fig. 1). 14 of the lines were Lyb-2^+ and uniformly Ly-5(B220)$^+$, or contained subpopulations of Ly-5(B220)$^+$ cells and were ThB$^-$, sIg$^-$, and Ia$^-$, and therefore corresponded to the large pre-B cell stage of differentiation. Two lines Y1C1 and HAFTL3, contained subpopulations of ThB$^+$ cells and Ia$^+$ cells. Finally, two lines (HAFTL1 and FE2NCI) were Ly-17^+, Lyb-2^+, Ly-5(B220)$^+$, and Ia$^{+/-}$, sIg$^-$, and ThB$^-$ a phenotype with no documented counterpart in studies of normal or malignant cells within the B lymphocyte lineage (25, 26). This finding raises the possibility that Ia may sometimes be expressed before ThB or sIg during normal B cell maturation.

TABLE II

Analysis of MuLV-infected Bone Marrow and Fetal Liver Cells

Virus‡	Cell line	Cell source	Cell surface antigens*												cμ‖	Ig gene organization¶	
			Thy-1	sIg§	Ia	ThB	Ly-5 (B220)	Lyb-8	Lyb-2	Ly-5	Ly-17	Ly-1	MAC-1	6C3		JH	JK
ABL	Y1C1	BM	−	−	−/+	−/+	−	+	++	+	++	−/+	−	+	−	G/R	G/G
	AAC6	BM	−	−	−	−	−/+	−	++	+	++	−/+	−	+	+	R/R	G/G
BMSV	BASC6	BM	−	−	−	−	−	+	++	+	++	+++	−	+	−	R/R	G/G
	BAC14	BM	−	−	−	−	+/−	+	++	+	++	+	−	+	−	R/R	G/G
	BAMC1	BM	−	−	−	−	(+)	+	++	+	+++	+++	−/+	−/+	−	R/R	G/G
FESV	FE2NC1	BM	−	−	+/−	−	+++	+	+++	++	+++	+	−	+	+	G/R	G/G
	FEMCL	BM	−/+	−	−	−	+	−	+	+	+	++	−	+	−	R/R**	G/G
	FEUC	BM	−	−	−	−	(+)	−	++	+	++	++	−	+	+	R/R**	G/R
HMSV	HAFTL3	FL	−	−	−/+	−/+	+++	+	++	+++	+++	+	−/+	+	−	R/R	G/G
	HS1C5	BM	−	−	−	−	+/−	+	++	+	++	+	−	+	−	R/R	G/G
	HRC3	BM	−	−	−	−	−	+	++	+	++	+	−	+	−	R/R	G/G
	HAFTL1	FL	−	−	−/+	−	+	+	++/−	+	+	+	−	−	−	G/R	G/G
	HAFTL2	FL	−	−	−	−	+	+	++	+	+	+++	−	−	−	R/R	G/G
KMSV	KFFTL1	BM	−	−	−	−	+	(+)	++	+	+	+++	−	−/+	−	R/R	G/G
MRSV	JP1	BM	−	−	−	−	+	−	++	+	++	−	−	+	−	R/R	G/G
	JP4-2	BM	−	−	−	−	+	++	+++	++	+++	+	−	+	−	R/R	G/G
	JP5	BM	−	−	−	−	(+)	+	+	+	++	−	−	+	−	R/R	R/−
	JP4-1	BM	+	−	−	−	+	++	++	+	++	−	−	+	−	R/R	G/G
	JP2	BM	−	−	−	−	+	+	++	+	+++	+	−	+	−	G/R	G/R
	JP7	FL	−	−	−	−	+	+	++	+	++	−	−	+	−	R/R	G/G

* FMF analysis in which observed fluorescence is related to staining intensity observed with normal spleen cells using the following scheme: −, nonreactive; +, reactive with a mean intensity more than 100 fluorescence units (FU) less than the mean intensity of normal cells; ++, reactive with a mean intensity equal to the mean intensity ± 100 FU of normal cells; and +++, reactive with a mean intensity more than 100 FU greater than the mean intensity of normal cells; (+), staining only slightly above unstained controls; +/−, heterogeneity of staining with >50% of the population reactive with the antibody; or −/+, <50% reactivity.

