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IL-3 AND GM-CSF INDUCE THE EXPRESSION OF THE INOSITOL TRISPHOSPHATE RECEPTOR IN K562 MYELOBLAST CELLS

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When treated with IL-3 plus GM-CSF, K562 myeloblast cells acquired the ability to mobilize nonmitochondrial stores of intracellular Ca^{2+} in response to added Ins(1,4,5)P_3. Untreated K562 cells are capable of sequestering intracellular Ca^{2+} but released none of this Ca^{2+} in response to Ins(1,4,5)P_3. Untreated K562 cells were shown to have no detectable specific [^{3}H]Ins(1,4,5)P_3 binding sites and no $InsP_3$ receptor mRNA as assayed by Northern blot and PCR. However, following IL-3 and GM-CSF treatment, both a single class of low nM K_D Ins(1,4,5)P_3 binding site and a 10 kb $InsP_3$ receptor mRNA were detectable. The results suggest that IL-3 and GM-CSF regulate the expression of the Ins(1,4,5)P_3 receptor gene.

Ins(1,4,5)P_3 is an established second messenger for intracellular Ca^{2+} mobilization (1,2). This activity is mediated through a specific $InsP_3$ receptor which has properties of a ligand-regulated Ca^{2+} channel (3,4). There is significant tissue and cell variation in the amount of $InsP_3$ receptor mRNA (5) and it is has been shown that the level of the $InsP_3$ receptor protein is specifically increased in some hormone-treated cells (6). From this, it is clear that regulation of second messenger Ca^{2+} signaling may reside, in part, at the level of expression of the $InsP_3$ receptor.

K562 is a cell line established from a patient with chronic myelogenous leukemia in blast crisis and has features of a pluripotent myeloid stem cell (7). High afffinity receptors for IL-3 and GM-CSF have been demonstrated on human pluripotent myeloid progenitor cells and the administration of IL-3 and GM-CSF to these

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Abbreviations: IL-3, recombinant human interleukin-3; GM-CSF, recombinant human granulocyte macrophage-colony stimulating factor; $InsP_3$, inositol 1,4,5-trisphosphate.

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cells has been shown to promote hematopoietic growth and maturation (8,9). Since the $InsP_3$ receptor appears to be critical for differentiated myeloid cell function, we examined whether $InsP_3$ receptor expression is regulated by growth and differentiation factors in K562 cells. In this report, we document that K562 cells have nearly undetectable $InsP_3$ receptor mRNA, $InsP_3$ receptor protein, and $InsP_3$-regulated Ca^{2+} mobilization, but acquire these macromolecules and functions after incubation with IL-3 and GM-CSF.

MATERIALS AND METHODS

[^{3}H]Ins(1,4,5)P_3 (17 Ci/mmol) was purchased from New England Nuclear. Human recombinant IL-3 and GM-CSF were purchased from US Biochemicals and Boehringer Mannheim, respectively. K562 cells were obtained from American Type Culture Collection and passaged in RPMI 1640 media supplemented with 4 mM glutamine, 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Determination of [Ca^{2+}] using fura-2 in permeabilized K562 cells

K562 cells were washed in phosphate-buffered saline and resuspended at 4 x 10^6/ml in an intracellular salts solution composed of 20 mM NaCl, 100 mM KCl, 1 mM $MgCl_2$, and 30 mM Hepes, pH 7.3. Two mls of cells were placed in quartz cuvettes along with digitonin (25 μM) and the fluorescent Ca^{2+} indicator fura-2 (2 μM) and maintained at 22°C. Oligomycin (10 μg/ml) and antimycin A (10 μM) were added to inhibit mitochondrial function and fluorescence measured using a Shimadzu RF-5000 spectrofluorometer. After 5 to 10 min, ATP (5 mM) was added to initiate uptake of ambient Ca^{2+} into nonmitochondrial vesicular stores. After 2 min, $InsP_3$ (3 μM) or ionomycin (1 μM) was added to initiate release of stored Ca^{2+}. Excitation wavelengths were 340 nm and 380 nm and emission was monitored at 500 nm. Ca^{2+} concentrations were calculated from 340/380 ratios after subtraction of autofluorescence (10).

Northern blot and PCR analysis of $InsP_3$ receptor mRNA

Poly A^+ RNA was prepared from cells by guanidinium thiocyanate homogenization followed by CsCl centrifugation and chromatography of the reprecipitated RNA over oligo dT cellulose columns. Poly A^+ RNA (3-5 μg) was electrophoresed through 1% agarose-formaldehyde gels and blotted onto Gene Screen Plus (NEN) nylon membranes. After blocking, membranes were probed with ^{32}P-labelled IP3R1 DNA. The IP3R1 DNA was derived by PCR amplification, subcloning, and purification of a 482 bp segment of the human $InsP_3$ receptor cDNA. This DNA was sequenced and confirmed to contain DNA corresponding to bp 489-970 of the mouse $InsP_3$ receptor cDNA (11). For polymerase chain reaction (PCR) analysis, poly A^+ RNA was reverse transcribed into DNA and amplified using specific primers corresponding to bases 410-434 and the complement to bases 946-970.

