Journal of Cellular Biochemistry 71:441–448 (1998)

Differentiation of HL-60 Promyelocytic Leukemia Cells Is Accompanied by a Modification of Magnesium Homeostasis

F.I. Wolf,[1]* V. Covacci,[1] N. Bruzzese,[1] A. Di Francesco,[1] A. Sacchetti,[2] D. Corda,[2] and A. Cittadini[1]

[1]Institute of General Pathology and Giovanni XXIII Cancer Research Center, Catholic University of Sacred Heart, 00168 Roma, Italy
[2]Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio Mario Negri Sud, 66030 S. Maria Imbaro (Chieti), Italy

Abstract Magnesium homeostasis in HL-60 promyelocytic leukemia cells was compared to that in neutrophyl-like HL-60 cells obtained by 1.3% DMSO treatment. Magnesium homeostasis was studied by the characterization of magnesium efflux, the identification of intracellular magnesium pools, and the regulation of intracellular ionized Mg^{2+}. In both undifferentiated and neutrophyl-like HL-60 cells, magnesium efflux occurred via the Na-Mg antiporter which was inhibited by imipramine and stimulated by db cAMP and forskolin. Receptor-mediated signals such as ATP, IFN-α, or PGE1, which can trigger cAMP-dependent magnesium efflux, were ineffective in undifferentiated HL-60 cells but induced 60–70% increase of magnesium efflux in neutrophyl-like HL-60 cells. Selective membrane permeabilization by the cation ionophore A23187 induced a large magnesium release when cells were treated with rotenone. In both cell populations, the addition of glucose to rotenone-treated cells restored magnesium release to the control level. Permeabilization by 0.005% digitonin provoked the release of 90% cell total magnesium in both cell types. Intracellular $[Mg^{2+}]_i$ was 0.15 and 0.26 mM in undifferentiated and neutrophyl-like HL-60 cells, respectively. Stimuli that triggered magnesium efflux, such as db cAMP in undifferentiated and IFN-α in neutrophyl-like HL-60 cells, induced a slow but consistent increase of $[Mg^{2+}]_i$ which was independent from Ca^{2+} movements. Overall, these data indicate that magnesium homeostasis is regulated by receptor-mediated magnesium efflux which was modified during differentiation of HL-60 cells. Stimulation of magnesium efflux is paralleled by an increase of $[Mg^{2+}]_i$ which reflects a release of magnesium from the bound cation pool. J. Cell. Biochem. 71:441–448, 1998.

Key words: proliferation; maturation; intracellular magnesium pools; receptor-mediated stimuli; cyclic-AMP; IFN-α; cell permeabilization; ionophore A23187; Na-Mg antiporter

Magnesium is recognized as an essential cation involved in crucial steps of proliferation and differentiation processes such as DNA replication, protein synthesis, and transphosphorylation reactions [Cowan, 1995]. While the biochemical interactions of magnesium have long been studied in vitro, knowledge about its homeostasis at a cellular level has been precluded by several factors. For example, specific and sensitive assays for magnesium are still lacking. Inasmuch as magnesium is very abundant and ubiquitously distributed, these technical limitations make it difficult to detect small changes of magnesium against a high background. Moreover, other cations and especially Ca^{2+} can interfere with magnesium measurements [Hurley et al., 1992]. The information available about cell magnesium homeostasis can be summarized as it follows. In the majority of cells studied, magnesium efflux is regu-

Abbreviations used: ATP, adenosine triphosphate; cAMP, cyclic-AMP; $[Ca^{2+}]_i$, intracellular ionized calcium concentration; db cAMP, dibutyryl cyclic-AMP; DMSO, dimethylsulphoxyde; EGF, epidermal growth factor; FCS, fetal calf serum; IFN-α, interferon alpha; IFN-γ, interferon gamma; LDH, lactate dehydrogenase; $[Mg^{2+}]_i$, intracellular ionized magnesium concentration; PBS, phosphate buffer saline; PGE1, prostaglandin E-1; PKA, protein kinase A; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PMA, phorbol 12-myristate 13-acetate.

Contract grant sponsors: MURST; Italian Association for Cancer Research; Italian National Council.

