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Coordinate Regulation of Myelomonocytic Phenotype by v-*myb* and v-*myc*

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Both avian myeloblastosis virus (by the action of v-*myb*) and avian myelocytomatosis virus MC29 (by the action of v-*myc*) transform cells of the myelomonocytic lineage. Whereas avian myeloblastosis virus elicits a relatively immature phenotype, cells transformed by MC29 resemble mature macrophages. When cells previously transformed by v-*myb* were superinfected with MC29, their phenotype was rapidly altered to that of a more mature cell. These superinfected cells expressed both v-*myb* (at a level similar to that found before superinfection) and v-*myc*. It therefore appears that the expression of v-*myc* can elicit certain properties of a more differentiated phenotype. In addition, unlike cells transformed by v-*myb* alone, the cells expressing both v-*myb* and v-*myc* could not be induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate to differentiate to fully mature macrophages. Cells with a morphology similar to that of the superinfected cells were elicited by simultaneously infecting yolk sac macrophages with avian myeloblastosis virus and MC29. Such cells expressed both v-*myb* and v-*myc*. These results indicate that expression of v-*myb* and v-*myc* in infected cells coordinately regulates myelomonocytic phenotype and that the two viral oncogenes vary in their ability to interfere with tumor promoter-induced differentiation. Our findings also sustain previous suggestions that the oncogenes v-*myb* and v-*myc* may not transform target cells by simply blocking differentiation.

Avian myeloblastosis virus (AMV) specifically transforms myelomonocytic cells in culture, whereas avian myelocytomatosis virus (MC29) transforms both myelomonocytic cells and fibroblasts (3–6, 13–15, 18, 25, 27, 29). In vivo, AMV induces myeloblastic leukemia; MC29 induces carcinomas, sarcomas, and myelomonocytic leukemia (3, 4, 15, 18, 25, 27, 29). The cellular genes from which the viral oncogenes v-*myb* and v-*myc* were derived have been termed c-*myb* and c-*myc*, respectively (4, 9). Both c-*myb* and c-*myc* have been found to be expressed in hematopoietic cells, c-*myb* primarily in immature hematopoietic cells and c-*myc* in dividing hematopoietic cells and in a variety of other proliferating cells such as fibroblasts and B-lymphocytes (4, 8, 12, 16, 17, 20–22). In normal chicken and mouse myelomonocytic cells, the expression of both cellular genes appears to decrease as the cells become more differentiated (10, 12, 16, 17, 35, 36).

Although both v-*myb* and v-*myc* cause transformation in the myelomonocytic lineage, each elicits a different characteristic phenotype. Cells transformed by AMV appear to be similar to myeloblasts, whereas those transformed by MC29 resemble macrophages (3, 5, 6, 13, 15, 18, 25, 27, 29). This characterization was based on the morphology of the cells, both in culture and after cytochemical staining. When other phenotypic properties were examined, however, it became clear that cells transformed by either AMV or MC29 did not have the phenotype of a particular cell type in the normal lineage (3, 5, 6, 13, 15, 25, 27, 29). AMV-transformed cells resemble myeloblasts in that they lack receptors for the C3 component of complement (C3 receptor) and the ability to phagocytose via the Fc receptor (Fc-immune phagocytosis). Unlike myeloblasts, however, they have membrane ATPase activity, and a reasonably high percentage of cells possess the receptor for the Fc component of immunoglobulin G (IgG) (Fc receptors). By contrast, MC29-transformed cells morphologically resemble macrophages, have a high level of Fc receptors, and are capable of Fc-immune phagocytosis. However, like less mature cells, they have a low level of membrane ATPase, and few cells possess C3 receptors (3, 5, 6, 13, 15, 25, 27, 29). Thus, neither transformed cell appears to represent a defined cell type in the normal lineage, although MC29-transformed cells are characterized as more mature than AMV-transformed cells.

It has been postulated that genes such as v-*myb* and v-*myc* function by blocking differentiation (18). However, the data outlined above have been taken to indicate that each gene causes a specific derangement of the expression of normal macrophage properties (5, 6, 13, 14). We sought to analyze the interaction of these two viral genes in myelomonocytic cells by comparing their combined and separate effects on the differentiated phenotype.