‡ Bone marrow or fetal liver cells were infected in vitro with ABL MuLV, BMSV, HMSV, or KMSV, FeSV or MRSV.

§ sIg detected with Goat anti-κ antibodies.

‖ Ig μ chain as determined by RIA.

¶ Rearrangement of Ig genes detected with ^{32}P-labeled probes specific for Ig heavy chain (J_H) and with light chain (J_K) genes. G, germline configuration; R, rearranged.

** Indicates hybridization of more than two rearranged heavy chain fragments with the J_H probe.

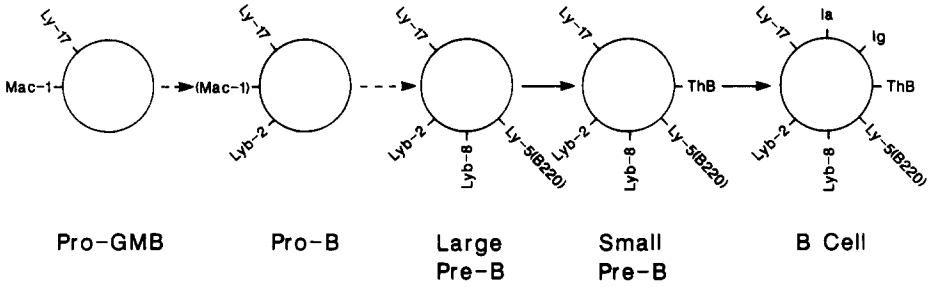

FIGURE 1. Scheme of B cell differentiation showing established (*solid arrows*) and proposed pathways (*broken arrows*).

The majority of the lines (16/20), including both of the pro-B cell lines, were Ly-1$^+$, a finding consistent with previous reports that expression of Ly-1 occurs very early during the development of the Ly-1$^+$ B cell lineage (27–30). In addition, all the cell lines but two expressed the antigen recognized by the mAb 6C3, recently shown to be expressed at low levels on some normal bone marrow cells (31) and at high levels in many MuLV-induced B and pre-B cell lymphomas (reference 20 and unpublished observations). The pattern of expression of Lyb-8.2 and Ly-5(B220) on these lines suggests that these antigens may be expressed simultaneously during B cell differentiation (see Fig. 1). The observation that all of the lines expressed Ly-17.1, recently shown to be a polymorphic determinant of the Fc γ receptor (14), is consistent with previous suggestions that this antigen may be expressed very early in B cell differentiation (23). It is noteworthy that two cell lines, FEMCL and JP4-1, expressed Thy-1, a cell surface antigen usually associated with the T cell lineage among murine hematopoietic cells, but that may also be expressed during myeloid differentiation (32, 33).

Two cell lines, HAFTL3 and BAMC1, were of particular interest. Cells from these lines were uniformly Lyb-2$^+$, Ly-5(B220)$^+$, and ThB$^-$, corresponding to the large pre-B cell stage of differentiation, yet both lines were Mac-1$^{-/+}$, i.e., contained subpopulations (10–15%) of Mac-1$^+$ cells (see Table II legend). Expression of Lyb-2 and Ly-5(B220) by BAMC1 was surprising, since this line was previously shown to be Mac-1$^+$ and to express other characteristics consistent with immature cells of the myeloid lineage (5).

Ig Gene Organization and Expression. High molecular weight DNA extracted from the cell lines was digested with Eco RI or Hind III and hybridized with the J_H or the J_κ probes, respectively. Results of hybridization with the J_H probe showed that all of the lines had undergone rearrangements of this locus on at least one of the two chromosomes bearing J_H genes (Table II and Fig. 2). Hybridization with the J_κ probe showed that only three cell lines exhibited rearrangements of κ light chain genes (Table II and Fig. 3). The results obtained with both probes yielded no evidence that any of the lines, in particular in HAFTL3 and BAMC1, were not monoclonal. Productive Ig gene rearrangements resulting in production of $c\mu$ molecules were observed only in three cell lines: AAC6, FE2NCI, and FEUC (Table II). No κ chain protein was detected in any of the lines, including those that had rearrangements at the κ gene locus.