*Correspondence to: Federica I. Wolf, Institute of General Pathology, School of Medicine, Catholic University, Faculty of Medicine, L.go F. Vito 1, 00168 Rome, Italy. E-mail: ibipg@rm.unicatt.it

Received 4 May 1998; Accepted 23 June 1998

lated by a secondary active mechanism, the Na-Mg antiporter, which exchanges intracellular Mg^{2+} with extracellular Na^+ [Flatman, 1991; Vormann and Günther, 1993]. Depending on cell type, both influx and efflux seem to be under the control of PKA or PKC [Wolf et al., 1996; Romani and Scarpa, 1995; Günther and Vormann, 1992]. Magnesium is compartmentalized within the cell; however, a clear-cut picture of its subcellular distribution and related transport mechanisms is not available at present [Romani et al., 1993; Rutter et al., 1990]. ATP is the most powerful chelator for magnesium but certainly not the only one, as many other molecules have a high affinity for magnesium. Overall, intracellular ionized magnesium, $[Mg^{2+}]_i$, is tightly buffered under physiological conditions [Romani and Scarpa, 1992]; hence, only a few cells such as neurones or myocardiocytes can significantly increase their $[Mg^{2+}]_i$ in response to stimulation by glutamate or EGF, respectively [Brocard et al., 1993; Grubbs, 1991].

Several observations suggest an interesting relationship between magnesium and cell proliferation and differentiation. It has been shown that magnesium restriction in culture media is able to induce phenotypic differentiation in virus-transformed fibroblasts [Rubin, 1981]. Other studies described different magnesium fluxes in proliferating vs. differentiated cells [Maguire, 1988; Rubin, 1982; McKeehan and Ham, 1978]. We have recently described that magnesium restriction induces granulocytic differentiation of HL-60 cells and that these cells displayed a decrease of magnesium content and an intracellular cation distribution similar to that of cells differentiated by chemical agents [Covacci et al., in press].

On the basis of these observations, we have extended our studies of magnesium homeostasis and regulation in undifferentiated cells as compared to their terminally differentiated counterpart. To this end, we have used HL-60 cells, a human promyelocytic leukemia cell line which can be easily induced to differentiate by treatment with various agents such as DMSO, retinoic acid, or PMA [Collins et al., 1978].

Our results show that magnesium homeostasis is influenced by receptor-mediated stimuli capable of inducing magnesium efflux. Furthermore, we show for the first time that stimulation of magnesium efflux accompanies with a rise of $[Mg^{2+}]_i$, probably reflecting a depletion of the pool of bound magnesium. These processes occur in neutrophyl-like but not in undifferentiated cells, which thus exhibit a larger pool of bound magnesium. We propose that changes in magnesium content and distribution are physiological events accompanying cell differentiation.

MATERIALS AND METHODS

The human promyelocytic leukemia HL-60 cell line was maintained in log phase by seeding twice a week at a density of 3×10^5/ml. Cells were cultured in RPMI 1640 medium (Bio Whittaker, Walkerville, MD) supplemented with 10% heat-inactivated FCS (Biological Industries, Rehovot, Israel) and 2 mM glutamine in a 5% CO_2 atmosphere at 37°C. HL-60 cells were induced to differentiate toward neutrophyl-like granulocytes by treatment with 1.3% DMSO for 7 days [Collins et al., 1978].

For experimental incubations, HL-60 cells were washed in serum-free RPMI and subsequently suspended in ice-cold Mg-free Ringer solution. Viable cells were counted after staining with trypan blue (0.04%). Final cell suspensions were usually 10×10^6 cells/ml, with a viability >95%. Incubations were carried out at 37°C in a Mg-free medium, referred to as *0-trans* incubations. Magnesium-free Ringer was composed of 120 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 20 mM Hepes-Na, pH 7.4, and 10 mM glucose. In Na-free incubations, Na was substituted with an equimolar amount of cholineCl, and Hepes was buffered with KOH. Where physiologic magnesium was required, 0.5 mM $MgSO_4$ was added to the incubation medium.

Magnesium efflux under *0-trans* conditions and cell total magnesium were measured on cell supernatants or pellets, respectively, by atomic absorption spectrometry (model 212; Perkin Elmer, Oak Brook, IL) as previously described [Wolf et al., 1996].