MATERIALS AND METHODS

Cells and cell culture. The BM2 cell line used here has been described previously (25, 27, 29, 34); it is a line of chicken nonproducer myeloblasts transformed by AMV. BM2 clone 3 was used in the present experiments. The growth medium and culture conditions used have been described previously (34). Yolk sac macrophages were purified and cultured as previously described (26). MC29-transformed quail cells were the Q8 cell line, a line of nonproducer fibroblasts (2, 20).

Viral infections. For superinfection of AMV-transformed

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cells with MC29 (subgroup A or B) or with the Schmidt-Ruppin subgroup A strain of Rous sarcoma virus, the following protocol was used. Cells (15×10^6) were pelleted, and 0.3 ml of virus-containing plasma was added. The cells were suspended and left at 4°C for 30 min to allow viral adsorption, followed by incubation at 37°C for 30 min. They were then plated in 3 ml of culture medium. After 24 h the medium was removed, and the cells were washed once and replated in 5 ml of fresh culture medium. After 2 weeks, productive infection of nonproducer BM2 cells was confirmed by assaying 2 ml of culture supernatant for reverse transcriptase activity (33). For infection of yolk sac macrophages, the following protocol was used. Secondary yolk sac macrophages were seeded at 5×10^5 cells per 25-cm^2 flask, and 0.3 to 0.5 ml of plasma containing AMV-B, MC29A, or both was added in a final volume of 4 ml of growth medium. The medium was changed after 24 h, and at day 4 the cells were split 1:4. They were then split as the growth rate required, the medium being changed every 3 to 4 days. In all cases, the cells were analyzed for oncogene expression and differentiation-associated properties approximately 2 weeks after infection.

Differentiation-associated properties. The differentiation-associated properties of Fc and C3 receptors, Fc-immune phagocytosis, and cellular morphology were examined as previously described (15, 24, 34). Briefly, Fc and C3 receptors were assayed by rosette formation with appropriately coated sheep erythrocytes. Fc-immune phagocytosis was assayed by determining the percentage of cells phagocytosing erythrocytes via the Fc receptor. Cellular morphology was determined with a Cytospin for cells growing in suspension and by growing adherent cells on cover slips (37). Cells were stained with May-Grunwald–Giemsa stain. Induction of differentiation by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was assessed as described previously (34).

[^{35}S]methionine labeling of cells and immunoprecipitations. The protocols for labeling suspended and adherent cells with [^{35}S]methionine and for immunoprecipitation have been described previously (21, 34). Immunoprecipitations were carried out with antisera raised to a portion of v-*myb*- (21) or v-*myc*- (2) encoded proteins. Blocking with bacterially expressed antigen was carried out as previously described (2, 21).

Indirect immunofluorescence. The method used has been described previously (22). Adherent cells were grown directly on glass cover slips, and cells growing in suspension were pelleted onto cover slips with a Cytospin. The cover slips were then washed with calcium- and magnesium-free phosphate-buffered saline (PBS) (this PBS formulation was used throughout). After fixation with 3.5% paraformaldehyde in PBS and brief washes with PBS, the cells were permeabilized by being treated with 0.2% Nonidet P-40 for 5 min at room temperature. The cells were then incubated with rabbit anti-*myb*, anti-*myc*, or normal rabbit serum (2, 21) at 1:50 dilution for 30 min at 37°C. After being washed extensively in PBS, the cells were stained with the same dilution of affinity-purified fluorescein-conjugated goat anti-rabbit IgG antibodies (Cappel Laboratories). Finally, they were rewashed with PBS, and the cover slips were mounted in 50% glycerol in PBS.

RESULTS

Superinfection of AMV-transformed cells with MC29. The cell line generally used in the present experiments, termed BM2, is a nonproducer line of AMV-transformed myeloblasts (25, 27, 29, 34). These cells grow in suspension and resemble myeloblasts. A clone of this line (BM2 clone 3) was used here. The expression of myeloid-associated properties of this line has been described previously (27, 34) and was confirmed here (Table 1); their morphology was that of myeloblasts, they had no C3 receptors, and they were not competent for Fc-immune phagocytosis. However, approximately 50% of the cells did have Fc receptors (Table 1). These cells expressed v-*myb*, the oncogene of AMV, producing a 45,000-molecular-weight protein termed p45$^{v\text{-}myb}$ (8, 21).

