Nuclear Oncoprotein Expression as a Function of Lineage, Differentiation Stage, and Proliferative Status of Normal Human Hematopoietic Cells

By Michael B. Kastan, Kelly D. Stone, and Curt I. Civin

Relative levels of the nuclear oncoproteins c-myb, c-myc, and c-fos were determined in selected subpopulations of normal human bone marrow (BM) cells using a flow cytometric assay which simultaneously detects a cell-surface antigen (as a marker of lineage and stage of maturation) and levels of an intracellular protein. At least two monoclonal antibodies directed against each oncoprotein and specific peptide inhibition controls were used for these determinations. Hematopoietic progenitor cells (CD34+) express the highest levels of c-myb and c-myc, whereas c-fos levels in CD34+ progenitor cells are similar to c-fos levels in mature monocytes and granulocytes. Granulocytes are the only hematopoietic cells examined which do not express detectable levels of c-myb and c-myc. The levels of these oncoproteins in these normal, unstimulated BM cell populations were more closely linked to lineage and maturation stage than to the proliferative status of the given population, as determined by either DNA staining or expression of the cell-cycle specific nuclear protein, Ki67. This flow cytometric assay helps in interpreting the significance of oncoprotein levels in leukemia cells by allowing direct comparisons of a leukemia with the phenotypically similar “normal counterpart control” cell population in normal BM.

MATERIALS AND METHODS

Antibodies and peptides. MoAbs directed against oncoprotein peptide sequences were obtained from Microbiological Associates (Bethesda, MD) (myb132, myb133, myc155, fos411, and fos416), Cambridge Research Biochemicals (Valley Stream, NY) (myc906), or Oncogene Science (Manhasset, NY) (myc9E10). Peptide sequences which inhibited myb132, myb133, myc155, fos411, fos416, and myc906 were obtained from Microbiological Associates. (The same peptide sequence was used to raise the myc155 and myc906 antibodies.) Phyoerythrin (PE)-conjugated or unlabeled MoAbs to CD10 (common acute lymphoblastic antigen), CD22 (Leu-14), CD5 (Leu-1), CD14 (Leu-M3), CD45 (HLe-1), CD11b (Leu-15), and CD15 (Leu-M1) were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA). Other MoAbs were obtained as follows: CD34 (ICH3, a gift from F. Katz, London), transferrin receptor (My29, developed in our laboratory), glycophorin (produced in our laboratory from the 10F7MN hybridoma, ATCC, Rockville, MD), CD36 (SF1, a gift from I. Bernstein, Seattle, WA), and blood group A antibody (developed in our laboratory). Ki67 was obtained from Dako (Carpinteria, CA). When unlabeled antibodies were used to stain cell-surface antigens, isotype-specific, PE-conjugated, affinity-purified goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) or monoclonal rat anti-mouse Ig antibodies (a gift from Dr David Buck, from The Johns Hopkins Oncology Center, Baltimore, MD).

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Address reprint requests to Michael B. Kastan, MD, PhD, Division of Pediatric Oncology, Johns Hopkins Oncology Center, Oncology 3-121, 600 N Wolfe St, Baltimore, MD 21205.

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Becton Dickinson) were used as secondary labeling reagents. For labeling of the nuclear antigens, isotype-specific, fluorescein isothiocyanate (FITC)-conjugated secondary antibodies from the same two sources were used.

**Immunofluorescence assay.** BM aspirates were obtained from consenting normal adult volunteers, as approved by our institutional review board and the Department of Health and Human Services. Low-density BM mononuclear cells were prepared by Ficoll-Hypaque density centrifugation (1.077 g/cm³, Pharmacia, Piscataway, NJ).