Analysis of Mac-1$^+$ and Mac-1$^-$ Subpopulations of HAFTL3 and BAMC1 Obtained

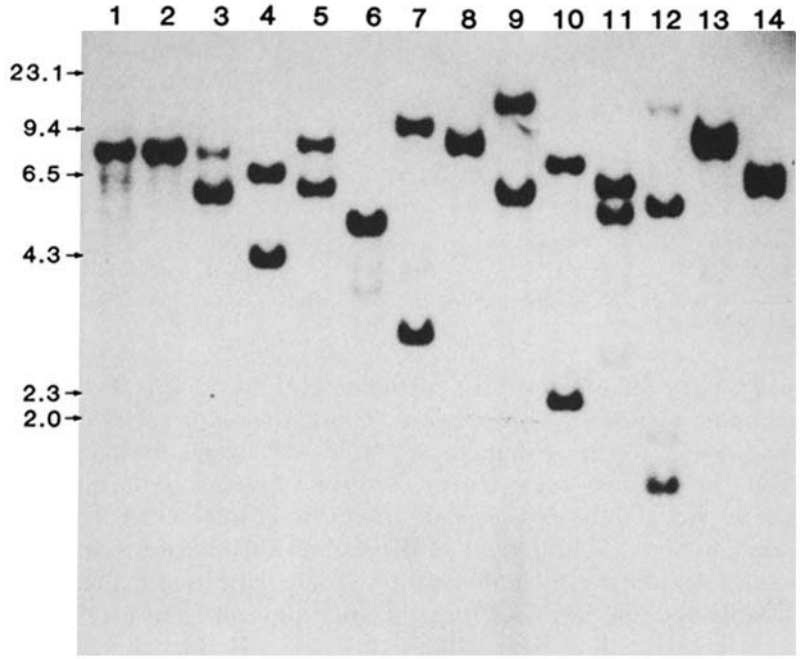

FIGURE 2. Southern blot of Eco RI–digested DNA from: NFS spleen (*1*), NFS liver (*2*), HAFTL1 (*3*), BAMC1 (*4*), JP2 (*5*), AAC6 (*6*), HAFTL2 (*7*), Y1C1 (*8*), JP7 (*9*), HS1C5 (*10*), HRC3 (*11*), FEUC (*12*), FE2NCI (*13*), and KFFTL1 (*14*) hybridized with the J_H probe.

by Cell Sorting. The observations that HAFTL3 and BAMC1 were uniformly positive for expression of the B lineage antigens Lyb-2 and Ly-5(B220) but Mac-$1^{-/+}$ (Table II) indicated that subpopulations of both cell lines simultaneously expressed both myeloid and B lineage cell-surface antigens. This was confirmed by two-color FMF analyses of HAFTL3 and BAMC1 for expression of Mac-1 in relation to Lyb-2.1 or Ly-5(B220) (data not shown). To determine whether the subpopulations of Lyb-2^+, Ly-5(B220)$^+$, Mac-1^-, and Lyb-2^+, Ly-5(B220)$^+$, Mac-1^+ cells in HAFTL3 and BAMC1 could be propagated as phenotypically stable cell lines, HAFTL3 and BAMC1 were sorted by FMF into nonoverlapping Mac-1^+ and Mac-1^- populations. After expansion in liquid culture, both the Mac-1^+-sorted and Mac-1^--sorted HAFTL3 lines were Mac-$1^{-/+}$ when analyzed by FMF. To determine whether the observed heterogeneity was due to contamination between sorted populations, HAFTL3 Mac-1^+ and Mac-1^- populations were single cell-cloned by FMF. Analysis of clones for the expression of Mac-1 using fluorescence microscopy revealed that at 1 wk after sorting (~500 cells per well), Mac-1^--cloned cells were uniformly Mac-1^-, whereas at 2 wk, 5 out of 5 clones analyzed were Mac-$1^{-/+}$. FMF analysis of Mac-1^--cloned lines after expansion in culture showed that 29 of 31 clones were Mac-$1^{-/+}$ and 2 were uniformly Mac-1^+. In contrast, Mac-1^+-cloned HAFTL3 cells were uniformly Mac-1^+ when analyzed by fluorescence microscopy at 1 and 2 wk after sorting. 12 of 13 lines, expanded in culture and analyzed by FMF, were uniformly Mac-1^+ and one was Mac-$1^{+/-}$. These results showed that Mac-1^+ HAFTL3 cells were derived from Mac-1^- cells. Moreover, hybridization of the J_H probe with DNA prepared from