TABLE 1. Effect of MC29 superinfection on the phenotype of AMV-transformed cells[a]

Cells	Rosettes[b] (% of cells)		Fc-immune phagocytosis (% of cells)	Morphology[c] (% of cells)		
	Fc	C3		Blast	Intermediate	Mature
BM2	52 ± 4	0	0	93 ± 4	7 ± 4	0
BM2-MC29	50 ± 7	20 ± 4	25 ± 3	3 ± 2	97 ± 2	0

[a] Myeloid-associated properties were determined for BM2 cells, a nonproducer line of AMV-transformed myeloblasts, and BM2-MC29 cells, the BM2 cell line superinfected with MC29-B. The BM2-MC29 cells were cloned by limiting dilution 4 weeks after infection, and the myeloid-associated properties of several clones were determined 3 to 5 weeks later. The data presented are means ± standard deviation for at least three independent experiments with one of these clones; other clones gave similar results.

[b] Binding of appropriately coated sheep erythrocytes; percentage of cells with functional Fc and C3 receptors.

[c] Morphology was determined by May-Grunwald–Giemsa staining. Cells were divided into blast, intermediate, and mature categories by the ratio of nucleus to cytoplasm and the color and appearance of the nucleus and cytoplasm. Mature cells were all macrophages.

To determine the effect of v-*myc* on the phenotype of BM2 clone 3, the cells were superinfected with MC29 subgroup B. It was observed that approximately 2 weeks after superinfection, the majority of cells appeared larger and the growth rate had increased. The doubling time of cells decreased from approximately 30 h for BM2 cells to approximately 18 h for the superinfected cells. These superinfected cells were cloned by limiting dilution, and a number of clones were expanded. Both before and after cloning, when phenotypic markers of the myeloid lineage were examined, a number of differentiation markers were consistent with a more mature phenotype in the superinfected cells (Table 1). In contrast to BM2 cells, these cells (termed BM2-MC29) were larger (Fig. 1) and were found to have C3 receptors and to be competent for Fc-immune phagocytosis (Table 1).

In contrast to the results obtained by superinfection with MC29, when BM2 cells were infected with the Schmidt-Ruppin A strain of Rous sarcoma virus no effects on cell morphology or differentiation parameters were observed, even though the protein encoded by v-*src*, pp60$^{v\text{-}src}$, was synthesized (assessed by [^{35}S]methionine labeling and immunoprecipitation with anti-*src* serum) (data not shown). A slight increase in growth rate was observed, however (data not shown).

Expression of v-*myb* and v-*myc* in superinfected cells. Two weeks after superinfection of BM2 cells with MC29, expression of v-*myb* and v-*myc* was analyzed by indirect immunofluorescence. It was found that as for BM2 cells, most (90 to 100%) of the cells expressed v-*myb*. As previously shown for BM2 cells (7, 22), in BM2-MC29 cells the *myb*-specific fluorescence was predominantly nuclear. Unlike BM2 cells, however, the superinfected cells also expressed v-*myc*. As