Intracellular free Mg ($[Mg^{2+}]_i$) determinations were carried out on mag-fura 2–/loaded HL-60 cells by a spectrofluorimeter (model CM1T11L, Spex Industries, Edison, NJ), with excitation at 335–370 nm and emission at 510 nm, as detailed previously [Wolf et al., 1997]. During free-magnesium measurements, a small but constant increase of signal was invariably detected. To correct for this background, data are reported as net $[Mg^{2+}]_i$ calculated by comparing coupled observations of control and treated cells. $[Ca^{2+}]_i$ was measured in fura2-loaded cells as described in Bizzarri and Corda [1994].

Proteins were measured by Bio-Rad (Richmond, CA) protein assay system using bovine serum albumin (BSA) as standard.

All analytical grade chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO). IFN-α (Intron A) was from Schering-Plough Pharmaceuticals (Berlin, Germany).

Deionized double distilled water (>18 MΩ) was used throughout.

For magnesium efflux experiments, data are presented as mean ± SE (number of experiments). When SEs are not provided, data represent a typical experiment repeated at least three times with similar results. In some cases, magnesium efflux is expressed as percent variation over control. In the case of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ measurements, vertical bars represent SD. Statistical analyses have been performed by an unpaired Student's *t*-test, with significance being considered when $P < 0.05$.

RESULTS

Magnesium efflux from HL-60 cells, determined by incubating cell suspensions under *0-trans* conditions (nominally Mg-free medium) is summarized in Figure 1. In control conditions, magnesium efflux from undifferentiated HL-60 cells averaged 15.4 ± 1.0 nmol/mg protein/30 min incubation (n = 18), and this value was assumed as 100% (Fig. 1A). In order to investigate the mechanism of magnesium efflux, we treated cells with imipramine, a well-known Na-channel blocker. This tricyclic antidepressant drug, which is recognized as the most effective inhibitor of the Na-Mg antiporter [Feray and Garay, 1988], elicited a 60% inhibition of magnesium efflux. Accordingly, substitution of extracellular Na with an equimolar amount of CholineCl determined inhibition of magnesium efflux identical to that obtained by imipramine. Figure 1A also reports the results of experiments aimed at investigating the regulation of magnesium efflux. Elevation of cell cAMP content by either the permeable analogue db cAMP or forskolin, a potent adenylcyclase activator, had a stimulatory effect leading to a 40% increase of magnesium efflux. To the contrary, receptor-mediated stimuli such as ATP, IFN-α, or PGE1, which we have previously shown to stimulate magnesium efflux from lymphocytes and Ehrlich tumor cells [Wolf et al., 1996, 1997], did not induce any significant in-

