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Transformation of Murine Bone Marrow Cells with Combined v-*raf*–v-*myc* Oncogenes Yields Clonally Related Mature B Cells and Macrophages

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Murine bone marrow cells infected with replication-defective retroviruses containing v-*raf* alone or v-*myc* alone yielded transformed pre-B cell lines, while a retroviral construct containing both v-*raf* and v-*myc* oncogenes produced clonally related populations of mature B cells and mature macrophages. The genealogy of these transformants demonstrates that mature myeloid cells were derived from cells with apparent B-lineage commitment and functional immunoglobulin rearrangements. This system should facilitate studies of developmental relationships in hematopoietic differentiation and analysis of lineage determination.

We have previously reported that individual mice infected with retroviral construct J2 containing combined v-*raf* and v-*myc* oncogenes developed a spectrum of neoplasms which represented a summation of the diseases induced with either v-*raf* or v-*myc* alone, including lymphomas of T- and B-cell lineages, and erythroleukemia, as well as sarcomas and carcinomas (7, 16, 23). Further studies showed that bone marrow cells infected with J2 in the absence of 2-mercaptoethanol (2-ME) yielded macrophage lines (4, 5). These observations suggested that synergistic interactions between *raf* and *myc* could promote transformations of all hematopoietic lineages and raised the question as to whether the tumors were derived from a common multipotent progenitor or from a series of more restricted progenitors. To evaluate this possibility, we compared the cell types obtained following in vitro infection of murine bone marrow cells with v-*raf*, v-*myc*, or v-*raf*–v-*myc* oncogenes in a transformation assay system which permits growth of both B- and myeloid-lineage cells (14, 19, 20).

These results indicate that whereas *raf* or *myc* alone induced transformation of pre-B cells, combined *raf-myc* oncogenes yielded clonally related transformants with characteristics of pre-B cells, mature B cells, macrophages, and a group of phenotypically undifferentiated cells. The demonstration of functional immunoglobulin gene rearrangements shared by related B-cell and macrophage clones supports the suggestion of close relationships between these lineages (1, 3, 6, 10, 12, 15, 19, 25, 28) and raises the possibility that oncogenes may affect lineage determination.

MATERIALS AND METHODS

Animals and cells. All animals used were 4- to 6-week-old NFS/N mice obtained from the small animal facility of the National Institutes of Health, Bethesda, Md. Bone marrow cells were obtained by flushing femurs and tibias with Dulbecco modified Eagle medium supplemented with 10% fetal calf serum.

Retrovirus stocks. Replication-defective recombinant retroviruses containing intact, combined v-*raf*–v-*myc* oncogenes (J-2), v-*myc* oncogene sequences (J-3), and v-*raf* containing retrovirus (3611-murine sarcoma virus [MSV]) were used. All retroviral stocks were passaged in NIH 3T3 fibroblast cultures and rescued with a molecularly cloned variant of Moloney murine leukemia virus (MuLV). For collection, the producer cell lines were grown to confluency and fresh medium was added. Supernatants from these producing stocks were collected the next day.

In vitro transformation. In vitro transformation was done as previously described (7, 8, 11). Briefly, freshly isolated NFS/N bone marrow cells from 2-week-old NFS/N mice were mixed with supernatant from NIH 3T3 cell cultures containing known titers of J2 or 3611 retroviruses pseudotyped with Moloney helper virus. The cells were incubated at 37°C for 2 h in the presence of 5×10^{-5} M 2-ME and 4 μg of Polybrene per ml. Infected cells were pelleted, suspended at 10^6 in RPMI 1640 with 10% fetal calf serum containing 5×10^{-5} M 2-ME, and plated in soft agar.

Following selection of growing colonies 10 days later, the transformants were expanded on bone marrow adherent cell feeder layers, and proliferating bulk cultures were subsequently expanded in liquid culture and subjected to cloning in soft agar.