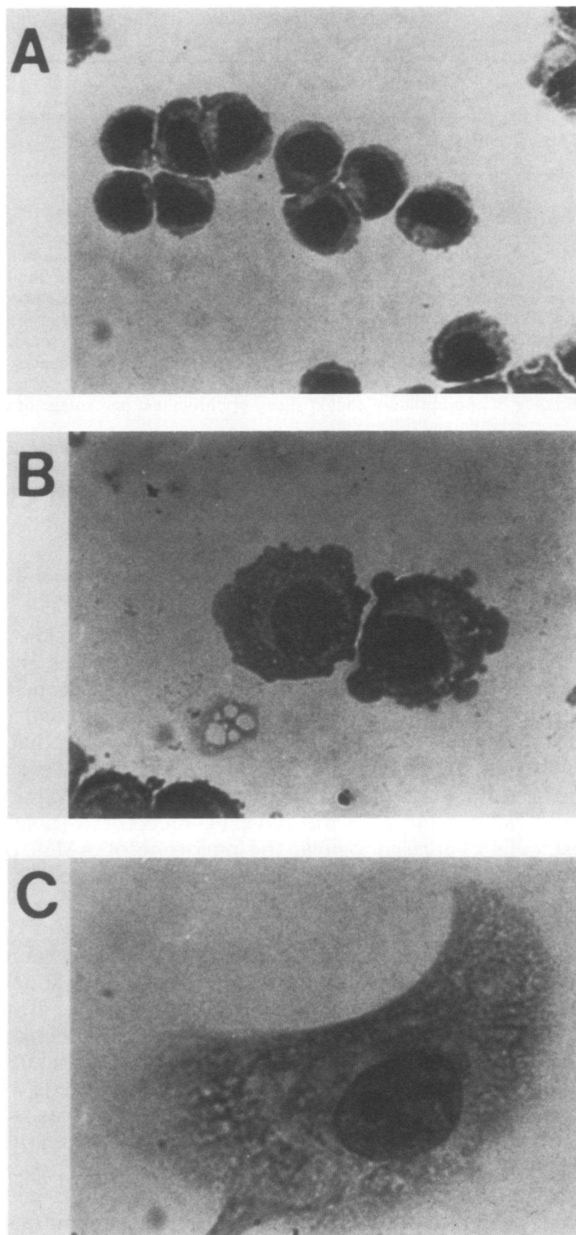

FIG. 1. Morphology of AMV-transformed myeloblasts before and after superinfection with MC29. (A) BM2 line of AMV-transformed myeloblasts; (B) BM2 cells superinfected with MC29; (C) normal macrophages derived from yolk sac. BM2 cells and yolk sac macrophages were cultured as described in Materials and Methods. The superinfected cells shown were stained 4 weeks after infection. Cells were stained with May-Grunwald–Giemsa and photographed with a Zeiss photomicroscope. The same magnification was used for all panels.

shown previously for other cell types (2, 11) this fluorescence was nuclear. By using anti-*myc* serum, it was found that approximately 80% of the cells exhibited nuclear fluorescence before cloning and 90 to 100% exhibited nuclear fluorescence after cloning (Fig. 2). Both before and after cloning, the cells expressing both viral oncogenes were larger than those expressing v-*myb* alone (Fig. 1 and 2). The *myb*-specific fluorescence, as shown previously for BM2 cells, was relatively weak compared with *myc*-specific fluorescence (Fig. 2). This may be due to different epitope presentation of the proteins within the nucleus.

To further test that the superinfected cells were indeed expressing the authentic v-*myc* gene and to determine its level of expression, BM2 and BM2-MC29 cells were radiolabeled with [^{35}S]methionine, and cell lysates were immunoprecipitated with anti-*myb*- or anti-*myc*-specific serum (Fig. 3). Two clones of BM2-MC29 cells were radiolabeled. It can be seen that (i) similar synthesis of v-*myb*-encoded protein occurred in BM2 and BM2-MC29 cells; (ii) this protein retained its characteristic apparent molecular weight of 45,000 in both cell types; and (iii) unlike BM2 cells, the superinfected cells expressed v-*myc* in addition to v-*myb*, synthesizing a protein of 110,000 apparent molecular weight. That this 110,000-M_r protein was indeed v-*myc*-encoded was confirmed by showing (i) its comigration with authentic p110$^{gag\text{-}myc}$ immunoprecipitated with anti-*myc* serum from lysates of the Q8 cell line of MC29-transformed fibroblasts (Fig. 3), (ii) the absence of a protein of this molecular weight from BM2 cell lysates immunoprecipitated with anti-*myc* serum (Fig. 3) and from BM2-MC29 cell lysates immunoprecipitated with either anti-*myb* serum (Fig. 3) or preimmune serum (data not shown), and (iii) the absence of this band from BM2-MC29 cell lysates immunoprecipitated with anti-*myc* serum that was pretreated with bacterially expressed *myc* antigen (data not shown). The other bands found (Fig. 3B, lanes 2 and 4) represent nonspecific immunoprecipitation; they were not blocked by *myc* antigen and they were also found after *myc*-specific immunoprecipitation of parental BM2 cells (Fig. 3). It was also found that the net synthesis of v-*myb*- and v-*myc*-encoded proteins was similar (Fig. 3).