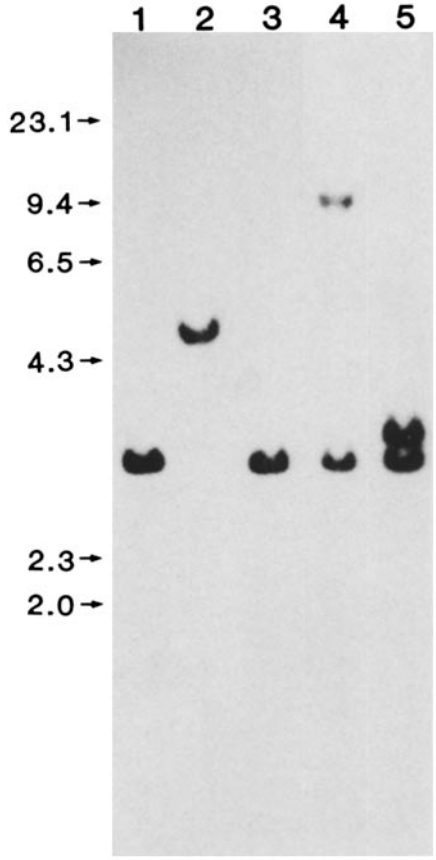

FIGURE 3. Southern blot of Hind III–digested DNA from: NFS liver (*1*), JP5 (*2*), BASC6C2 (*3*), JP2 (*4*), and FEUC (*5*), hybridized with the J_k probe.

the parental HAFTL3 line and the uniformly Mac-1^+ subclone HAFTL3-A, showed that both lines had identical J_H rearrangements (Fig. 4).

Two HAFTL3 Mac-1^+ subclones, HAFTL3-A and -3D10, were analyzed further for expression of Lyb-2, Ly-5(B220), and Ia; for the macrophage-lineage antigens Mac-2 and Mac-3; for production of lysozyme and nonspecific esterase (NSE); and for their ability to phagocytose latex beads (Table III). Both subclones were uniformly Ia^+, Lyb-2^+, and Ly-5(B220)$^+$; produced high levels of lysozyme and NSE; and were phagocytic. HAFTL3-A, in comparison with HAFTL3-3D10, produced higher levels of both enzymes, was more actively phagocytic, and also expressed Mac-2, an antigen normally found on thioglycollate-elicited peritoneal macrophages (18). Both lines were Mac-3^-. Consistent with these phenotypic and functional characteristics, HAFTL3-A had morphological features typical of a mature macrophage (Fig. 5*b*). In contrast, the parental HAFTL3 cells had a distinctive lymphoid morphology (Fig. 5*a*).

BAMC1 was originally described as a myeloid-lineage cell line (5), but attempts to obtain stable uniformly Mac-1^+ cell lines from BAMC1 by single cell-cloning were unsuccessful. FMF analysis of 24 subclones generated from both Mac-1^--

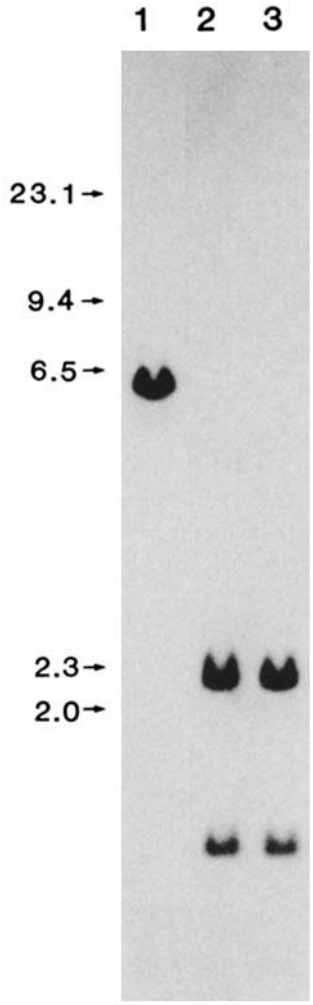

FIGURE 4. Southern blot of Eco RI–digested DNA from: C3H liver (*1*), HAFTL3-A (*2*), and HAFTL3 (*3*), probed with the J_H probe.

cloned and Mac-1^+-cloned BAMC1 showed that they were either Mac-1^- or Mac-$1^{+/-}$. However, one Mac-1^+-cloned line, BAMC1-4E10, which was Mac-$1^{+/-}$, had twofold higher levels of lysozyme production than the parental BAMC1 line, and could be induced to produce higher levels of lysozyme and become >80% Mac-1^+ after stimulation with the phorbol ester, PMA (data not shown).

