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Constitutive c-*ets*2 Expression in M1D+ Myeloblast Leukemic Cells Induces Their Differentiation to Macrophages

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The expression of c-*ets*2 is rapidly induced in a variety of myelomonocytic cell lines as they differentiate into macrophages. We find that constitutive expression of c-*ets*2 in the M1D+ myeloblast leukemic cell line (M1*ets*2) is sufficient to push these cells to a more differentiated state. The expression of several differentiation-specific genes is upregulated in M1*ets*2 cells, including those encoding macrophage-specific lysozyme M and tumor necrosis factor alpha, which are involved in bacteriolytic and inflammatory processes, respectively. Transcription factors c-*jun* and *jun*B, previously shown to induce partial macrophage differentiation when overexpressed in myelomonocytic leukemia cell lines, are also upregulated in M1*ets*2 cells. The upregulation of *jun*B is the result of a direct interaction of Ets2 with *ets* binding sites of the *jun*B promoter, since transient or constitutive Ets2 expression in M1D+ cells activates *jun*B transcription via *ets* binding sites. In addition, transfection of a dominant negative mutant of Ets2, devoid of its transcriptional activation domain, greatly reduces transcriptional activities of the *jun*B promoter in M1*ets*2 cells. Finally, unlike their parental M1D+ counterparts, M1*ets*2 cells secrete the macrophage colony-stimulating factor, CSF-1, and are able to phagocytize. Taken together, these results show that when the immature myeloid M1D+ cell line constitutively expresses c-*ets*2, these cells acquire different functions of mature macrophages.

c-*ets*2 was first identified (5, 12, 49, 50) by its sequence identity to v-*ets*, found in the genome of the avian E26 retrovirus (18, 32). Ets2 is a member of a large family of transcription factors with over 35 members described to date. The most highly conserved domain of approximately 85 amino acids, first identified by sequence comparisons between different Ets family members (5, 49), was shown to be the DNA binding domain (6), also known as the Ets domain (48). The Ets domains of the closely related Ets2 and Ets1, the progenitor of v-*ets*, are 98% identical, but Ets1 and Ets2 are more distantly related (identity of 35%) to another family member, PU.1/Spi.1 (reference 48 and references therein). All members of the Ets family bind to a core GGA sequence. However, the specificity of the different Ets members is provided by differences found in both the Ets domain and the DNA binding sequences flanking the GGA core (reference 48 and references therein). Ets1 is abundantly expressed in B and T lymphocytes (9, 13, 33, 34) but is not abundant in myelomonocytic lineages (12), although the c-*ets*1 transcript has been detected in chicken myelomonocytic cells (43). Ets2 is abundantly expressed in monocytes and macrophages of the myeloid lineage but not in granulocytes (7, 12). PU.1/Spi.1 is upregulated early during commitment of multipotential progenitors to myeloid lineages and is expressed in granulocytes, monocytes, macrophages, and B lymphocytes (reviewed in reference 25; see also references therein). Defects in PU.1/Spi.1 function result in defective development of myeloid and lymphoid progenitors (40, 47).

c-*ets*2 RNA and Ets2 protein are rapidly induced during myelomonocytic cell differentiation into macrophages in response to a variety of factors, some dependent upon activation of specific membrane receptors (7, 12). Ets2 expression appears to be regulated transcriptionally (7). The functional significance of the rapid increase of c-*ets*2 mRNA and Ets2 protein in myeloid cells remains to be elucidated. Since expression is activated in more mature myeloid cells rather than in immature myeloblast cells or in cells as they differentiate toward granulocytes, Ets2 may play an important role in the onset of macrophage differentiation.

The M1D+ murine leukemic myeloblast cell line (38) can differentiate into adhering macrophages able to phagocytize in the presence of the cytokine leukemia inhibitory factor (LIF) (11). This cell line was used to investigate the possible role of Ets2 in initiating the macrophage differentiation program.

MATERIALS AND METHODS

Cell culture and establishment of M1*ets*2 cells. M1D+ cells were maintained in Dulbecco's modified Eagle's medium containing 15% horse serum (Gibco-BRL). When LIF was added to M1D+ cells, concentrations of LIF ranged from 0.1 to 30 ng/ml as indicated below. BAC1.2F5 cells (27) were cultured in Dulbecco's modified Eagle's medium and 20% L-cell-conditioned medium as a source of colony-stimulating factor 1 (CSF-1) (44).

The complete human c-*ets*2 cDNA was inserted into the murine retroviral vector pLXSN containing the *neo* gene for selection (24). The murine ecotropic retrovirus packaging cell line PE501 (24) was transfected with this retroviral construct by calcium phosphate coprecipitation (51, 52). A viral supernatant was obtained 24 h posttransfection and was used to infect M1D+ cells. A *neomycin*-resistant pooled population of M1D+ cells constitutively expressing *ets*2 was obtained upon selection with 200 μg of active G418 (Geneticin; Sigma) per ml.