Cell-surface antigen and nuclear oncoprotein fluorescence labeling was performed as previously described. All procedures were performed at 4°C. Cell-surface antigens were labeled, directly or indirectly, with PE-conjugated antibodies. The cells were then fixed in 1% paraformaldehyde in phosphate-buffered saline and permeabilized with 0.1% Triton X-100 (Sigma, St Louis) in IFA buffer (10 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl, 4% newborn calf serum, 0.1% NaN₃). The permeabilized cells were incubated with 0.1 mL nuclear protein antibody (neat hybridoma supernatant for myb132, myb133, myc155, fos411, and fos416; myc9E10 IgG diluted 1:5 in IFA buffer; myc906 ascites diluted 1:400 in IFA buffer; Ki67 diluted 1:10 in IFA buffer; all of these dilutions are volume per volume and represent optimal specific staining for each antibody; titration data not shown) for 1 hour, followed by incubation with FITC-conjugated, purified isotype-specific, monoclonal rat anti-mouse IgG (diluted 1:100 in IFA buffer) or affinity-purified goat anti-mouse IgG (diluted 1:300 in IFA buffer) for 30 minutes. Washes between each step were performed with IFA buffer containing 0.1% Triton X-100.

Two-color flow cytometric analysis was performed on a FACScan flow cytometer (Becton Dickinson) after appropriate compensations of the FITC and PE channels using Calibrite beads (Becton Dickinson) according to the manufacturer’s instructions. Controls for nonspecific cell-surface labeling (with and without detergent exposure) and nuclear protein staining were run for each antibody in every experiment. Antibodies to surface markers and nuclear proteins were chosen so that pairs of antibodies with different isotypes were always used in a given sample. Otherwise, the secondary FITC-conjugated antibody, intended to mark the nuclear protein specifically, could bind also to the cell-surface antibody. Because all nuclear oncoprotein antibodies used in this study were IgG1, except for fos411 (IgG2b), most of the cell-surface markers used were IgG2a or IgG2b. Antibody controls demonstrated that none of the antibody combinations used in these experiments exhibited this type of crossreactive binding (eg, Fig 1C and D). For peptide inhibition studies, 2 μg peptide (this amount of peptide is in excess for maximal specific inhibition of antibody staining) was incubated with the oncoprotein antibody (at least 1 hour at 4°C) before antibody incubation with the permeabilized PE-labeled cells. Nonspecific blocking serum (2% to 5% human and goat or rat (as appropriate) serum) was present during each antibody incubation. All antibodies used resulted in specific nuclear (excluding nucleolar) staining in all cell types as assessed by both fluorescence microscopy and immunoperoxidase staining (data not shown).

**RESULTS**

**Flow cytometric assessment of oncoprotein levels.** We recently described a two-color flow cytometric assay which permits simultaneous identification of a cell surface marker, delineating hematopoietic cell lineage and/or developmental stage, and relative quantitation of an intracellular protein. Quantitation of nuclear oncoproteins with MoAbs, rather than the polyclonal antibodies described in the earlier study, results in much lower control antibody binding levels (Fig 1C and D) and improves quantitative assessment of oncoprotein levels in different cell types. After cell-surface labeling, fixation, and detergent permeabilization, the cells were incubated with MoAbs to one of the nuclear proteins (c-myc, c-myc, c-fos, or Ki67) or with control antibody (either a nonspecific MoAb, MOPC-21 IgG1, or a nuclear oncoprotein antibody after a preincubation with specific blocking peptide). The cells were then incubated with an FITC-conjugated isotype-specific secondary antibody to identify the nuclear protein. Two-color flow cytometric analysis was performed to quantitate nuclear protein staining levels (relative to controls) in each hematopoietic cell subtype (defined by the PE-conjugated cell-surface markers).

Examples of this analysis are shown in Fig 1. Low-density BM leukocytes surface labeled with PE for the progenitor cell-specific surface antigen, CD34 (Fig 1A and C), or a monocyte-specific cell surface antigen, CD14 (Fig 1B and D), were incubated with MoAb to the c-myc oncoprotein, with (Fig 1C and D) or without (Fig 1A and B) the blocking c-myc peptide. Progenitor cells (CD34-PE⁺; Fig 1A) and monocytes (CD14-PE⁺; Fig 1B) have easily detectable levels of c-myc protein. Preincubation of the c-myc antibody with the c-myc peptide used to raise the antibody abolishes all nuclear fluorescent staining in these cell populations (Fig 1C and D). Therefore, all nuclear staining detected with this antibody is a result of binding of the c-myc antibody to this epitope.