Induction of differentiation in superinfected cells by TPA. It has previously been shown that BM2 cells can be induced to differentiate to fully mature nondividing macrophages by treatment with the phorbol ester TPA (30, 34), and this was confirmed here (Table 2). By contrast, treating BM2-MC29 cells with TPA induced differentiation of the cells but not to fully mature macrophages (Table 2). After treatment with TPA, BM2-MC29 cells morphologically resembled macrophages, and when compared with macrophages, a similar percentage of cells possessed Fc receptors. But certain properties were not expressed to the same degree as in mature macrophages: the percentage of cells expressing the C3 receptor was far lower (approximately 20% versus approximately 80% for mature macrophages), and unlike mature macrophages, the cells were only loosely adherent. In addition, unlike BM2 cells, BM2-MC29 cells treated with TPA did not cease dividing. The fact that Fc-immune phagocytosis did not increase in the BM2-MC29 cells after TPA induction is probably because TPA has an inhibitory effect on this activity (30, 34). These properties are characteristic of MC29-transformed macrophages (see following section and Table 3), indicating that unlike the parental BM2 cells, BM2-MC29 cells are induced by TPA to differentiate only to the phenotype characteristic of myelomonocytic cells transformed by MC29.

Infection of yolk sac macrophages with AMV and MC29. Macrophages cultured from the chicken yolk sac can be transformed by both AMV and MC29 (6, 13, 14, 25, 27). It has previously been shown (3, 13–15, 18, 25, 27) and was confirmed here (Table 3) that such macrophages are transformed by these two viruses to different phenotypic states: AMV elicits the outgrowth of cells resembling myeloblasts, whereas MC29 evokes the growth of a relatively mature cell resembling a macrophage. Uninfected macrophages have a