Assay of phagocytosis. Latex beads (1.1 μm) (LB-11; Sigma) resuspended in 1× phosphate-buffered saline (1× PBS) were added for 12 h to medium containing untreated M1D+, M1*ets*2, or BAC1.2F5 cells or to medium containing M1D+ or M1*ets*2 cells at the end of the various treatments; then cells were photographed. The latex beads are visualized as large black aggregates in cells.

Northern (RNA) hybridization analysis. Cells were left untreated or were treated with LIF for the times indicated. Cells were washed twice with 1× PBS. Cells were then lysed in RNA Insta-Pure (Eurogentec) as described by the manufacturer. Five micrograms of total RNA was loaded and electrophoresed on a 2.2 M formaldehyde–1% agarose gel and then transferred to a nylon membrane (Amersham) as described by the manufacturer. Full-length, purified c-*ets*2, lysozyme M, tumor necrosis factor alpha (TNF-α), c-*jun*, *jun*B, PU.1/Spi.1, Egr-1, and S26 cDNA fragments were used as probes. High-specific-activity probes were generated by using a Stratagene Prime-It kit as described by the manufacturer. Prehybridization and hybridization procedures were carried out at 42°C in a solution of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–0.5% sodium dodecyl sulfate (SDS)–50% formamide con-

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taining 20 μg of denatured salmon sperm DNA per ml. Normal-stringency wash procedures were performed at 50°C with 0.1× SSC–0.1% SDS. All mRNA transcripts were visualized after a 4- to 12-h exposure to BIOMAX film (Kodak) at −80°C with Dupont Quanta Fast intensifying screens.

Transactivation studies. The thymidine kinase (tk) promoter, alone or together with the *ets* binding sites (EBS) of the *jun*B promoter (10), was subcloned in the promoterless pXP1 vector upstream of the luciferase gene (31) to generate ptk-Luc or pEBS-tk-Luc, respectively. Ets2 or a dominant negative mutant of Ets2, Δ1-328Ets2, was cloned into pRK5 (39) to generate pRK5-Ets2 or pRK5Δ1-328Ets2, respectively. 293 cells were transfected by the calcium phosphate coprecipitation method in six well dishes with 0.5 μg of pEBS-tk-Luc in the presence of pRK5-Ets2 (0.5 μg) or pRK5Δ1-328Ets2 (2.5 μg) or both (0.5 and 2 μg, respectively) and 0.1 μg of pCMV-βGal as an internal control for transfection efficiency. All experiments using 293 cells were performed at least four times in duplicate (see Fig. 7 legend). M1 or M1*ets*2 cells (1×10^7/ml in 800 μl of 1× PBS) were transfected by electroporation (450 V, 500 μF) with 5 μg of pCMV-βGal, 5 μg of pEBS-tk-Luc, and 15 μg of pRK5-Ets2 or pRK5Δ1-328Ets2. Two different preparations of DNA were used for each independent experiment, and each experiment was repeated four times. Cell lysates were prepared as previously described (1). Briefly, 36 to 48 h after transfections, cells lysates were prepared in 25 mM Tris, pH 7.5–10% glycerol–1% Triton X-100–2 mM dithiothreitol and analyzed for luciferase and β-galactosidase activities as described by the manufacturers, Promega (luciferase assay) and Tropix (Galactolight β-galactosidase assay).

RESULTS

M1D+ cells were incubated with 30 ng of LIF per ml in order to investigate the level of c-*ets*2 expression as these cells differentiate into macrophages. Total RNA was isolated from the cells at various time periods after LIF treatment, and following electrophoresis and transfer to a nylon membrane, the RNAs were hybridized to c-*ets*2 as a probe. We found that the level of c-*ets*2 expression is low in the M1D+ cell line and that c-*ets*2 is not induced early in LIF-induced M1D+ differentiation (data not shown). This is in agreement with our previous observations with other myeloblast cell lines and in contrast to the increase of c-*ets*2 expression upon induction of differentiation of more mature myeloid lineages, including monoblasts, monocytes, and macrophages (7, 12). The Ets family member PU.1/Spi.1 is also expressed as myeloid cells differentiate into macrophages but is expressed as well in multipotent progenitor cells as they become committed to myeloid lineages (25, 47). PU.1/Spi.1 expression was already upregulated in untreated M1 cells (Fig. 1B) and remained upregulated over the 10-day LIF treatment period (data not shown). Therefore, this cell line was useful for investigating whether forced expression of c-*ets*2 in myeloblast M1D+ cells is sufficient to push these cells toward a more mature phenotype.