Several different cell populations identified by PE-labeled

![Fig 1. Examples of flow cytometric analysis of oncoprotein levels in normal BM cells. Normal human BM cells were labeled on the surface with PE-conjugated CD34 (A and C, identifying pluripotent progenitor cells) or CD14 (B and D, identifying monocytes) and in the nuclei with myc155 antibody with (C and D) or without (A and B) preincubation with myc155 peptide. Dashed lines defining quadrants designate values used for positive PE and FITC fluorescence. The population of cells remaining in the right quadrant in the control samples (C and D) are granulocytes (CD15⁺; data not shown; discussed in text). Irrelevant peptides were not inhibitory (data not shown).](https://www.bloodjournal.org/content/102/6/1518/F1)

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cell-surface markers, including CD34 (multilineage progenitor cells), CD10 (early B-lymphoid cells), CD22 (mature B cells), CD5 (T cells), CD14 (bright on monocytes), and CD15 (bright on granulocytes) were analyzed for oncoprotein levels. Erythroid precursors were identified by antibodies to transferrin receptor, glycophorin, CD36, blood group A or by lack of expression of a combination of both CD45 and CD11b. Oncoprotein levels were virtually identical in the populations identified by each of these different erythroid surface markers (data not shown). Since mature red blood cells are lysed during this assay, they are not evaluable.

Oncoprotein levels in each of these cell-surface antigen-defined populations were characterized with two different MoAbs to c-myb, three different MoAbs to c-myc, and two different MoAbs to c-fos (described in the Materials and Methods section). Each of these MoAbs had been raised against conserved peptide sequences from the appropriate oncoprotein. In each cell type examined, preincubation of the oncoprotein antibody with its corresponding peptide abolished binding of the antibody (Figs 1 and 2). No inhibition was observed when an irrelevant peptide was preincubated with the antibodies (data not shown).

The flow cytometer allows us to evaluate the level of an oncoprotein (FITC) in a selected hematopoietic cell population (identified by the PE-labeled cell surface marker). Examples are shown of c-myb (Fig 2A and B), c-myc (Fig 2C and D), and c-fos (Fig 2E and F) levels in progenitor cells (CD34\(^+\); Fig 2A, C, and E) or monocytes (CD14\(^+\); Fig 2B, D, and F). Nuclear staining controls (nonspecific MOPC-21 IgG\(_2\), and specific peptide inhibition) demonstrate the epitope specificity of each oncoprotein antibody in these two hematopoietic cell populations (Fig 2). This specificity was observed in each hematopoietic cell population examined.

Relative oncoprotein levels in different cell populations. We compared relative oncoprotein levels in the various hematopoietic cell types. In all cases, the different MoAbs directed against a particular oncoprotein reflected similar relative levels in the various cellular subtypes. Relative c-myb levels with both myb132 (Fig 3) and myb133 (data not shown) were: progenitor cells (CD34\(^+\)) > monocytes (CD14\(^+\)) > B-lymphoid cells (both CD10\(^+\) and CD22\(^+\) subsets) > T-lymphoid cells (CD5\(^+\)) > late erythroid progenitors [(CD45 + CD11b) negative]; c-myc levels were heterogeneous in this erythroid progenitor population.

The differential pattern of c-myc expression in the various cell populations with myc155 (Fig 3), myc906, and myc9E10 (data not shown) was similar to c-myb: progenitor cells > monocytes > B-lymphoid cells (again CD10\(^+\) progenitors > CD22\(^+\) mature B cells) = T-lymphoid cells > some late erythroid progenitors (a very heterogeneous population). Relative c-fos levels with both fos416 (Fig 3) and fos411 (data not shown) were: progenitor cells > monocytes > B- or T-lymphoid cells > many late erythroid progenitors (again a very heterogeneous population).

Such direct comparisons of relative oncoprotein levels in different cell subtypes is valid only if antibody control binding levels are similar in each of the cellular subtypes. In Fig 1C and D, the population of cells which remained to the right of the vertical quadrant despite peptide inhibition of myc staining, is the granulocytes (CD15 brightly labeled). Binding of MOPC-21 IgG\(_2\) showed similar high background levels only in granulocytes (data not shown). All other cell types had background control FITC staining levels within the left quadrant. Because of this high background level, granulocytes could not be directly compared with the other cell types in the analysis we have described.