Discussion

The results of this study show that in vitro infection of bone marrow or fetal liver cells with retroviruses containing the *bas*, H-*ras*, K-*ras*, *src*, *abl*, and *fes* oncogenes induces the transformation of early B lineage cells. Analyses of these cell lines for expression of cell-surface antigens and Ig gene organization showed that they had remarkably similar phenotypes, regardless of whether the viruses

TABLE III
Characterization of Mac-1$^+$ Sublines of HAFTL3

Line	Cell surface antigen*				Function		
	Ia	Mac-1	Mac-2	Mac-3	Lysozyme‡	NSE§	Phagocytosis‖
HAFTL3	−/+	−/+	−	−	<0.1	−	ND
HAFTL3-A	+	+	+	−	12.5	++	+++(97%)
HAFTL3-3D10	+	+	−	−	5.0	+	+(5%)

* Determined by FMF.
‡ $\mu g/10^6$ cells/24 h.
§ Relative amounts of reaction product as compared with a negative control.
‖ Average number of latex beads in cytoplasm of positive cells according to the following scheme: +++, >75 beads per cell; +, between 5 and 25 beads per cell. Numbers in parentheses indicate the percentages of cells with >5 beads per cell.

used for infection contained oncogenes coding for tyrosine-specific kinases or GDP-binding proteins (Table II). These data confirm reports of transformation of pre-B cells by *abl* and *fes* (1, 2, 34) and significantly extend previous phenotypic characterization of *ras*- and *src*-induced hematopoietic cell transformants (4–6). It was previously shown that infection of bone marrow or fetal liver cells with *ras*- or *src*-containing retroviruses induced the transformation of cells that were assumed to be lymphoid progenitor cells because they were sIg$^-$, cμ^-, but expressed high levels of TdT (4, 6). *Ras*-containing viruses also induced alterations in the growth and differentiation of myeloid cells, although only one continuous cell line, BAMC1, was isolated from these cultures (5). In the present study, extensive analyses of the *ras*- and *src*-transformants have shown that all had phenotypes characteristic of pre-B cells and that most corresponded to the large pre-B cell stage of differentiation, i.e., were Lyb-2$^+$, Ly-5(B220)$^+$, ThB$^-$, and sIg$^-$. However, two cell lines, HAFTL3 and BAMC1, contained subpopulations of Lyb-2$^+$, Ly-5(B220)$^+$, Mac-1$^+$ cells that differentiated or could be induced to differentiate into cells with mature macrophage characteristics after single-cell FMF cloning.

These results raise the question as to the nature of the initial cell transformed by these oncogene-containing viruses. The target cell may be a totipotent stem cell that, under the culture conditions employed, preferentially differentiates within the B cell lineage. Although most leukemia-derived cell lines show a restricted pattern of differentiation, a transformed cell line with the capacity to differentiate within the T, myeloid, and erythroid lineages has been established from a patient undergoing treatment with deoxycoformycin, an adenosine deaminase inhibitor (35). An alternative hypothesis is that a cell with a more restricted differentiation potential may be targeted in this system. In this regard, the characteristics of most of the cell lines examined are consistent with the transformation of an early B cell progenitor giving rise to these lines.

In the clinical literature, there are numerous examples of leukemias and lymphomas that, similar to HAFTL3 and BAMC1, coexpress markers usually restricted to distinct hematopoietic cell lineages. These observations have led to two divergent interpretations. McCulloch has argued that cells with these characteristics are decidedly abnormal and that genetic misprogramming during the production of leukemic cells results in the coexpression of markers usually