[^{3}H]Ins(1,4,5)P_3 binding assay

Preparation of K562 cell membranes and assay of [^{3}H]Ins(1,4,5)P_3 binding were performed essentially as described for HL-60 cells (6). Nonspecific binding was determined in the presence of 10 μM Ins(1,4,5)P_3. Data were analyzed by the ELF/EDBA LIGAND program.

RESULTS AND DISCUSSION

When permeabilized with digitonin, both untreated K562 cells and K562 cells treated for two days with GM-CSF (10 pM) plus IL-3 (167

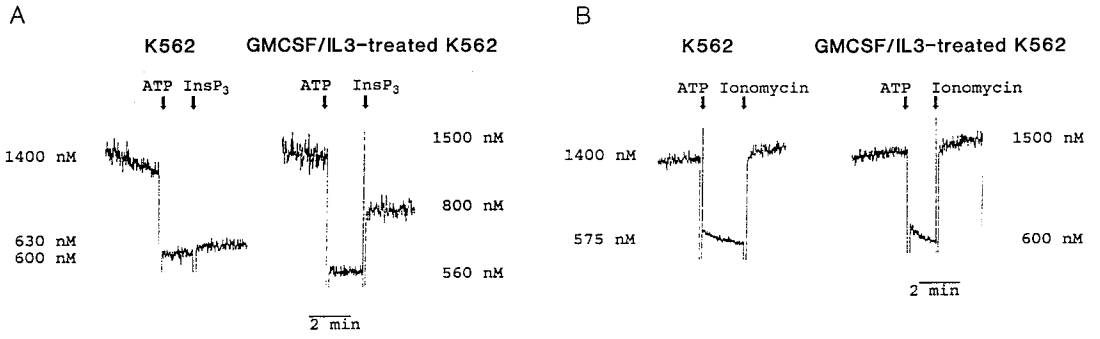


Figure 1. **Effect of GM-CSF and IL-3 treatment on (A) $InsP_3$-induced and (B) ionomycin-induced Ca^{2+} release from permeabilized K562 cells.** Untreated and 3-day GM-CSF/IL-3 treated K562 cells were permeabilized with digitonin in the presence of fura-2 and mitochondrial inhibitors. ATP and then either $InsP_3$ (3 μM) or ionomycin (1 μM) were added as indicated and changes in Ca^{2+} concentration monitored by fluorescence. Ordinates show calculated Ca^{2+} concentrations. Duplicate measurements from three different passages of cells showed similar results.

pM) sequester ambient Ca^{2+} into nonmitochondrial vesicular stores in response to ATP. However, only the GM-CSF/IL-3-treated cells can mobilize this stored Ca^{2+} in response to added $InsP_3$ (Figure 1A). The fact that both untreated and GM-CSF/IL-3-treated cells took up Ca^{2+} to the same extent and that ionomycin released the entire ATP-dependent Ca^{2+} store (Figure 1B) suggests that both cell types have similar intracellular Ca^{2+} pumping capabilities and that the storage capacities of these cells are approximately equal. The amount of Ca^{2+} released from GM-CSF/IL-3-treated cells after the addition of 10 μM $InsP_3$ was no different than that released by 3 μM $InsP_3$, indicating that a saturating amount of $InsP_3$ was used in these experiments. The results suggest that there is an absolute increase in functional $InsP_3$ receptors following GM-CSF/IL-3 treatment of K562 cells.

The $InsP_3$ receptor content of cells was assessed independently by [^{3}H]$InsP_3$ binding studies. Using membranes from K562 cells, no specific binding could be detected over a [^{3}H]$InsP_3$ concentration range of 0.3-20 nM. However, specific binding was detectable in membranes from GM-CSF/IL-3-treated K562 cells. The data in Table 1 summarize [^{3}H]$InsP_3$ binding parameters derived from Scatchard analysis of GM-CSF/IL-3-treated K562 cell membranes. The B_{MAX} (29 fmol/mg) is less than that observed in other cell membrane preparations but the K_D (ca. 12 nM) is similar to previous reports (12,13). The results confirm that treatment of K562 cells with GM-CSF plus IL-3 increases the content of $InsP_3$ receptor.