To make valid comparisons of oncoprotein levels in granulocytes relative to other cell types, we calculated a "corrected mean fluorescence intensity" (MFI) for each cell type by subtracting the MFI of the peptide inhibition control sample from the MFI of the oncoprotein antibody sample for each cell type. This corrected MFI represents the mean amount of epitope-specific oncoprotein FITC staining in each cell population, and the analysis allows comparisons of oncoprotein levels in all cell types by correcting for any variations in background binding. The results of these calculations for the antibodies myb132, myc155, myc906, and fos416 are shown in Fig 4. There was virtually no specific staining of granulocytes for c-myb (Fig 4A) or c-myc (Fig 4B and D). However, c-fos levels (Fig 4C) appeared to be as high in granulocytes as they were in CD34\(^+\) progenitor cells and monocytes. Relative oncoprotein levels in the other cell types reflected the histogram data (Fig 3), since there was little variability in the background binding in these other cell types. However, the wide range in oncoprotein staining in the late erythroid
progenitors, which was so evident in the histogram analysis (Fig 3), was obscured by this type of calculation. Therefore, both types of assessments provide useful information about relative oncoprotein levels. The numerical value for the MFI of a given oncoprotein can vary slightly from one experiment to another, but the relative oncoprotein levels in each hematopoietic cell population was constant in all BM samples evaluated.

Cell cycle status of the different cell populations. In several experimental systems, changes in the levels of c-myb, c-myc, and c-fos have been associated with mitogenic stimulation and cell cycle status. To examine a potential relationship between cell cycle and oncoprotein levels in normal, unstimulated hematopoietic cells in vivo, we characterized the proliferative status of these hematopoietic cell populations. Previous studies had demonstrated that early B cells (CD10+) are more highly proliferative than CD34+ progenitor cells, which in turn are more highly proliferative than maturing and mature B cells (CD20+). In contrast to this timing of proliferation in B-cell development, CD34+ cells have the highest levels of all three oncoproteins and CD10+ B cells have oncoprotein levels similar to those of more mature B cells (Figs 3 and 4). Propidium iodide staining done in conjunction with some of the above experiments also indicated that of the other cell populations evaluated for oncoprotein expression in this study (T cells, monocytes, granulocytes, and late erythroid progenitors) only the erythroid progenitors have a substantial number of cycling cells (data not shown).

As another approach to characterizing the proliferative status of these cell populations, we analyzed expression of Ki67, a nuclear antigen present in all cycling cells and absent...
in G<sub>0</sub> cells. It is convenient to use Ki67 as an indicator of the proliferation status of a cell population of interest, since it uses the same two-color flow cytometric assay used to analyze oncoprotein levels, providing direct comparisons of the oncoproteins to a nuclear protein known to vary with cell cycle status. As predicted by the DNA staining results, Ki67 levels were clearly different from the oncoprotein staining patterns with: B-lymphoid progenitors (CD10<sup>+</sup>) > late erythroid progenitors > CD34<sup>+</sup> progenitors > mature B cells (CD22<sup>+</sup>; there are occasional strongly positive cells, presumably activated B lymphocytes) > T cells (CD5<sup>+</sup>) > monocytes (CD14<sup>+</sup>) > granulocytes (CD15<sup>+</sup>; no staining above MOPC-21 IgG<sub>1</sub> background) (Fig 5). The differences between oncoprotein and Ki67 levels is probably best exemplified by the high level of Ki67 staining in CD10 cells as compared with CD22<sup>+</sup> B lymphoid cells (Fig 5), but similar levels of c-myc staining in these two populations (Fig 4D).