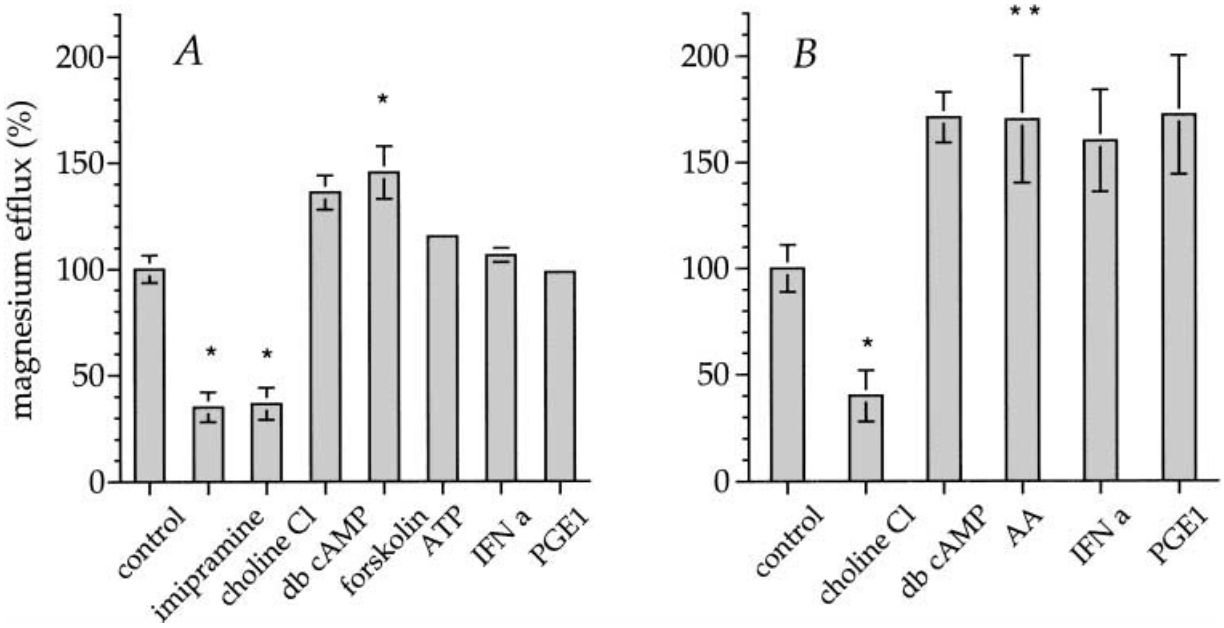

Fig. 1. Magnesium efflux from undifferentiated and neutrophyl-like HL-60 cells. HL-60 cells were incubated under *0-trans* conditions; magnesium efflux was evaluated after 30 min at 37°C. **A:** Undifferentiated HL-60 incubated with imipramine (0.3 mM), 130 mM choline Cl which substitutes NaCl, db cAMP (0.1 mM), forskolin (50 μM), ATP (50 μM), IFN-α (1,500 IU/ml), PGE1 (20 nM). **B:** Same experiments performed with neutrophyl-like HL-60 cells. AA, arachidonic acid (10 μM). Magnesium efflux is expressed as % variations from the control. Data represent mean of five separate experiments ± SE. When vertical bars are not visible, SE falls within the line. *$P < 0.05$ vs. control. **$P < 0.005$ vs. control.

crease of magnesium efflux from undifferentiated HL-60 cells.

Figure 1B reports parallel data obtained on neutrophyl-like HL-60 cells. Control magnesium efflux, which was of 4.5 ± 0.5 (m = 13) nmol/mg protein/30 min (100%), was inhibited by 60% by extracellular Na substitution with CholineCl, confirming that also in these differentiated cells the Na-Mg antiporter was mostly responsible for magnesium extrusion. However, magnesium efflux was substantially stimulated not only by db cAMP but also by arachidonate, IFN-α, and PGE1. In particular, we were interested in further investigating the effect of IFN-α on magnesium efflux from HL-60 cells, and the results of these studies are reported in Figure 2. It is evident that 50–1,500 IU/ml of IFN-α increased magnesium efflux from neutrophyl-like HL-60 cells but not from undifferentiated HL-60 cells. In these latter, IFN-α above 1,500 IU/ml seemed to inhibit magnesium efflux.

Magnesium content and efflux were therefore quantitatively and qualitatively different in undifferentiated and neutrophyl-like HL-60 cells. In an attempt to better characterize this different pattern, we studied the effect of selective permeabilization of the two cell populations incubated under different metabolic conditions. To this end, cells were incubated with or without 6.7 μM rotenone, a well-known inhibitor of mitochondrial respiration. After 30 min incubation, we added the cation ionophore A23187 which, under the *0-trans* condition, induces Mg^{2+} release in exchange with extracellular Ca^{2+}. Based on titration experiments, a concentration of 20 mM A23187 was chosen for this study. Figure 3A shows that the addition of A23187 to rotenone-treated undifferentiated HL-60 cells substantially increased the release of magnesium; however, this effect could not be observed in cells coincubated with rotenone plus glucose. Inasmuch as tumor cells can efficiently produce ATP by anaerobic glycolysis [Wolf and Cittadini, 1988], these data indicate that the release of magnesium by A23187 is inversely related to the cell ATP content. Similar results were obtained with neutrophyl-like HL-60 cells (Fig. 3B). It is interesting to note that when glucose was added to rotenone-treated cells, magnesium release by A23187 was the same as in control conditions, showing that these phagocytic cells can equally form ATP by anaerobic glycolysis.