The human c-*ets*2 cDNA was subcloned into the pLXSN retroviral vector containing the *neo* gene as a selectable marker (24). After transfection into the ecotropic helper cell line PE501 (24), a viral supernatant was obtained and was used to infect murine M1D+ cells. Upon G418 selection, a mixed population of *neomycin*-resistant M1D+ cells was obtained. These cells spontaneously adhered to the bottom of the culture flasks early during G418 selection. Northern analysis showed that there is indeed c-*ets*2 mRNA (with the retroviral *ets*2 transcript migrating at approximately 5 kbp) expressed in these adherent M1D+ cells, and therefore we refer to these cells as M1*ets*2 cells (Fig. 1A). The level of expression of the viral *ets*2 transcript is roughly equivalent to the level of c-*ets*2 expression we observed following treatment with growth factors or protein kinase C activators (7). The endogenous 3.5-kb c-*ets*2 mRNA transcript is weakly visible upon a much longer exposure of RNA from M1D+ cells (data not shown).

Macrophages secrete lysozyme in their defense against bacterial infection. Since M1D+ cells synthesize and secrete lysozyme as they differentiate into macrophages (16), we compared the level of expression of the macrophage-specific lysozyme M transcript in control M1D+ and M1*ets*2 cells. We found that lysozyme M mRNA expression is low in untreated M1D+ cells and that its expression increases upon an 8-h LIF treatment of these cells (Fig. 1A). However, the level of the macrophage differentiation marker lysozyme M mRNA is upregulated in untreated M1*ets*2 cells.

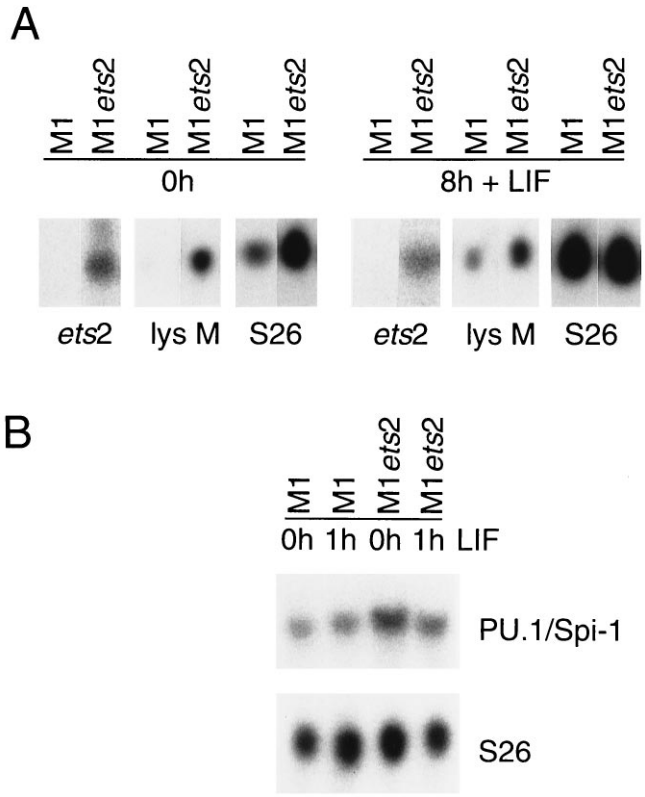

FIG. 1. Northern analyses. M1D+ (M1) or M1*ets*2 cells were left untreated (0 h) or treated with 30 ng of LIF per ml for 8 h (A) or 1 h (B). Five micrograms of total RNA isolated from these cells was electrophoresed, transferred to a nylon membrane, and hybridized to c-*ets*2 (*ets*2), lysozyme M (lys M), PU.1/Spi.1, or S26 (control for RNA quantification) probes.

Since macrophages are highly phagocytic cells, we investigated the ability of M1*ets*2 cells to phagocytize. Untreated control M1D+ cells are unable to phagocytize in the absence of LIF; however, upon LIF treatment M1D+ cells become phagocytic (Fig. 2A and B). We found that even in the absence of LIF treatment, M1*ets*2 cells are able to phagocytize. The BAC1.2F5 macrophage cell line (27) was used as a positive control for phagocytosis. These results show that the M1*ets*2 cells are able to perform a key function of macrophages, phagocytosis.

Since M1*ets*2 cells appear more mature than M1D+ cells, and since LIF induces M1D+ differentiation, we wanted to investigate whether M1*ets*2 cells would be more responsive to LIF treatment than M1D+ cells. Control M1D+ cells respond to LIF first by attaching to the bottoms of the culture dishes and second by becoming flattened and elongated. We compared M1*ets*2 cells and control M1D+ cells exposed to a range of LIF concentrations. M1D+ cells responded to a range of 0.1 to 30 ng of LIF per ml by forming adherent macrophage cells of an elongated, flattened shape, with optimal response at 10 to 30 ng/ml (data not shown). We found that at concentrations as low as 0.01 ng/ml, a small percentage of M1*ets*2 cells became elongated (data not shown). We compared the responses of M1D+ and M1*ets*2 cells to 0.1 ng of LIF per ml for 10 days.