Flow microfluorometry. The cell lines obtained following in vitro transformation were analyzed by standard staining procedures. All fluorescence data were collected on a Becton Dickinson FACS II (Becton Dickinson and Co.) by using logarithmic amplification on viable cells as determined by forward light scatter and propidium iodide. Monoclonal antibodies used for phenotyping included: Lyb-2(10-D2), Ly-5(B220) (RA3-B2), Thy-1 (Dupont, NEN Research Products), Ly-1 (Becton Dickinson), PC-1, Ia^k(10.3-6), Mac-1(M1/70), ThB(53-9.2), Ly-17(2.4G2), 6C3, Mac-2(M3/38), immunoglobulin M (IgM) (Bet 2), and alloantisera specific for mouse κ chain (Southern Biotechnology).

DNA analysis. High-molecular-weight DNA was prepared

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TABLE 1. In vitro transformation of lymphoid and myeloid procursors with 16311-MSV, J2, and J3

Virus	Colonies (per 2 × 10^6 cells)[a]	
	+2-ME[b]	−2-ME
3611-MSV	8	0
J2	114	12
J3	4[c]	0
Moloney MuLV	0	0
Control (uninfected)	0	0

[a] 2 × 10^6 bone marrow cells from 2-week-old NFS/N mice infected with 5 × 10^5 focus-forming units of 3611-MSV, J2, J3 (20, 21), or Moloney MuLV helper virus alone were plated in semisolid agarose, and colonies were quantitated 10 days after infection (20). Results, except of J3, are representative of three assays in which bone marrow cells were used and one assay in which fetal liver cells were used. Only compact lymphoid colonies emerged in the presence of 2-ME, and diffuse myeloid colonies emerged in its absence.
[b] Semisolid culture containing 5 × 10^{-5} M 2-ME.
[c] Observation from one experiment only; no colonies in other assays.

from 3611-MSV-, J-2-, and J-3-derived transformants and the panel of J-2-derived subclones. Digestion of 10 μg of each DNA sample was done by using *Eco*RI, *Bam*HI, or *Hin*dIII, followed by electrophoresis on 0.8% agarose gels, and the samples were transferred onto nitrocellulose. Baked membranes were hybridized to nick-translated probes specific for IgJH (J11), κ (pECk), and v-*myc* sequences and were washed under stringent conditions before analysis by autoradiography.

RNA analysis. Poly(A)$^+$ RNA was prepared from all 3611-MSV-, J-2-, and J-3-derived transformants and was separated in 1% agarose–6% formaldehyde gels by established techniques. All probes utilized for analysis were purified inserts and included sequences specific for v-*myc*, c-*myc*, c-*raf*, c-*fos* (18), *myb* (27), μ heavy chain, and κ light chain (pECk).

RESULTS

Isolation of transformed lines. NFS/N bone marrow cells were infected in the presence of 2-ME and Polybrene with Moloney MuLV pseudotypes of 3611-MSV expressing v-*raf* alone, a retroviral construct (J-3) expressing an intact v-*myc* oncogene as well as a nonfunctional v-*raf* gene, and a recombinant retroviral construct (J-2) that expresses both v-*raf* and v-*myc* oncogenes. The infected cells were plated in soft agar in the presence of 2-ME, which permits outgrowth of lymphoid and myeloid lineages (14, 20), or in the absence of 2-ME, which has been reported to suffice for the outgrowth of myeloid cells (4). 3611-MSV stimulated moderate growth of lymphoid cells in cultures containing 2-ME but was ineffective in transforming cultures lacking 2-ME. Colony formation following infection of bone marrow cells with J-3 was generally very poor in cultures containing 2-ME (Table 1). In contrast, J-2 stimulated the production of numerous colonies in 2-ME-containing cultures. Moloney MuLV alone did not induce transformation under the conditions of this assay.

Proliferating colonies were selected and transferred onto bone marrow feeder layers. Vigorously growing cultures were expanded and subjected to cloning in soft agar. Soft agar colonies were selected and designated 3611 or J3 for transformants arising following 3611-MSV or J-3 infections, respectively. The J-2-induced transformants were designated J2C3, J2C5, and J2C9. Initial isolates appeared as immature lymphocytes, which grew to high density in suspension and did not require supplementation by exogenous growth factors. The 3611 and J3 cultures retained a homogeneous lymphoid morphology, while the J2 cultures gave rise to larger pleiomorphic cells which adhered to the culture dishes. In the case of J2C9, it was possible to separate the nonadherent and adherent cells by selective passages, establishing two distinct cell lines, J2C9 lymphoid and J2C9 myeloid, which exhibited homogeneous cell morphologies.