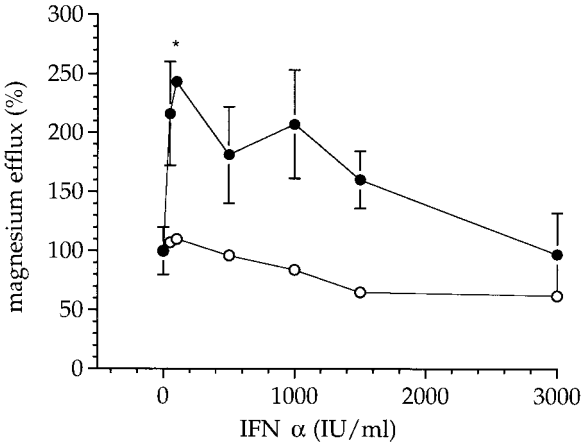

Fig. 2. Regulation of magnesium efflux by IFN-α in undifferentiated and neutrophyl-like HL-60 cells. Results, expressed as % of the control, represent magnesium efflux after 30 min at 37°C in the presence of different concentrations of IFN-α. Open circles, undifferentiated cells; closed circles, neutrophyl-like HL-60. Data are mean ± SE from three to five experiments. *$P <$ 0.0001 vs. control (without IFN-α).

A different kind of membrane permeabilization can be obtained with digitonin [Grubbs et al., 1984]. In keeping with other studies, 0.005% digitonin was shown to induce a 90% release of cell LDH, representative of selective plasma membrane permeabilization. Figure 4 shows that the addition of 0.005% digitonin caused a rapid (1 min) and complete release of magnesium from both undifferentiated and differentiated cells, accounting for 90% of cell total cation (118.6 vs. 133.4 and 94.6 vs. 104.5 nmol/mg protein, respectively). Provided that cells were not exposed to metabolic inhibitors that affect ATP levels, these data indicate that digitonin treatment allows for a complete release of ATP-Mg from both cell populations.

In order to further investigate intracellular magnesium homeostasis in undifferentiated and neutrophyl-like HL-60 cells, we measured $[Mg^{2+}]_i$ on mag-fura 2AM–loaded cells. The $[Mg^{2+}]_i$ was 0.15 ± 0.03 (m = 11) and 0.26 ± 0.07 (m = 10) mM in undifferentiated and neutrophyl-like HL-60 cells incubated under *0-trans* conditions, respectively ($P < 0.0001$). These results confirm previous data showing that $[Mg^{2+}]_i$ is higher in differentiated than in undifferentiated cells [Covacci et al., in press]. Parallel measurements of $[Ca^{2+}]_i$ on fura/2– loaded cells showed that under the same experimental conditions $[Ca^{2+}]_i$ was 168.0 ± 18.5 (m = 3) and 152.3 ± 13.7 (m = 3) nM in undifferentiated and neutrophyl-like HL-60 cells, respectively.