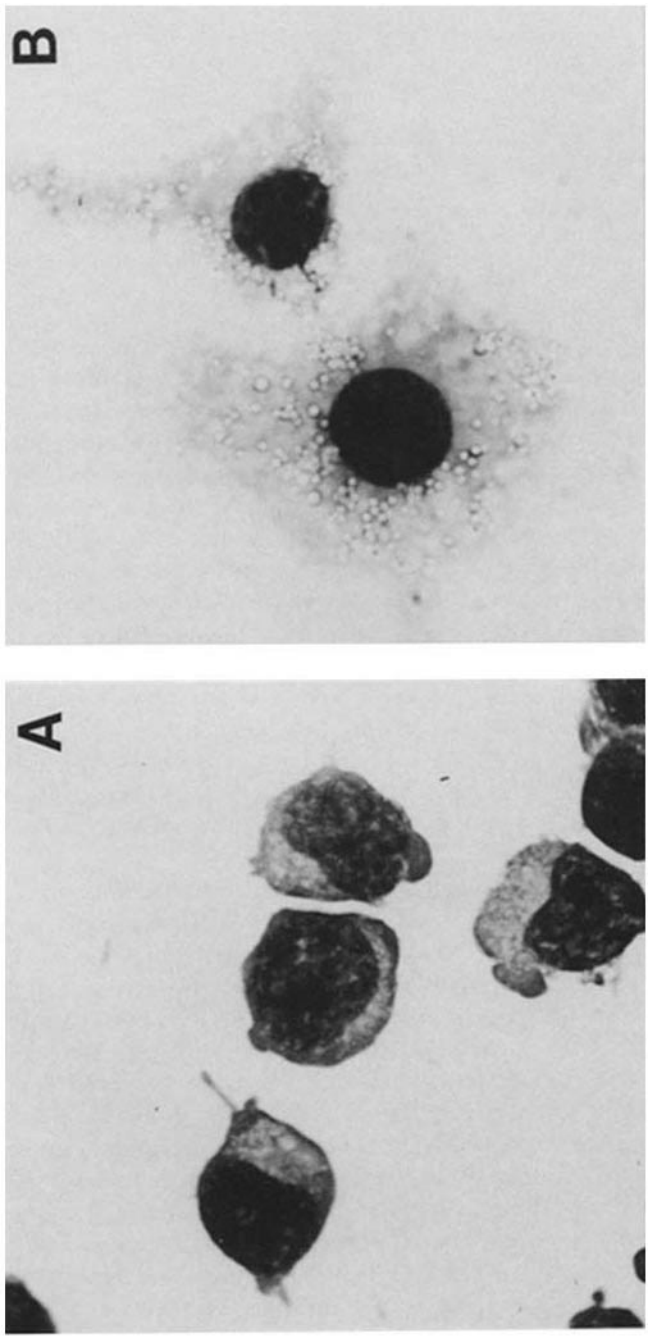

FIGURE 5. (*A*) Cytocentrifuge preparation of the HMSV-infected HAFTL3 line. (*B*) Mac-1$^+$ subclone HAFTL3-A after incubation in chamber slides with latex beads. Stained with Wright-Giemsa. × 1,000.

restricted to distinct hematopoietic cell lineages. He has coined the term "lineage infidelity" to describe this process (36). In contrast, Greaves et al. (37) suggest that these leukemic phenotypes reflect the persistent expression by progeny leukemic blasts of genes usually expressed only transiently by their bipotential or multipotential progenitors. They refer to this pattern of phenotypic expression in normal differentiation and leukemia as lineage promiscuity. Till (38) has raised similar arguments with respect to normal hematopoiesis.

Earlier studies of a large number of murine leukemias and lymphomas led us to propose models of normal hematopoietic differentiation (39) and more particularly of B cell differentiation (Fig. 1 and References 23, 24, 40). These models were based, in part, on the assumption that neoplasms with characteristics not represented among known normal cell phenotypes may provide important clues to lineage relationships. This view is similar to that supported by Greaves and coworkers (37), and leads to the suggestion that the results presented here and elsewhere support the hypothesis of a close developmental relationship between the B cell and myeloid lineages.