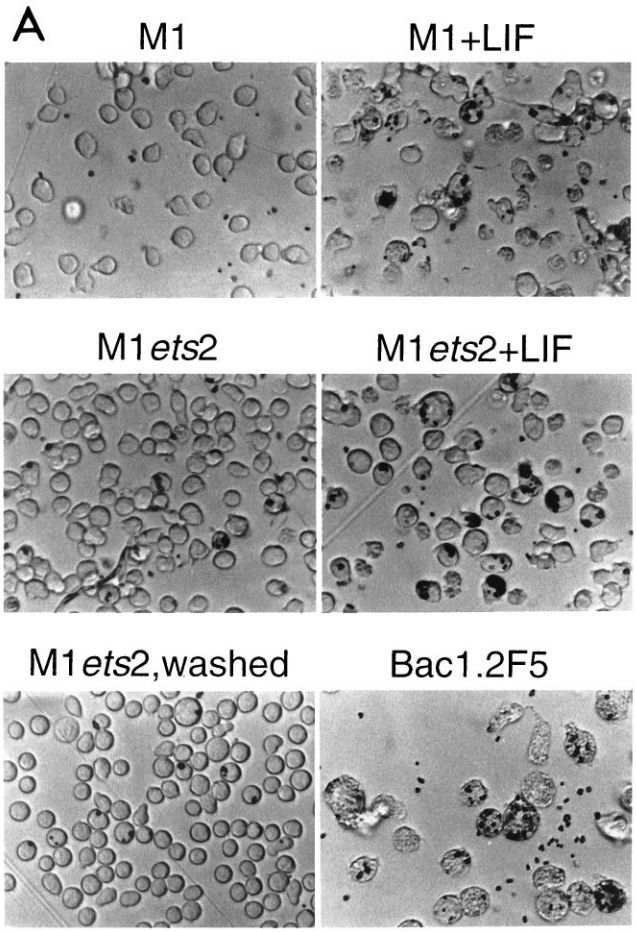

FIG. 2. Phagocytosis. (A) M1D+ (M1) or M1*ets*2 cells were untreated or treated with 30 ng of LIF per ml for 4 days. Control macrophage cells (Bac1.2F5) were maintained in conditioned medium containing CSF-1. Cells were then scraped and transferred to new culture dishes prior to addition of 1.1-μm latex beads. Beads were added to cells for 12 h, and cells were then photographed. M1*ets*2 cells were then washed several times with 1× PBS and rephotographed to demonstrate that the beads were indeed in the cells. Large aggregates of beads are apparent as large black particles in cells. (B) Cells photographed in this and other experiments were counted, and the percentage of cells that were phagocytic was calculated and is represented here graphically.

After incubation with LIF, cells were washed and maintained for 14 days in its absence. As can be seen in Fig. 3, unlike control M1D+ cells, M1*ets*2 cells maintain a differentiated phenotype. This phenotype was irreversible over a test period of 5 weeks (data not shown). This shows that LIF treatment induces a differentiated phenotype and that once this phenotype is obtained, it is maintained in M1*ets*2 cells. These differentiated cells were shown to be macrophages, as determined by their increased ability to phagocytize latex beads (data not shown).

It was shown that long-term LIF treatment of M1D+ cells results in upregulation of c-*fms* mRNA (36, 46), which encodes the CSF-1 receptor (CSF-1R) (41). We have shown that CSF-1 and LIF act synergistically to induce M1D+ differentiation (2). We therefore asked whether CSF-1 is secreted from M1*ets*2 cells, which could account for the maintenance of the differentiated macrophage phenotype of M1*ets*2 cells following LIF treatment. Cells of the BAC1.2F5 macrophage cell line are dependent on CSF-1 for their growth and survival (27). When media conditioned by M1D+ or M1*ets*2 cells were added to these cells, conditioned media from M1*ets*2 cells, but not conditioned media from M1D+ cells, supported BAC1.2F5 cell growth (Fig. 4A). When a neutralizing anti-mouse CSF-1 antibody (44) was included with the conditioned media from M1*ets*2 cells, the BAC1.2F5 cells died (Fig. 4B), whereas an antibody to an unrelated antigen (anti-USF18-105 [35]) had no blocking effect on BAC1.2F5 proliferation (data not shown). These results show that CSF-1 is secreted from M1*ets*2 cells and that M1*ets*2 cells resemble macrophages in their secretory functions.