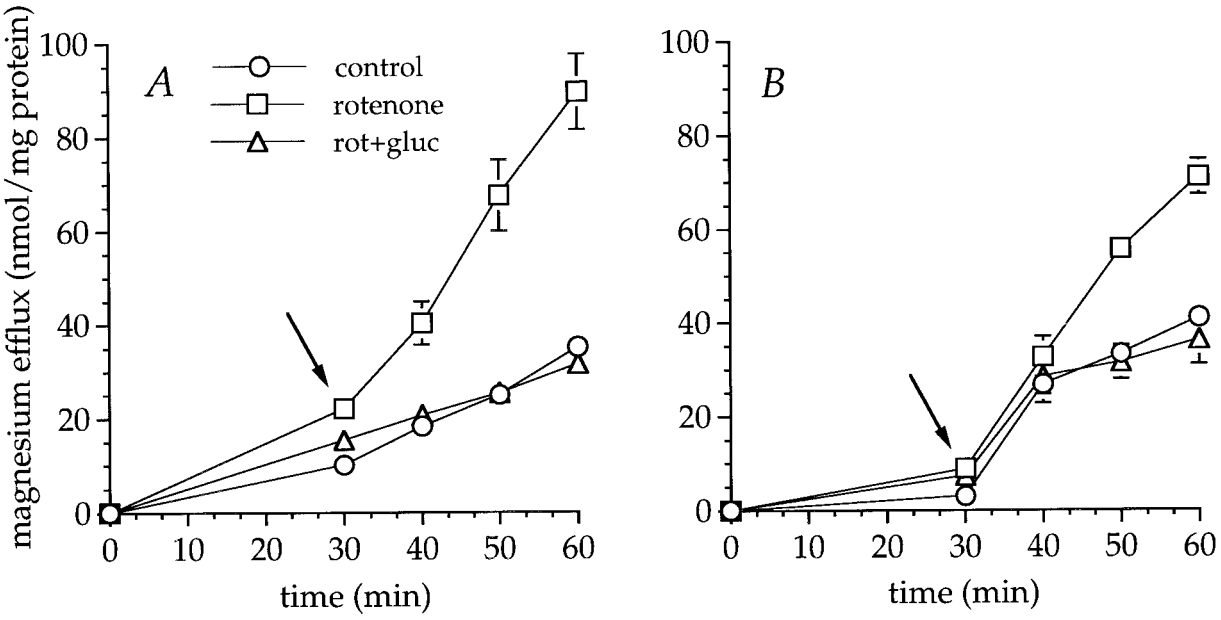

Fig. 3. Effect of A23187 on magnesium release from undifferentiated and neutrophyl-like HL-60 cells. **A:** Undifferentiated HL-60 cells were incubated in control condition and in the presence of rotenone (rot) (6.7 μM) ± 10 mM glucose (gluc). At 30 min, 20 μM A23187 was added (arrow). **B:** Same experiments performed in neutrophyl-like HL-60 cells. Arrow, 20 μM A23187 added at 30 min. Data are mean ± SE of three separate experiments.

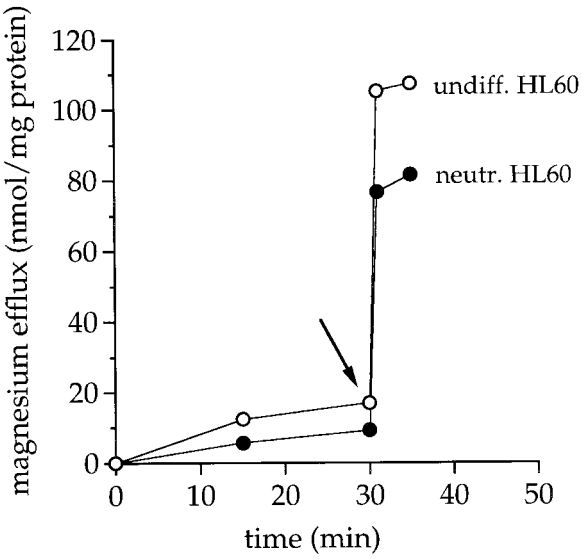

Fig. 4. Effect of digitonin upon magnesium release from undifferentiated and neutrophyl-like HL-60 cells. Cells were incubated under *0-trans* conditions at 37°C and magnesium efflux evaluated at 30 min. At this time (arrow), 0.005% digitonin was added. Typical experiments repeated at least three times with similar results.

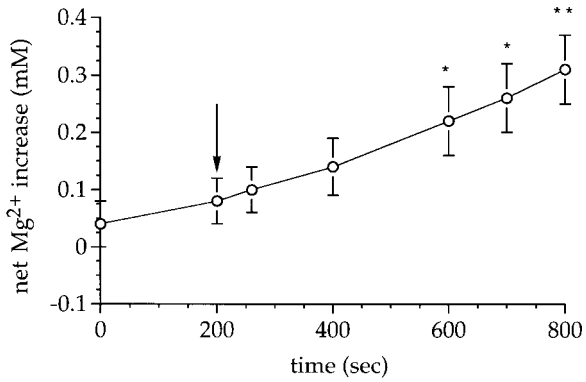

Fig. 5. Effect of db cAMP on $[Mg^{2+}]_i$ in undifferentiated HL-60 cells. Data represent net $[Mg^{2+}]_i$ increase obtained by comparing three coupled measurements of control and db cAMP-treated cells. Vertical bars represent SD. Arrow, addition of db cAMP, 0.1 mM. *$P < 0.05$ vs. values at 200 sec. **$P < 0.005$ vs. values at 200 sec.