Isolation and characterization of transformants. 3611-MSV- and J-3-induced transformants exhibited phenotypic features characteristic of pre-B cells (8, 9, 13) in being Lyb-2, Ly-5(B220), and ThB positive but were Mac-1, κ light chain, and surface IgM (sIgM) negative (Table 2). In addition, the J3 cultures were Ia$^+$. Both lines expressed cytoplasmic μ chains, as determined by radioimmunoassays.

In contrast, J2C3, J2C5, and J2C9 exhibited phenotypes associated with B-lymphoid and myeloid differentiation. All expressed Ly-5(B220) and contained subpopulations expressing κ light chain, sIgM, varying levels of Ly-1, and the

TABLE 2. Phenotypic analysis of transformed cell lines

Virus	Monoclonal antibody[a]												
	Thy-1	IgM	K	Ia	ThB	B220	Lyb-2	Ly-17	PC 1	Ly-1	Mac-1	Mac-2	Ly-24
3611	−	−	−	−	+	+	++	ND	ND	−	−	ND	ND
J3	−	−	−	+	+	++	++	ND	ND	−	−	ND	ND
J2C3 parent	−	−/+	−/+	ND	−	++	−	ND	ND	+	−/+	ND	ND
Subclone 1	−	−	−	−	−	−/+	−	+	−	−	−	−	ND
Subclone 2	−	−	−	−	−	−	−	−	−	−	−	−	+
Subclone 3	−	−	−	−	−	−	−	−/+	−	−	−	−	+
Subclone 4	−	+	+	−/+	+	+	−	+	+	−/+	−	−	ND
Subclone 5	−	+	+	−/+	+	+	−	+	−	−/+	−	−	ND
Subclone 6	−	−	−	−/+	−	−	−	−/+	−	−/+	−/+	−/+	ND
J2C5 parent	−	−/+	−/+	ND	−	++	−	ND	ND	+	−/+	ND	ND
Subclone A	−	−	−	−	−	−	−	−	−	−	−	−	+
Subclone E	−	−	−	−	−	−	−	−	−	−	−	−	+
J2C9 parent	−	−/+	−/+	ND	−	++	−	ND	ND	+	−/+	ND	ND
Subclone C9-1	−	+	+	−	−	+	−	+	−	−	−	−	ND
Subclone C9-2	−	−	−	−	−	−	−	−	−	−	−	−	+

[a] Reactivity of indicated monoclonal antibodies relative to staining on normal NFS/N spleen. −, Nonreactive; −/+, negative and positive subpopulations with less than 30% of cells positive; +, reactive with an intensity equal to normal spleen cells; ++, reactive with an intensity greater than normal spleen cells; ND, not determined.

putative macrophage marker, Mac-1. Since sIg$^+$ and Mac-1$^+$ subpopulations were observed within the progeny of individual soft agar colonies, it was unclear as to whether both markers were coexpressed on the surface of B-lineage transformants or whether two independent lineages, B lymphoid and myeloid, had arisen from a single soft agar colony. If this were true, it would suggest that a common progenitor cell could give rise to both lymphoid and myeloid lineages and that this progenitor may have been the target for J2-induced transformation. The J2 lines were cloned by limiting dilution and analyzed for expression of lineage-specific determinants.

Transformation by v-*raf*–v-*myc* establishes mature B- and myeloid-lineage cell lines. All of the subclones resulted from seedings at 0.3 cell per well or 1.0 cell per well, which resulted in growth frequencies of less than 25% (data not shown). Approximately 30 subclones were analyzed, and of these, six J2C3 subclones (1 through 6), two J2C5 subclones (A and E), one J2C9 lymphoid subclone (C9-1), and one J2C9 myeloid subclone (C9-2) were characterized extensively by flow microfluorometry (Table 2).

Four distinct phenotypes were obtained following transformation with combined v-*raf*–v-*myc* oncogenes. A pre-B cell isolate, represented by J2C3 subclone 1, was Ly-17(FcR)$^+$, sIg$^-$, and Ly-5(B220)$^+$. The detection of a cytoplasmic μ chain but not a κ light chain by radioimmunoassay and its nonadherent lymphoid morphology in culture was consistent with its designation (radioimmunoassay data not shown). The second group exhibited a phenotype characteristic of mature B cells (subclones 4, 5, and J2C9 lymphoid subclone C9-1). All were Ly-5(B220)$^+$, IgM$^+$, κ light chain positive, Ly-17(FcR)$^+$, and PC-1$^+$ and expressed varying levels of Ly-1 and Ia. The presence of IgM heavy and κ light chains was substantiated by radioimmunoassay.