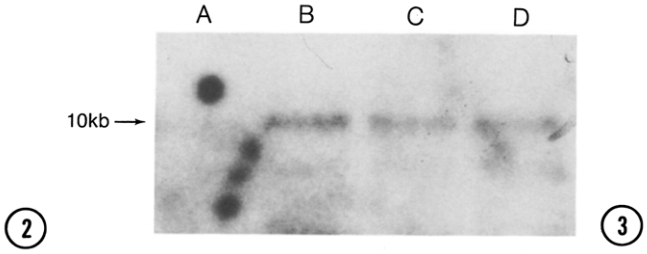
TABLE 1. Effect of GM-CSF and IL-3 Treatment of K562 Cells on

K562 Cell Treatment	K_D	B_{MAX}
	nM	fmol/mg
Untreated	ND[a]	ND[a]
GM-CSF/IL-3	11.6	29.0

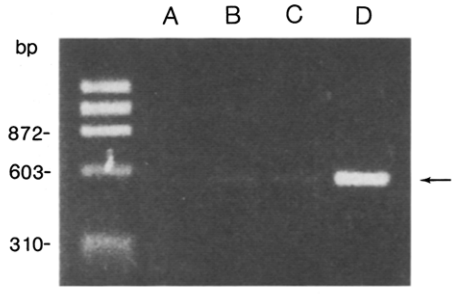
[a]ND= no detectable specific binding.

[^{3}H]InsP$_3$ Binding Parameters. Binding of [^{3}H]InsP$_3$ to membranes derived from untreated K562 cells and from cells treated for 3 days with GM-CSF (10 pM) and IL-3 (167 pM) was performed as described in Materials and Methods. Values shown are from a single experiment representative of two seperate cell preparations.

Northern blot and PCR analysis reveal the probable basis of these findings. Using a radiolabelled probe specific for InsP$_3$ receptor mRNA, the data in Figure 2 show that there was very little or undetectable 10 kb InsP$_3$ receptor mRNA in K562 cells. However, following 2 days treatment with either IL-3 or GM-CSF, InsP$_3$ receptor mRNA was significantly elevated and treatment with the combination of GM-CSF and IL-3 gave more InsP$_3$ receptor mRNA than either agent alone. β-Actin mRNA levels were unchanged by IL-3 and GM-CSF treatment (data not shown). PCR analysis of reverse transcribed mRNA confirmed both the deficiency of InsP$_3$ receptor



(2)



(3)

Figure 2. **Northern blot analysis of poly A$^+$ RNA from (A) untreated, (B) GM-CSF/IL-3-treated, (C) GM-CSF-treated, and (D) IL-3-treated K562 cells.** K562 cells were incubated with cytokines for 2 days, poly A$^+$ RNA prepared, electrophoresed, blotted, and the blot probed with an InsP$_3$ receptor specific probe. 10 kb InsP$_3$ receptor size is shown.

Figure 3. **PCR analysis of cDNA from (A) untreated, (B) IL-3-treated, (C) GM-CSF-treated, and (D) IL-3/GM-CSF-treated K562 cells.** cDNA from 2-day treated K562 cells was amplified with specific primers corresponding to bases 410-434 and the complement to bases 946-970. The product DNA was separated on agarose gels along side of standard-sized DNA fragments as shown. The predicted PCR product is 560 bp, shown by arrow.

mRNA in K562 cells as well as the enhancement in $InsP_3$ receptor mRNA following GM-CSF/IL-3 treatment (Figure 3).

From these studies it can be concluded that there is a relative deficiency of functional $InsP_3$ receptor in K562 cells compared to cells treated with IL-3 and GM-CSF. The lack of $InsP_3$-mediated Ca^{2+} mobilization in K562 cells is consistent both with the lack of $InsP_3$ receptor protein as determined by radioligand binding studies and with the undectable level of $InsP_3$ receptor mRNA, as determined by both Northern blot and PCR analyses. Treatment of K562 cells with GM-CSF and IL-3 rapidly increased the expression of $InsP_3$ receptor mRNA, $InsP_3$ receptor protein, and $InsP_3$ receptor mediated Ca^{2+}-mobilization.

These results are significant because they represent the first demonstration of regulation of $InsP_3$ receptor expression at the nucleic acid level and suggest that part of the pathological dysfunction of the K562 myeloid leukemic cell may reside in a deficiency in $InsP_3$-mediated Ca^{2+} signalling. Furthermore, the results suggest that signal transduction processes initiated at cell surface GM-CSF and IL-3 receptors on K562 cells culminate in nuclear events which allow enhanced expression of the $InsP_3$ receptor gene. Further study of $InsP_3$ receptor gene transcription in these and normal myeloid cells may elucidate specific genetic mechanisms by which hematopoietic hormones effect cell growth and differentiation.

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