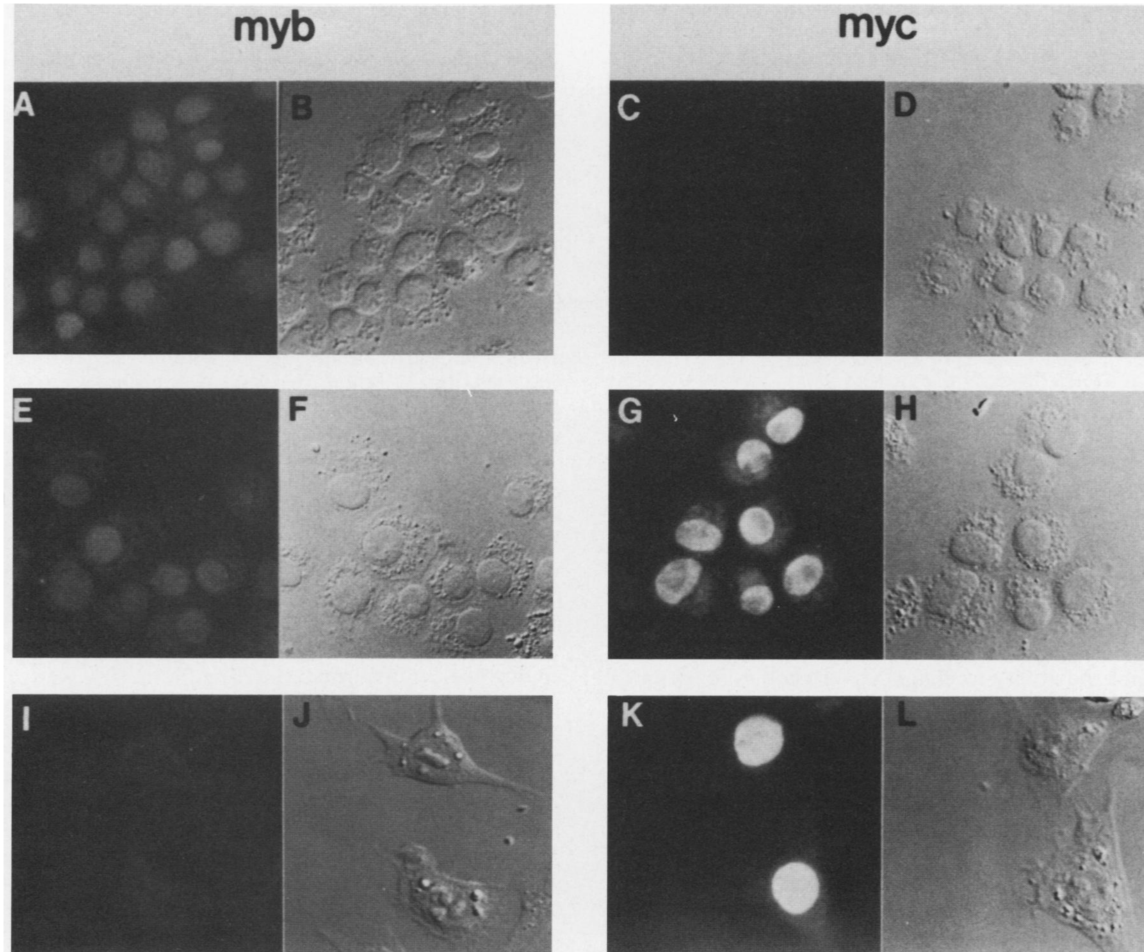

FIG. 2. Indirect immunofluorescence of cells expressing v-*myb* and v-*myc*. A–D, BM2 cells; E–H, BM2-MC29 cells; I–L, Q8 cells. Cells were stained with anti-*myb* (A, E, I) or anti-*myc* (C, G, K) serum. Nomarski differential interference contrast microscopy of cells treated with anti-*myb* (B, F, J) or anti-*myc* (D, H, L) serum. Cells were photographed with a Zeiss photomicroscope. The same magnification was used for all panels.

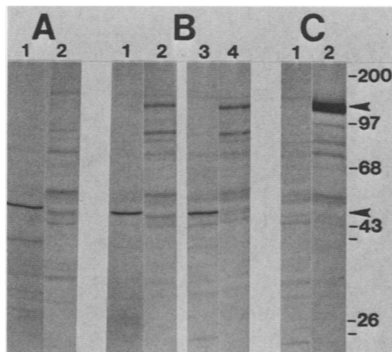

FIG. 3. Synthesis of p45$^{v\text{-}myb}$ and p110$^{gag\text{-}myc}$ in BM2 and BM2-MC29 cells. Cells were radiolabeled with [^{35}S]methionine, lysed, and immunoprecipitated with anti-*myb* or anti-*myc* serum. Cell extracts from approximately 3×10^6 cells (BM2 and BM2-MC29) or 1×10^6 cells (Q8) were run on a 10% sodium dodecyl sulfate-polyacrylamide gel and visualized by autoradiography. (A) BM2 cells; (B) BM2-MC29 clones 4 (lanes 1 and 2) and 7 (lanes 3 and 4); (C) Q8 cells. Lanes: 1 and 3, anti-*myb* serum; 2 and 4, anti-*myc* serum. Arrowheads, Position of p45$^{v\text{-}myb}$ and p110$^{gag\text{-}myc}$. Molecular weight (in thousands) is indicated at the right.

characteristic morphology (Fig. 1), and this was not significantly altered by infection with MC29 (Table 3) (3, 13–15, 25, 27). In addition, a high percentage (80 to 90%) of macrophages transformed by MC29 possessed Fc receptors and

TABLE 2. Induction of differentiation by TPA[a]

Cells	Inducer	Rosettes (% of cells)		Fc-immune phagocytosis (% of cells)	Morphology (% of cells)		
		Fc	C3		Blast	Intermediate	Mature
BM2	None	52 ± 4	0	0	93 ± 4	7 ± 4	0
	TPA	78 ± 6	70 ± 3	22 ± 4	0	10 ± 7	90 ± 7
BM2-MC29	None	50 ± 7	20 ± 4	25 ± 3	3 ± 2	97 ± 2	0
	TPA	84 ± 9	17 ± 5	29 ± 5	0	2 ± 2	98 ± 2
Mature macrophages	None	97 ± 4	78 ± 5	92 ± 5	0	0	100

[a] Cells (BM2 or BM2-MC29) were incubated with or without the tumor promoter TPA (250 μg/ml) for 4 days. TPA was added daily until day 3, and myeloid-associated properties were assayed on day 4. The cells and assays used are as described in Table 1, footnotes *a*, *b*, and *c*.