Since the M1*ets*2 cells we selected were adherent, we wanted to rule out the possibility that the newly acquired characteristics of these cells were simply the result of their adherence and to show that these characteristics were due to a bona fide effect of *ets*2 expression in these cells. To this end, we obtained an M1D+ adherent (M1ad) cell population by selecting for adherent cells during continuous culture of M1D+ cells in liquid media in the absence of LIF. This process took 8 weeks for M1D+ cells but only 2 weeks for M1*ets*2 cells. First we tested the ability of M1ad cells to respond to LIF. After a 4-day treatment with 30 ng of LIF per ml, a small percentage of M1ad cells became elongated (Fig. 5A and B). Under the same conditions, there was a higher percentage of elongated M1*ets*2 cells (Fig. 5A and B), suggesting that these cells are more sensitive to induction of differentiation by LIF. RNA was extracted from untreated M1ad cells and untreated M1*ets*2 cells and was subjected to Northern analysis with probes for lysozyme M, c-*jun*, *jun*B, and TNF-α (Fig. 6). Several differences in mRNA expression were observed between these two lines. First, lysozyme M mRNA was not detected in M1ad cells. Therefore, although lysozyme M upregulation coincides with adherence in LIF-treated M1D+ cells, this expression is not a consequence of adherence. Second, c-*jun* and *jun*B have been implicated in the LIF signaling pathway and LIF-induced differentiation of M1D+ cells, since both c-*jun* and *jun*B mRNA levels increase rapidly in response to LIF and remain high (20). We found that both of these RNAs are upregulated in M1*ets*2 cells compared with M1ad cells. Finally, we found that the

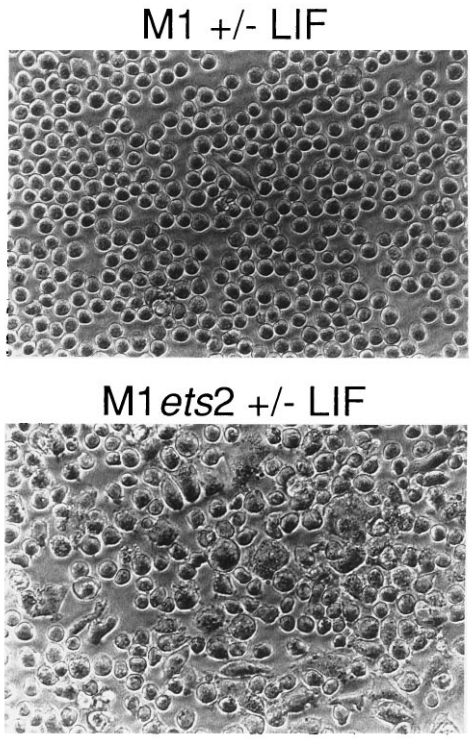

FIG. 3. Cell morphology after LIF removal. M1D+ (M1) and M1*ets*2 cells were treated with 0.1 ng of LIF per ml for 10 days. Cells were then maintained in culture in the absence of LIF for an additional 14 days and photographed.

expression of TNF-α mRNA is upregulated in M1*ets*2 cells compared with M1ad cells. TNF-α is secreted from macrophages and plays an important role in inflammatory responses. Taken together, the morphological responses to LIF and the upregulation of TNF-α, lysozyme M, and the transcription factors c-*jun* and *jun*B in M1*ets*2 cells are not just consequences of adherence but are due to the constitutive expression of *ets*2 in these cells.

Coffer et al. (10) have shown that Ets2 can bind to the EBS at position −147 to −124 in the *jun*B promoter and activate transcription. An EBS-tk promoter fragment (10) was subcloned into a promoterless vector upstream of the luciferase gene to generate pEBS-tk-Luc, and its ability to be transactivated by cytomegalovirus-driven Ets2 in transient transfection studies using 293 cells was investigated (Fig. 7). These cells are known to be easily transfected and are thus a convenient system for monitoring Ets2 transactivating potential. In the following transactivation experiments, pCMV-βGal was used to correct for variations in transfection efficiency. The luciferase activity obtained by using the empty expression vector (Ø) and ptk-Luc was set at 1 arbitrary unit. In agreement with previous studies (10), Ets2 strongly activated transcription via this EBS, with luciferase activities increasing approximately 1,300-fold. However, increases as measured by luciferase activities are much higher than those previously published (1,300-fold versus 14-fold). These differences are most likely due to the higher sensitivity of luciferase assays compared with chloramphenicol acetyltransferase assays, as well as to the different cell types used in both studies. The observed stimulatory effect is indeed the result of Ets2 interacting with its target sequences, since no significant activation was detected in the absence of EBS (Fig. 7A, Ets2/tk-Luc). We constructed a dominant negative mutant of Ets2, pRK5Δ1-328Ets2, containing the DNA binding domain in the absence of the transcriptional activation domain. When pRK5Δ1-328Ets2 was coexpressed with full-length pRK5-Ets2, the transcriptional activation was reduced by more than 50% (Fig. 7A).