In contrast, the third category detected by this analysis included mature macrophages and monocytes (10, 13, 14). J2C3 clone 6 had a heterogeneous morphology in culture, exhibiting flat adherent cells and nonadherent clusters. Phenotypically, this culture contained a subpopulation with characteristics of a mature activated macrophage (11). This subpopulation was positive for Ly-17(FcR), Ly-1, Mac-1, Mac-2, PC-1, and Ia. Further characterization of this line demonstrated typical macrophage characteristics, including lysozyme production, latex bead ingestion, and α-naphthyl esterase activity. Radioimmunoassay did not detect IgM heavy or κ light chains. Clone 3 was uniformly positive for Ly-24, contained a subpopulation of Ly-17(FcR)$^+$ cells, produced lysozyme, and exhibited a heterogeneous morphology. It was therefore classified as myeloid.

A fourth category was detected in which only the panleukocyte antigen Ly-24 (13) was expressed (J2C3 subclone 2, J2C5 subclones A and E, J2C9 myeloid subclone C9-2). Collectively, these lines did not express antigens recognized by any other monoclonal antibodies used in this analysis. This phenotypic pattern has not been reported previously in extensive phenotyping of normal cells or neoplasms and did not permit assignment to a particular lineage. No change in cell surface phenotype was observed in any one of these subclones following culture in lipopolysaccharide or phorbol myristate acetate and rat spleen concanavalin A supernatants for periods of up to 12 months (M. A. Principato, unpublished observations). Finally, all of the v-*raf*, v-*myc*, and v-*raf*–v-*myc* transformants were negative for the gp160^{6C3} antigen, which is expressed on most B-lineage tumors, except those containing deregulated v-*myc* or c-*myc* (17), and the J2 transformants were also negative for Lyb-2, which is expressed on normal pre-B and B cells, and all B lineage lymphomas analyzed by this laboratory (9, 13, 14). Normal pre-B or B cells of this phenotype have not been described.

These data provide strong evidence for the synergistic interaction in vitro of a serine-threonine kinase (v-*raf*) and the nuclear oncogene (v-*myc*) and provide a striking parallel to earlier observations which showed that in vivo inoculation with activated v-*raf*–v-*myc* oncogenes resulted in the rapid induction of multiple hematopoietic tumors (7, 16, 23).

Further, these observations indicated that the sIg$^+$ Mac-1$^+$ phenotype obtained in the parental J2C3 soft agar clone represented the existence of discrete subpopulations. Whether these populations represented independent transformation events or represented the progeny of a singular transformation was evaluated by a comparison of retroviral insertion sites and immunoglobulin gene rearrangements (Fig. 1).

J2-derived B and myeloid lineages are clonally related. DNA from the cultures was digested with *Bam*HI and hybridized to a v-*myc* probe which detects MC29 v-*myc* sequences incorporated into the J2 and J3 retroviral constructs but does not cross-hybridize with endogenous c-*myc* sequences (Fig. 1A). Each J2C3-derived subclone exhibited two major hybridizing bands; the more intense band was noted at 6 kilobases (kb), while a weaker hybridizing species of approximately 9.6 kb was detectable. The J2C5 family shared a single hybridizing band of 7.2 kb, while the J2C9 families all shared a major hybridizing species of approximately 8.3 kb. These results indicated that all of the subclones were clonally related to the original parental soft agar clone and, therefore, represented a single transformation event. Variations in the intensities of bands hybridizing with the v-*myc* probe were taken to reflect reinfections during culture.

In order to confirm the clonality of the transformants, the organization of the immunoglobulin loci was analyzed by hybridization of *Eco*RI-digested genomic DNA to a JH-specific probe. As shown in Fig. 1B, all of the J2C3 subclones contain identical JH rearrangements, as evidenced by the loss of the germ line fragment found in NFS/N liver DNA. A comparison of the J2C5 and J2C9 clones and their subclones also demonstrated identical rearrangements within each family (Fig. 1B). Collectively, these data demonstrate that all of the cells obtained, including pre-B, B, and myeloid lineages, were derived from a single transformed cell.