TABLE 3. Phenotype of yolk sac-derived macrophages infected with AMV, MC29, or both[a]

Infecting virus	Rosettes (% of cells)		Fc-immune phagocytosis (% of cells)	Morphology (% of cells)		
	Fc	C3		Blast	Inter-mediate	Mature
None	97 ± 4	78 ± 5	92 ± 5	0	0	100
AMV	60 ± 7	0	0	98 ± 1	2 ± 1	0
MC29	87 ± 5	15 ± 4	82 ± 7	0	7 ± 5	93 ± 5
AMV and MC29	69 ± 7	18 ± 2	45 ± 5	8 ± 3	58 ± 7	34 ± 2

[a] Myeloid-associated properties of secondary yolk sac-derived macrophages uninfected or infected with AMV-B, MC29A, or both viruses. In each case properties were determined 2 to 3 weeks postinfection. The assays used are described in Table 1, footnotes *a*, *b*, and *c*.

were functional for Fc-immune phagocytosis. This level was similar to that of uninfected macrophages (Table 3) (3, 13–15, 25, 27). Unlike uninfected macrophages, however, the majority of MC29-transformed macrophages did not possess C3 receptors (Table 3). By contrast, AMV-transformed macrophages had a myeloblast morphology, approximately 60% possessed Fc receptors, and they possessed neither C3 receptors nor the ability to phagocytose via the Fc receptor (Table 3). Thus, AMV-transformed macrophages resemble the BM2 cell line described previously (Table 1).

When yolk sac macrophages were infected simultaneously with both viruses, a heterogeneous population of cells was obtained (Table 3) within which the majority of cells (approximately 60%) morphologically resembled BM2-MC29 cells. Approximately 10% resembled myeloblasts and approximately 30% were morphologically macrophages. When these cells were examined by indirect immunofluorescence with anti-*myb* and anti-*myc* sera, it was found that the cells resembling BM2-MC29 cells expressed both v-*myb* and v-*myc*. Of the others, the cells resembling myeloblasts were found to express v-*myb* but not v-*myc*, whereas those resembling macrophages expressed either v-*myc* alone or neither (data not shown). In each case the fluorescence (when present) was nuclear (data not shown). The percentage of cells possessing Fc receptors and C3 receptors and capable of Fc-mediated phagocytosis is shown in Table 3.

DISCUSSION

Coordinate regulation of phenotype by v-*myb* and v-*myc*. The viral oncogenes v-*myb* and v-*myc* both transform myelomonocytic cells in vitro and in vivo, but they elicit characteristic and different phenotypes (3–6, 13–15, 18, 25, 27, 29, 30). AMV-transformed cells morphologically resemble myeloblasts, and yet a high percentage of these cells possess Fc receptors and have a high level of membrane ATPase, properties of more mature cells. By contrast, MC29-transformed cells morphologically resemble macrophages, but a low percentage of cells have C3 receptors and the cells have a low level of membrane ATPase, properties of less mature cells. Thus, in both cases a cellular phenotype is elicited which is not that of a single cell within the developmental lineage. In fact, it can be envisaged that transformation by v-*myb* and v-*myc* is accomplished by the conferral of an inappropriate phenotype on the target cell that does not allow the cell to respond to normal growth and differentiation signals in vitro or in vivo.

When both viral oncogenes were expressed, the resulting phenotype was again abnormal, although in this case aspects of the phenotypes induced by each oncogene alone were expressed. Thus, neither gene appears to completely suppress the action of the other; rather, they coordinately regulate cellular phenotype. Epistasis is a term referring to interference by one allele with the phenotypic expression of a nonallele. This is what appears to be happening here: the phenotype elicited by v-*myb* is interfered with by the expression of v-*myc*, and vice versa. Each gene is thus epistatic to the other for certain phenotypic properties (Table 4).