Once optimal conditions were determined in 293 cells, we investigated the effects of Ets2 in transactivation experiments

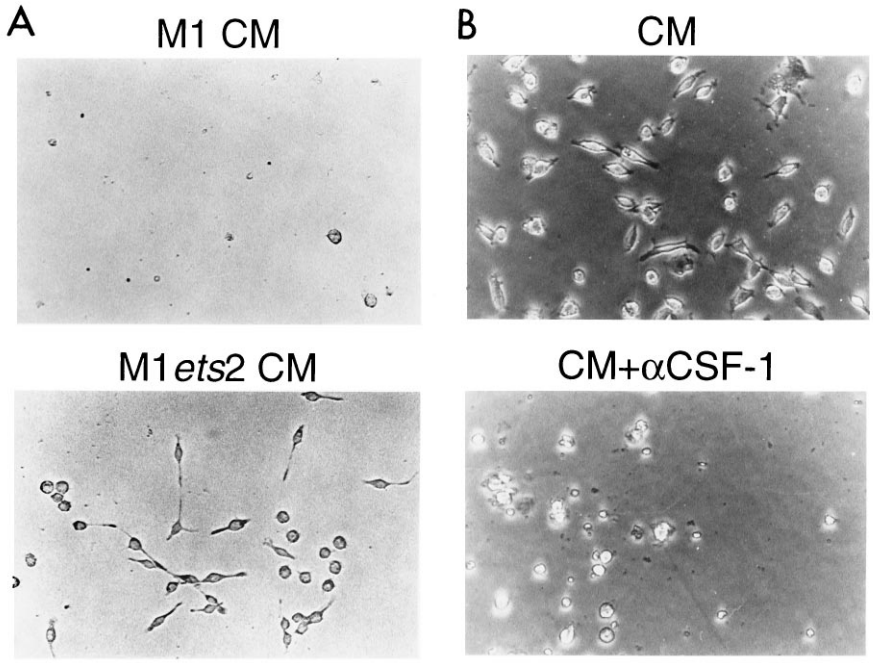

FIG. 4. BAC1.2F5 cell survival. (A) Conditioned media from M1D+ cells (M1 CM) or M1*ets*2 cells (M1*ets*2 CM) were added directly to BAC1.2F5 cells for 5 days; then BAC1.2F5 cells were photographed. (B) Conditioned media from M1*ets*2 cells were added to BAC1.2F5 cells for 5 days in the absence (CM) or presence (CM+αCSF-1) of a neutralizing anti-mouse CSF-1 antibody; then cells were photographed.

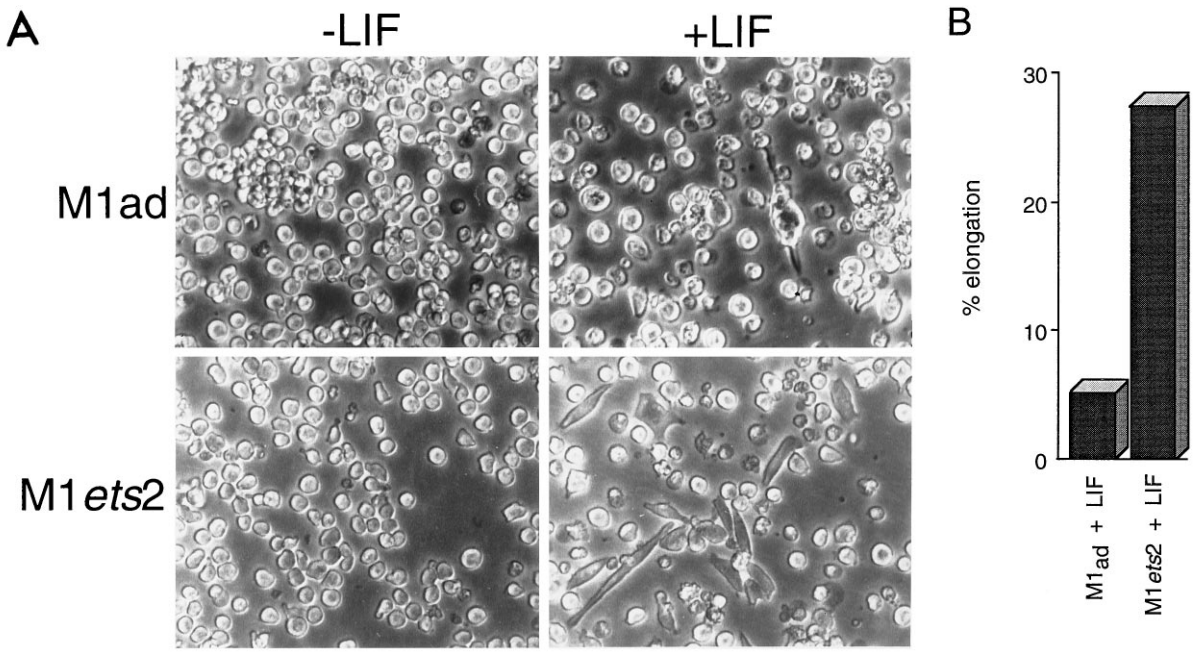

FIG. 5. LIF-induced morphological changes. (A) Photographs of adherent M1D+ (M1ad) and M1*ets*2 cells grown in the absence or presence of 30 ng of LIF per ml for 4 days. (B) Percentage of elongated cells determined from this and other experiments.