κ light-chain gene rearrangements in myeloid-lineage cultures. The status of the κ light-chain locus was assessed by using *Hin*dIII (Fig. 1C) and *Bam*HI digests (Fig. 1D) and hybridizing with pECk.

The lack of κ light-chain gene rearrangements in 3611 confirmed its designation as a pre-B cell. The J3 line, classified as a small pre-B, exhibited one rearranged allele (8). A diversity of κ light-chain gene rearrangements was evident in the J2C3 subclones. Thus, J2C3 subclone 1 did not exhibit any rearrangements of this locus, affirming its designated pre-B cell phenotype (Fig. 1C and D, J2C3, lane 4), while rearrangements were noted in all lines classified as mature B lineage (Fig. 1C and D, lanes 7, 8, 13, and 14).

Of particular interest was the detection of a shared submolar band of approximately 7.0 kb in the *Hin*dIII digest of the J2C9 myeloid and J2C9 lymphoid cultures and the C9-1 subclone (Fig. 1C). This particular observation was confirmed by using a *Bam*HI digest of the cultures, which also permitted some additional interesting observations. First,

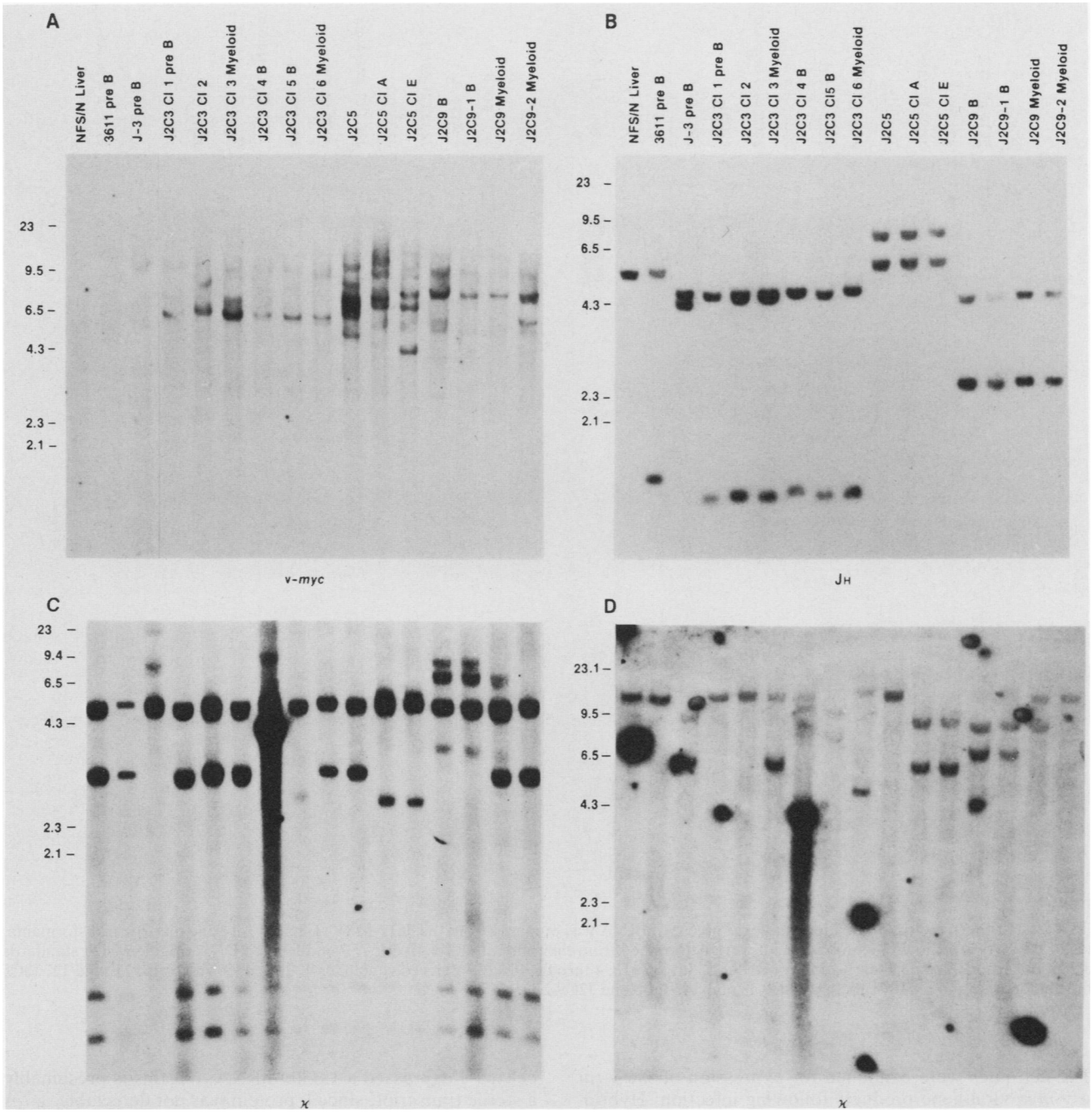

FIG. 1. Southern blot hybridization of v-*myc* integrations and immunoglobulin heavy- and light-chain gene organization. (A) *Bam*HI digests with a v-*myc*-specific probe. (B) *Eco*RI-digested DNA hybridized with a JH-specific probe (J11). (C) κ light-chain gene organization as observed following hybridization of *Hin*dIII digests with the