**Oncoprotein levels in leukemia cells.** Previously, assessment of the significance of measured protooncogene levels in leukemia cells was difficult due to the lack of an appropriate normal cell control value for comparison. This difficulty also arises because normal BM is a heterogeneous population of hematopoietic cells. Our flow cytometric assay circumvents this difficulty by permitting definition of oncoprotein levels in a BM cell population which is phenotypically similar to the leukemia cells of interest. For example, c-myb protein levels in a CD10<sup>+</sup> B-lineage acute lymphocytic leukemia specimen are virtually identical to c-myb levels in CD10<sup>+</sup> normal BM cells (Fig 6A), whereas c-fos protein levels in a CD11b<sup>+</sup> acute nonlymphocytic leukemia (FAB M4) case are slightly higher than c-fos levels in CD11b<sup>+</sup> normal BM cells (Fig 6B). FAB M4 and M5 acute nonlymphocytic leukemias have been reported to express c-fos mRNA levels higher than those of other acute leukemias.

**DISCUSSION**

The nuclear oncoproteins c-myb, c-myc, and c-fos appear to participate in lymphohematopoietic cell replication and/or differentiation pathways. For example, mitogenic stimulation or chemically induced differentiation of certain cell lines results in changes in the levels of these protooncogenes. Furthermore, recent experiments showed that inhibition of oncoprotein synthesis by specific antisense oligonucleotides for c-myb<sup>36</sup> or c-myc<sup>31,37,38</sup> can alter lymphohematopoietic cell growth and/or differentiation.

Protooncogenes have been difficult to study in normal human hematopoietic cells because of the cellular heterogeneity of normal human BM. We addressed this problem by using a flow cytometric assay which permits quantitative assessment of relative levels of a given intracellular protein in normal BM cells.
specific normal hematopoietic cell populations identified by lineage and differentiation stage-specific cell-surface antigen expression. We report relative bevels of the nuclear oncoproteins c-myb, c-myc, and c-fos in defined populations of normal human hematopoietic cells using MoAbs directed against these oncoproteins in a two-color flow cytometric assay. Hematopoietic progenitor cells (CD34+) express the highest relative levels of c-myb and c-myc. Levels of c-fos in CD34+ cells are similar to c-fos levels in mature monocytes and granulocytes. Granulocytes constitute the only hematopoietic cell population examined that does not express detectable levels of c-myb and c-myc. The late erythroid progenitor population examined in this study was heterogeneously stained for all three oncoproteins. We suspect that the more mature erythroid cells have relatively low levels of each of the oncoproteins (probably undetectable for c-myb and c-fos, Fig 3).

Our finding that normal hematopoietic progenitor cells have the highest relative levels of c-myb and c-myc is in agreement with predictions from earlier work with leukemic cell lines that demonstrated decreasing c-myb and c-myc mRNA levels with increasing cell maturation (chemically induced) and with earlier work with normal BM cells from our laboratory using two polyclonal antibodies directed against c-myb. In addition, our results are consistent with previous observations that c-myb and c-myc mRNA levels decrease with increasing erythroid maturation and are not detectable in mature granulocytes. The similar patterns of c-myb and c-myc expression that we observed were also expected from previous studies. However, the finding that mature lymphocytes and monocytes expressed detectable levels of c-myb and c-myc oncoproteins was surprising, since some previous studies had suggested that unstimulated mature hematopoietic cells do not express detectable levels of these protooncogenes. There are several potential explanations for this apparent discrepancy.

First, we evaluated oncoprotein levels, whereas many of the earlier studies examined protooncogene mRNA levels. Complex relationships may exist between protooncogene mRNA and protein levels in various cell types. Second, most of the earlier studies suggesting the absence of protooncogene expression in mature hematopoietic cells was performed in chemically induced leukemia cell lines, which may not accurately reflect physiologic changes which occur in normal differentiation. For example, normal hematopoietic cell differentiation occurs in the presence of ongoing cellular replication, whereas TPA treatment of HL-60 or ML-1 cells causes morphologic cell maturation while inhibiting cell division; c-myb and c-myc mRNA levels remain elevated for at least 48 hours during maturation of WEHI-3B cells to monocytes induced by a combination of actinomycin D and GM colony-stimulating factor (which probably allows some cell replication to continue during morphologic maturation).