using M1 or M1*ets*2 cells (Fig. 7B). M1 cells, refractory to calcium phosphate coprecipitations, were electroporated with pEBS-tk-Luc and either pRK5 or pRK5-Ets2. pCMV-βGal was used as a control of transfection efficiency. The β-galactosidase activities obtained indicated that the efficiency of transfection was quite low (data not shown). However, a fourfold increase in luciferase activity was detected (Fig. 7B, M1 + Ets2) over that observed for the empty expression vector, pRK5 (Fig. 7B, M1 + Ø). Similar results were obtained when M1*ets*2 cells were transfected with the pEBS-tk-Luc reporter construct and pRK5 (Fig. 7B, M1*ets*2 + Ø). The dominant negative mutant, pRK5Δ1-328Ets2, greatly reduced luciferase activity in M1*ets*2 cells (Fig. 7B, M1*ets*2 + ΔEts2). These results demonstrate that Ets2 can activate transcription of the *jun*B promoter via the EBS in M1*ets*2 cells, albeit at low levels, which are likely to reflect the limitation of the M1D+ and M1*ets*2 cell transfections.

DISCUSSION

Blood progenitor cells become committed to one of several lineages in the presence of a variety of cytokines, growth factors, and hormones. Disruption of the balance between these molecules and the respective responses of progenitor cells results in aberrant cell growth. Several Ets family members have been implicated in leukemias. The E26 retrovirus, which expresses the viral gag-Myb-Ets fusion protein, results in erythroid and myeloid leukemias in chickens (15, 28, 29). The Ets family members PU.1/Spi.1 and Fli-1 are activated in Friend erythroid leukemias in mice due to retroviral insertion of spleen focus-forming virus or murine leukemia virus, respectively (3, 26). In humans, several different translocations involve Ets family members, including Ets2 (17), Erg (42), and TEL (8, 14), in myeloid leukemias. We have previously shown that Ets2 is rapidly upregulated in a variety of myelomonocytic cell lines as they differentiate into macrophages (7, 12), and we have proposed that Ets2 may play a role in macrophage differentiation (7).

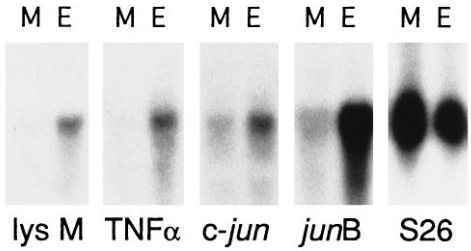

FIG. 6. Northern analysis using different macrophage differentiation markers. Total RNA (5 μg each) was isolated from M1ad (M) and M1*ets*2 (E) cells. Following electrophoresis and transfer to a nylon blot, these RNAs were hybridized to the following cDNA clones used as probes: lysozyme M (lys M), TNF-α, c-*jun*, and *jun*B. S26 was used as a control of quantification.

Macrophages play an important role in host defense. These cells are phagocytic and highly secretory. Products secreted from macrophages include proteases and lysozyme as well as modulatory factors like TNF-α, which is important in inflammatory responses. In this report, we show that constitutive expression of c-*ets*2 in the leukemic myeloblast M1D+ cell line results in the adherence of cells, the upregulation of mRNAs of different macrophage-specific markers, and the ability of these cells to phagocytize and to secrete CSF-1. These results show that M1*ets*2 cells, unlike their parental counterparts, can perform functions of mature macrophages in the absence of a differentiation factor. We and others have shown that CSF-1 alone induces adherence and some phagocytosis of M1D+ cells (2), that Ets2 can transcriptionally activate the CSF-1R promoter in a macrophage cell line (37), and that CSF-1R mRNA expression and promoter activity increase during LIF-induced M1D+ cell differentiation (36). Since constitutive *ets*-2 expression in M1D+ cells induces the secretion of CSF-1, one possible mechanism might be that Ets2 transactivates the expression of the CSF-1R and that via CSF-1/CSF-1R signaling, adherence and phagocytic capacities are induced in these