pECK probe. (D) *Bam*HI-digested DNA hybridized with pECK. In panels C and D, the intense hybridization seen with DNA from J2C3 subclone 4 represents contamination with pBR DNA. Lanes: 1, NFS liver; 2, 3611; 3, J3; 4 through 9, J2C3-derived subclones 1, 2, 3, 4, 5, and 6; 10 through 12, J2C5 parental (lane 10) with subclones A and E (lanes 11 and 12); 13 and 14, parental J2C9 lymphoid culture (lane 13) with subclone J2C9-1 (lane 14); and 15 and 16, parental J2C9 myeloid culture (lane 15) with its subclone J2C9-2 (lane 16).

the rearrangement noted in the J2C9 myeloid culture was confirmed in the *Bam*HI digest (Fig. 1D) as a 7.8-kb band and was detected below the 12-kb germ line band. The C9-2 subclone, derived from the J2C9 myeloid culture, retained one germ line allele and appeared to have deleted the second allele. Additionally, the rearrangement of a single κ allele in myeloid J2C3 subclones 3 (5.2 kb) and 6 (4.1 kb) is easily evident (Fig. 1D). The J2C5 family also exhibited some variability, and it was interesting to note that while the parental cloned population had not rearranged its allele, the undifferentiated subclones contained at least one rearrangement. The significance of these rearrangements with respect to lineage relatedness will be discussed below.

Molecular characteristics. Poly(A)$^+$-selected mRNA was prepared from 3611, J-3, and the panel of J2 clones and subclones and subjected to Northern (RNA) blot analysis.