The mechanism of this interference is not clear but in each case may involve interaction of the two gene products with targets that are involved in the regulation of growth and differentiation—specifically, in the determination of myelomonocytic phenotype. A similar, albeit more subtle, effect has been shown for v-*erb*-A, which appears to confer a more transformed phenotype (in terms of susceptibility to spontaneous differentiation) on avian erythroblastosis virus-transformed erythroblasts (4, 19). Other types of coordinate gene regulation previously reported are (i) the ability of v-*mil* to release v-*myc*-transformed myeloid cells from growth factor dependence (1), (ii) the ability of v-*ets* (in concert with v-*myb*) to confer an erythroid or myeloid phenotype (depending on culture conditions) on E26-transformed hematopoietic cells (28, 31), and (iii) the ability of genes such as v-*myc* and E1a to complement activated c-*ras* genes in rat embryo cell transformation (23, 32).

It has been proposed that v-*myb* and v-*myc* function by blocking otherwise normal differentiation (18). However, as outlined above, the transformed phenotype elicited by each of these viral oncogenes is not that of a defined cell type of the myelomonocytic lineage. This has been interpreted as indicating that v-*myb* and v-*myc* suppress different macrophage properties, thereby causing specific derangements of normal macrophage differentiation (5, 6, 13, 14). Our data support this proposition and extend it by illustrating that these two genes are epistatic to one another for the expression of certain of these macrophage properties.

TPA-induced differentiation of v-*myb*- and v-*myc*-transformed cells. It has previously been shown that v-*myb*-transformed myelomonocytic cells can be induced to differentiate to fully mature macrophages by the tumor promoter TPA (22, 30, 34). By contrast, in the experiments reported here it was not possible to induce cells transformed by the combination of v-*myb* and v-*myc* beyond the characteristic v-*myc*-transformed phenotype. These results indicate that whereas the transforming effect of v-*myb* can be overcome

TABLE 4. Epistatic effects of v-*myb* and v-*myc*

Property	Phenotype[a] of myelomonocytic cells transformed by:		
	v-*myb*	v-*myb* and v-*myc*	v-*myc*
Sustained division	*myb*/*myc*	*myb*/*myc*	*myb*/*myc*
Doubling time	*myb*	*myc*	*myc*
Cell size	*myb*	D	*myc*
Morphology	*myb*	D	*myc*
Adherence	*myb*	*myb*	*myc*
Fc receptors	*myb*	*myb*	*myc*
C3 receptors	*myb*	*myc*	*myc*
Fc-immune phagocytosis	*myb*	D	*myc*

[a] *myb*, Property is that of a v-*myb*-transformed cell; *myc*, property is that of a v-*myc*-transformed cell; *myb*/*myc*, property is the same for both v-*myb*- and v-*myc*-transformed cells; D, property is distinctive from those of v-*myb*- and v-*myc*-transformed cells. Data are derived from Tables 1 and 3 and the text.

by TPA, that of v-*myc* cannot. As the mechanism of TPA-induced differentiation is not known, we can offer no explanation at present for the different ability of v-*myb* and v-*myc* to interfere with this differentiation process.

c-*myb* and c-*myc* in myelomonocytic differentiation. The expression of the two proto-oncogenes c-*myb* and c-*myc* in normal myelomonocytic development appears to be different. Both are expressed in normal immature myeloid cells (12, 16, 17), but as these cells differentiate to mature nondividing macrophages, the expression of c-*myb* appears to decrease before that of c-*myc* (16, 17). c-*myc* expression appears to be sustained in macrophages until replication ceases (16); a pattern of expression that appears to be analogous to the results presented here for v-*myb* and v-*myc*, i.e. a high level of v-*myb* is associated with an immature phenotype and a high level of v-*myc* with a relatively mature phenotype, but when both oncogenes are expressed in roughly equal amounts, the phenotype is intermediate. In light of the findings for the effects of v-*myb* and v-*myc* on myelomonocytic phenotype, an interpretation of the previously reported findings for the cellular genes might be that both c-*myb* and c-*myc* regulate myelomonocytic division, but c-*myc* is involved in the division of immature to relatively mature cells and c-*myb* in the division of relatively immature cells only. This hypothesis is presently being tested.

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