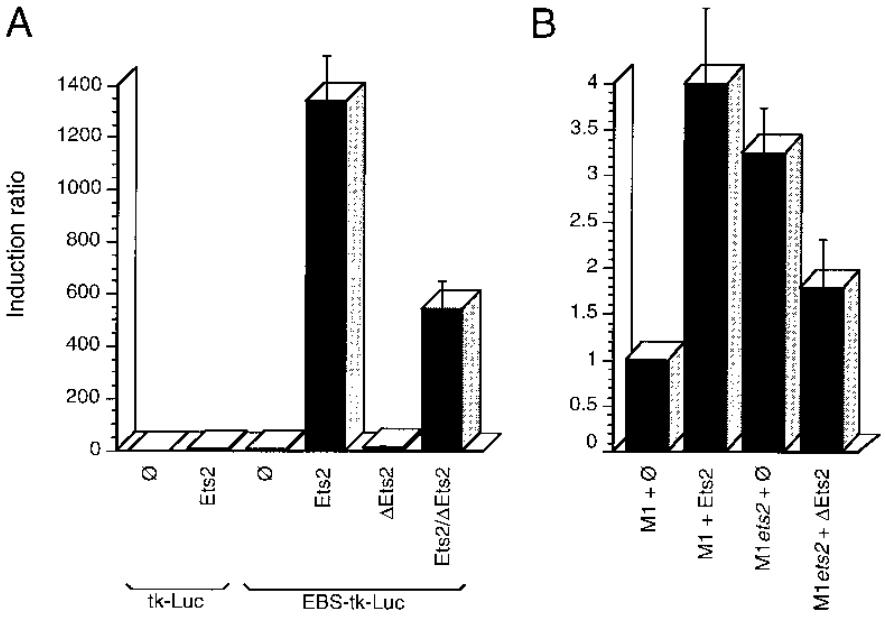

FIG. 7. Transactivation studies. (A) 293 cells were transfected with pEBS-tk-Luc (EBS-tk-Luc) in the presence of pRK5-Ets2 (Ets2) or pRK5Δ1-328Ets2 (ΔEts2) or both (with ΔEts2 used at a 4-fold excess with respect to Ets2) and pCMV-βGal as an internal control for transfection efficiency. The induction ratios were obtained from six independent experiments performed in duplicate. The luciferase activity of 1 arbitrary unit was determined after transfection of 293 cells with ptk-Luc (tk-Luc) and pRK5 (Ø). All cotransfections of 293 cells with ptk-Luc and either pRK5 or pRK5-Ets2 were performed four times in duplicate. (B) M1 or M1*ets*2 cells were transfected by electroporation with pCMV-βGal, pEBS-tk-Luc, and either pRK5, pRK5-Ets2, or pRK5Δ1-328Ets2. Experiments shown in panel B were performed four times, and two different preparations of DNA were used for each experiment.

cells. M1*ets*2 cells, like M1D+ cells, change morphology following a short-term, low-dose treatment with LIF. However, M1*ets*2 cells are more responsive to LIF than are M1D+ cells: a higher percentage of M1*ets*2 cells change morphology, this change in morphology is observed with lower concentrations of LIF, and once LIF is removed, this macrophage morphology is maintained in M1*ets*2 cells. We have shown that CSF-1 acts synergistically with LIF to induce M1D+ cell differentiation (2). Since CSF-1 is secreted from the more mature M1*ets*2 cells and not from M1D+ cells, it is possible that the addition of LIF results in higher transcriptional activation of the CSF-1R, which induces further M1*ets*2 morphological changes via CSF-1/CSF-1R signaling.

Several transcription factors are upregulated as M1D+ cells differentiate into macrophages. Egr-1 is one such factor, and Egr-1 antisense oligomers in the culture media were shown to block macrophage differentiation of M1D+ cells (30). Since Egr-1 contains potential EBS in its promoter (23), we investigated the expression of Egr-1 in M1*ets*2 cells by Northern analysis. Egr-1 was not upregulated in untreated M1*ets*2 cells, and the increase in Egr-1 expression in LIF-treated M1*ets*2 cells was similar to that observed in LIF-treated M1D+ cells (data not shown), indicating that Egr-1 is not a direct target of Ets2. Transcription factors c-*jun* and *jun*B have been shown to be rapidly and stably induced upon LIF treatment of M1D+ cells (20). Several laboratories have shown that constitutive expression of either c-*jun* or *jun*B results in partial macrophage differentiation (19, 21, 45). These results suggest that there is some redundancy in *jun* family members and that they play a role in inducing initial steps in macrophage differentiation. We observed that constitutive expression of *ets*2 in M1D+ cells results in the upregulation of both c-*jun* and *jun*B mRNA. Ets2 can bind to EBS in the *jun*B promoter and activate *jun*B transcription (10). Here we showed that either transient or constitutive expression of Ets2 in M1D+ cells results in transcriptional activation of the *jun*B promoter via the EBS. These results demonstrate that the upregulation of *jun*B is a result of direct interaction of Ets2 upon the *jun*B promoter.

c-*ets*2 is expressed in a wide variety of tissues, as determined by in situ hybridization, Northern, and immunoprecipitation analyses, and therefore c-*ets*2 has been proposed to be involved in cell proliferation and differentiation (4, 7, 22). In this report, we showed that forced expression of c-*ets*2 in immature M1D+ leukemic myeloblasts is sufficient to do the following: (i) upregulate the expression of c-*jun* and *jun*B (following promoter activation, at least in the case of *jun*B), previously shown to partially induce macrophage differentiation; (ii) upregulate the expression of several macrophage-specific genes, including lysozyme M and TNF-α; (iii) induce the secretion of CSF-1, a macrophage growth factor which induces survival, growth, and differentiation of mononuclear phagocytes; and (iv) induce phagocytosis, a key function of macrophages. Thus, these data clearly demonstrate that c-*ets*2 indeed plays a key role in macrophage differentiation.

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