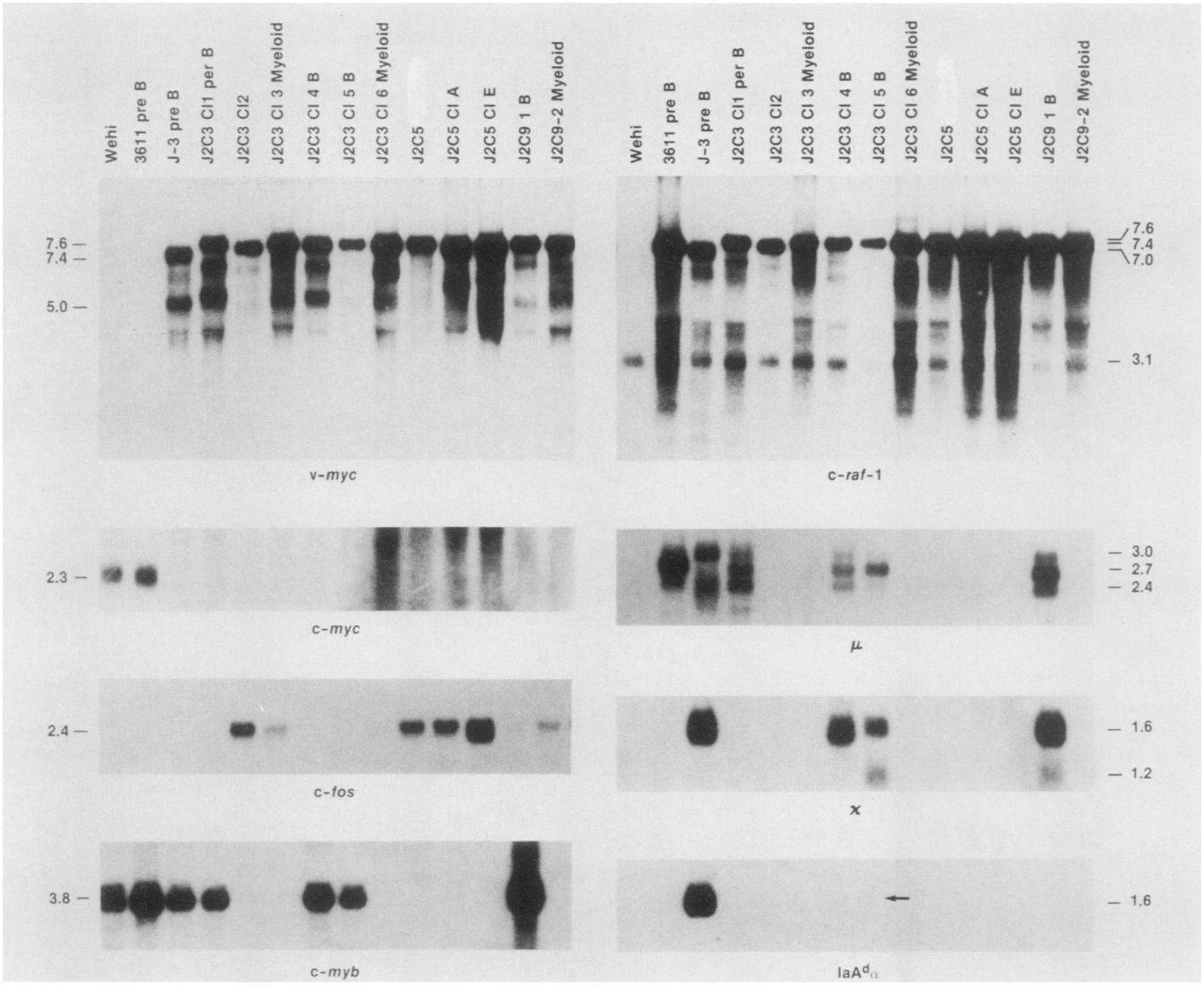

FIG. 2. Northern blot hybridization studies. Poly(A)$^+$ mRNA was prepared from the 3611-MSV, J-3, and J-2 bone marrow transformants. Sizes of mRNAs are shown in kilobases and were determined from their migration relative to ^{32}P-end-labeled DNA fragment-size standards and 28S and 18S rRNAs. Lanes: 1, control RNA; 2, 3611; 3, J3; 4 through 9, J2C3-derived subclones 1, 2, 3, 4, 5, and 6; 10, 11, and 12, J2C5 with subclones A and E; 13, subclone J2C9-1; and 14, subclone J2C9-2.

The cell lines were examined for expression of the v-*raf* and v-*myc* viral gene products following infection. Hybridization with the v-*myc* probe demonstrated that the J3 and J2 transformants expressed the functional subgenomic RNA of 6.1 and 6.3 kb, respectively (Fig. 2). High levels of v-*myc* expression have been reported to suppress endogenous c-*myc* transcription, and, accordingly, transcripts were detected only in the 3611-MSV pre-B cell culture and uninfected and 3611-MSV-infected NIH 3T3 fibroblast cultures. Hybridization with the c-*raf* probe demonstrated high levels of v-*raf* transcripts in 3611 pre-B cells, the fibroblast producer line, and J2-infected cultures, while lower levels of endogenous 3.1-kb c-*raf* mRNA were detected in all cell lines (7, 23). Transcripts for μ (Fig. 2) or δ heavy chain (not shown) were detected in the B-lineage cells, while none were detected in any of the undifferentiated or mature myeloid cell lines. Hybridization with a κ light-chain probe revealed that expression of rearranged κ genes was restricted to sIg$^+$ B cells. J-3 expressed a 1.6-kb mRNA, but this is presumably a sterile transcript, since κ protein was not detectable. c-*fos* expression, which has been reported to become prominent during myelomonocytic differentiation (18), was noted in those cultures classified as myeloid.