HAFTL3 and BAMC1 have characteristics thought to represent committment to the B cell lineage, including the expression of Lyb-2 and Ly-5(B220) and the rearrangement of Ig heavy chain genes. However, these lines have retained the capacity to differentiate within the macrophage lineage. Although this may not reflect a normal differentiation pathway, it may indicate that the transformation event occurred in a common B cell/myeloid progenitor cell. We have previously suggested that this cell type be termed a pro-GMB cell, to indicate its potential for producing cells in the granulocyte, macrophage, and B cell lineages, and predicted that it would have a Ly-17$^+$, Mac-1$^+$ phenotype (Fig. 1 and reference 23). Cells coexpressing Lyb-2 and Mac-1, and therefore corresponding to the putative pro-B cell (Fig. 1) make up a large percentage of spleen and bone marrow cells of newborn mice (24). In addition, neonatal liver cells expressing Ly-5(B220) and possessing peroxidase positive granules have been described (41). Studies are in progress to determine the potential of these cells to differentiate within the B or myeloid lineages.

Coexpression of B lineage and myeloid antigens, similar to that seen in HAFTL3 and BAMC1, has been reported for other cell lines that were induced with murine leukemia viruses or with chemical carcinogens (23, 24, 40, 42). Among these is an Abelson MuLV-induced pre-B cell line, ABLS 8.1, which was induced to become a macrophage line with the addition of the demethylating agent, 5-azacytidine (42). Similar reports were obtained when the phorbol ester, PMA, was added to a B lineage cell line established from a patient having a t(4;11) translocation (43). In both of these cases, the original tumor cell line had rearranged Ig genes. The differentiation potential of HAFTL3 was realized without the addition of inducing agents, whereas earlier passages of BAMC1 (5) and the Mac-1$^+$-cloned line, BAMC1-4E10, showed increased expression of Mac-1 and lysozyme in response to PMA stimulation.

Studies of human leukemias also provide support for the existence of a common B/myeloid progenitor cell. The most well documented is the occurrence, in 30% of patients, of a lymphoid blast crisis (of pre-B cell phenotype) in the acute phase of chronic myelogenous leukemia (44, 45). The Philadelphia chromosome t(9;22)

translocation (46, 47) and G6PD isoenzymes (47) have been used as markers for clonality of cells involved in the lymphoid blast crisis and in the chronic phase of myelogenous leukemia. In addition, there have been reports of patients with acute leukemia in which the leukemia was either composed of a mixture of both myeloid and lymphoid cells (48), or there was a conversion from acute lymphoblastic leukemia to acute myeloid leukemia (48), or the leukemia was composed of cells that coexpressed both B lineage and myeloid markers (49, 50). Other examples of the coexpression of B- and myeloid-associated antigens by human leukemic cells have recently been reviewed (37).

If our interpretation of these data is correct, it would be expected that cell lines that display clear, albeit early, markers of committment to either the B cell or myeloid pathways of differentiation might also exhibit potential to mature within either lineage. Evidence for this comes from recent studies of single-cell cloned progeny of HAFTL1 (Table II). Stimulation of some clones with LPS results in induction of multiple J_H rearrangements and the sequential expression of ThB and Ia, events associated with maturation within the B cell lineage (Fig. 1). By comparison, other clones develop characteristics of mature macrophages (Davidson, W. F., J. H. Pierce, S. Rudikoff, and H. C. Morse, III; manuscript in preparation).

In conclusion, these studies indicate that an unexpectedly wide range of *onc* genes can induce B lineage lymphomas in vitro. The availability of cell lines with both myeloid and B cell differentiation potentials provides a unique opportunity to explore the molecular and biochemical events that define irrevocable committment to these distinct hematopoietic lineages.

Summary

In vitro infection of bone marrow or fetal liver cells with retroviruses containing *fes*, *abl*, *ras*, or *src* oncogenes resulted in the transformation of early B lineage cells. All cell lines tested possessed rearrangements at the Ig heavy chain locus and some had rearrangements at the κ chain locus. The majority of the lines corresponded phenotypically to Lyb-2^+, Ly-5(B220)$^+$, ThB$^-$ large pre-B cells, although some were classified as pro-B cells because of their Lyb-2^+, Ly-17^+, Ly-5(B220)$^-$ phenotype. We identified two cell lines that contained subpopulations of cells that coexpressed the B lineage antigens Lyb-2 and Ly-5(B220) and the myeloid lineage antigen Mac-1. Single-cell FMF cloning of these subpopulations showed that Mac-1^+ cells were derived from Mac-1^- cells and that these Mac-1^+-cloned cells further differentiated into cells with phenotypic and functional characteristics of mature macrophages.

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