Normal monocytes and lymphocytes in vivo are not "end-stage" cells. Indeed, the only cell population evaluated in this study which cannot undergo further cell division (as stimulated lymphocytes might do) or maturation (as monocytes which mature to macrophages or B cells which mature to plasma cells would do) are the granulocytes. The only cell population which had no detectable c-myb and c-myc oncoprotein staining above background in this assay was the granulocytes. Thus, since normal differentiation occurs in the presence of cell replication and normal monocytes in vivo are not true end-stage cells, the mature monocyte population in normal BM or peripheral blood may be more analogous to the 48-hour GM-colony-stimulating factor/actinomycin D-induced WEHI-3B monocyte than to a TPA-induced HL-60 macrophage.

One further explanation for our detection of these oncoproteins in mature normal monocytes and lymphocytes is the sensitivity of the flow cytometric assay. With many other assays, such as Northern blots for mRNA, Western blots for protein, or even immunoperoxidase assays for protein, the detection threshold can be manipulated to change the sensitivity of the assay and is usually manipulated to minimize background levels. Thus, the assay itself helps define undetectable protooncogene levels. The levels of nuclear fluorescence for these oncoproteins using the MoAbs are not even visible in most cell types by immunofluorescence microscopy (partially because of the diffuse nature of the nuclear staining), but are easily measured by the flow cytometer. In addition, controls such as the peptide inhibition provide a sensitive, quantitative definition of a zero background.

Data in the literature suggesting selective c-fos expression in the monocyte lineage contrast with other data suggesting selective expression in neutrophils. Our data, in agreement with findings of another recent study, suggest the presence of c-fos in both mononuclear and neutrophilic lineages. Indeed, c-fos is the only nuclear protein we evaluated that is expressed at detectable levels in granulocytes. In addition, the normal progenitor cell population has c-fos protein levels similar to both of these mature populations. Thus, in unstimulated, normal mononuclear cells, there appears to be a relatively constant level of c-fos, irrespective of maturation stage. However, changes in c-fos levels may occur only transiently in small cell subsets at various maturation stages. Future studies using various methods of stimulating these normal hematopoietic cells might detect changes in c-fos levels in such rare cell types. Last, the relatively high levels of c-fos in mononuclear cells (relative to lymphoid or erythroid) may signify some lineage-specific function of this oncoprotein.

Both c-myb and c-myc levels have been linked to cell
proliferation status in many studies. However, at least in some situations, c-myc and c-myc levels may be more closely linked to differentiation stage than proliferation status. Our results (in agreement with some other reports in the literature analyzing steady-state oncoprotein levels in unstimulated, normal human BM cells reveal no simple correlation of c-myc, c-myc, or c-fos levels with proliferative status. With the same assay, levels of the cell cycle-specific nuclear protein Ki67 were consistent with DNA staining results, with high Ki67 levels in proliferating cells versus low levels in nondividing cell types. In contrast, c-myc and c-myc levels, though paralleling each other, did not correlate with relative levels of Ki67 in these cell populations. Thus, c-myc and c-myc levels were more closely linked to lineage and maturation stage than to the proliferative status of these populations of normal, unstimulated BM cells. Granulocytes (which are quiescent in terms of both proliferation and further differentiation) were the one cell population with virtually undetectable levels of these three nuclear proteins. Because stimulation of normal cells in vitro increases (at least transiently) expression of these oncogenes, there is probably a relationship between levels of these oncogenes and proliferative status that will be superimposed on the lineage and developmental stage-specific levels which we find. Future experiments using both in vivo and in vitro stimulated normal hematopoietic cells, should begin to elucidate these issues.

The similar oncoprotein levels in certain leukemias and phenotypically similar normal BM cells further supports the concept that the relationship between cell proliferative status and levels of these oncoproteins is not a simple one. Our current data on leukemias are too preliminary to allow conclusions about the significance of levels of oncoprotein expression in malignant cells, but future experiments will address this issue. This assay should be useful in improving understanding of the significance of oncoprotein levels in leukemias because it provides a more rational normal control cell for comparison of oncoprotein levels. Further use of this assay to examine changes in oncoproteins in hematopoietic cells with mitogenic stimulation, in leukemia cells and, eventually, during the process of malignant transformation (eg, potentially in transgenic mice), should continue to provide insights about the significance of these proteins in normal and abnormal hematopoiesis.

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REFERENCES


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