All transformants expressed detectable levels of H2-K and β2-microglobulin mRNA (latter not shown). As expected from the flow microfluorometry results, the J3-derived line contained a message for IA, while low levels were detected in clones 4 and 5. c-*myb* mRNA was detected only in pre-B and sIg$^+$ B cell clones, suggesting a relative state of immaturity, while the absence of c-*myb* RNAs in the undifferentiated lines suggested that these cells are not early hematopoietic cells (11). The macrophage lines were analyzed for expression of CSF1 receptor with a *fms* probe but found to be negative. No transcripts were detected for growth factors interleukin 3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and IL-6 in any of the trans-

formants. Transcripts for λ5 and v-pre-B mRNA were detected only in J2C3 clone 1 (S. Bauer, H. C. Morse, and U. R. Rapp, unpublished data).

DISCUSSION

The most striking feature of these studies is the derivation of clonally related undifferentiated progenitors, pre-B cells, B cells, and macrophages following in vitro infection of murine bone marrow cells with combined v-*raf*–v-*myc* oncogenes. Further, J-2-induced in vitro transformation did not preclude differentiation of the target cells, as the immunoglobulin heavy- and light-chain gene rearrangements observed in the isolates were functional. As has been reported in previous transformations in which single oncogenes were used (14, 19, 21, 24), the phenotype obtained in our experiments following transformation with either v-*raf* or v-*myc* produced cell lines of pre-B cell phenotype.

Lyb-2, presumptively involved in the triggering of B-cell proliferation (26), may be absent from the B-lineage J2 transformants due to the influence of v-*raf*–v-*myc*. Expression of *raf* and *myc* may obviate the need for this molecule by providing an alternative intrinsic activation pathway, similar to the effects of *abl* and *myc* on IL-3-dependent cell lines (22). The effect of these genes does not appear to reflect induction of an autocrine loop for any of the J2 clones, as they were uniformly negative for IL-3, IL-4, IL-6, and GM-CSF transcripts (data not shown).

Past analyses have suggested a close developmental relationship between the B cell and macrophage (6, 19, 25), and the derivation of myeloid cells from pre-B cells provides strong support for this hypothesis (3, 6, 10, 15). However, the lack of concomitant immunoglobulin expression by the pre-B cells in these reports suggested that the rearrangements were atypical and implied that differentiation into the myeloid lineage was a consequence of aberrant immunoglobulin rearrangements, possibly representing a salvage pathway (10). It was of interest to note that J2C3 subclones 3 and 6 exhibited similar IgH gene rearrangements which were productively expressed in clonally related pre-B and B cells. If these rearrangements were to be shown to be identical by sequencing, this would suggest that the crossover from the B to myeloid lineages reflects the existence of a physiological pathway. The rearrangements observed in clones 3 and 6 are not likely to be independent events, since immunoglobulin genes were not expressed in these cells, and expression is thought to be a prerequisite for rearrangement (2).

Further, we suggest that the κ gene rearrangements exhibited by the J2C3 and J2C9 families were indicative of a divergence of the B and myeloid lineages following JH rearrangements and subsequent to κ light-chain rearrangements. The latter possibility would indicate that the regulatory processes involved in lineage determination occurred at a later stage in differentiation than previously suggested. Indeed, morphological and phenotypic analyses have substantiated the spontaneous conversion of pre-B J2C3 clone 1 into a sIg^- $Mac\text{-}1^+$ population (M. A. Principato, M. Shapiro, and H. C. Morse, unpublished observations). This may also reflect an influence by the introduced oncogenes on regulatory elements mediating lineage determination. The mechanisms responsible for lineage commitment in hemopoiesis remain obscure, but the results of this and another study (15) clearly indicate that they can be influenced by the combined expression of *raf* and *myc*. Introduction of conditional oncogene expression vectors into immature pre-B single oncogene transformants will allow us to test this possibility.

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