In order to investigate the effect of the stimulation of magnesium efflux on $[Mg^{2+}]_i$, we treated HL-60 cells with db cAMP, which was able to increase magnesium efflux from undifferentiated HL-60 cells. Results in Figure 5 show that, after db cAMP addition, $[Mg^{2+}]_i$ increased slowly and constantly, approaching a net increase of 0.25 mM in 13 min. In principle, the positive D values shown in Figure 5 at time 0 and 200 sec might suggest that cAMP-treated cells have basal $[Mg^{2+}]_i$ higher than controls. However, this was not the case, since the mean value for the two sets of samples lies within the SD, as indicated by the absolute $[Mg^{2+}]_i$ value reported above (0.15 ± 0.03 mM). In addition, magnesium efflux from neutrophyl-like HL-60

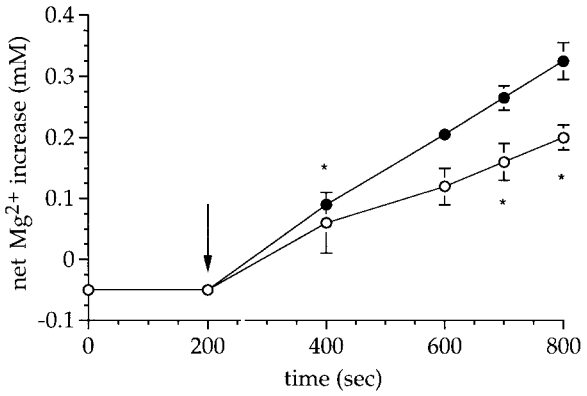

Fig. 6. Effect of db cAMP and IFN-α on $[Mg^{2+}]_i$ in neutrophyl-like HL-60 cells. Data represent net $[Mg^{2+}]_i$ of three coupled measurements of control and treated cells. Vertical bars represent SD. The arrow indicates the addition of IFN-α, 1,500 IU/ml (closed circles) or db cAMP, 0.1 mM (open circles). $*P < 0.05$ vs. values at 200 sec.

was also sensitive to several receptor-mediated stimuli, including IFN-α. Figure 6 shows that treatment of neutrophyl-like HL-60 cells with db cAMP increased $[Mg^{2+}]_i$ more slowly than observed in undifferentiated HL-60 cells (+0.14 mM in 13 min). Stimulation by IFN-α, in turn, induced a faster rise of $[Mg^{2+}]_i$ which increased by 0.27 mM in 13 min (+50%). In this particular set of experiments, basal $[Mg^{2+}]_i$ was lower in treated than in control cells. As underlined earlier, this difference represents sample variability defined by SD. Together, data on $[Mg^{2+}]_i$ indicate that stimuli activating magnesium efflux via the Na-Mg antiporter correlate with a slow increase of $[Mg^{2+}]_i$ which becomes significant toward the end of the observation time. To ensure that $[Ca^{2+}]_i$ movements were not interfering with our determinations of $[Mg^{2+}]_i$, we measured $[Ca^{2+}]_i$ under directly comparable conditions. The results demonstrated that neither IFN-α nor db cAMP affected $[Mg^{2+}]_i$ in neutrophyl-like HL-60 cells (not shown). This confirmed that, in our experimental conditions, data obtained on $[Mg^{2+}]_i$ were free from interference by $[Ca^{2+}]_i$.

DISCUSSION

Several reports have provided indirect or fragmentary evidence for a different regulation of magnesium in proliferating vs. resting cells [McKeehan and Ham, 1978; Rubin, 1982; Grubbs et al., 1984; Covacci et al., 1998]. The experiments described in this paper were intended to address such difference(s) in depth, taking advantage of an experimental model which allows for a direct comparison between two populations of HL-60 cells. Magnesium homeostasis has been examined from three different viewpoints: 1) the regulation of magnesium efflux from plasma membrane, 2) the release of magnesium from permeabilized cells, and 3) the co-regulation of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$.

As far as the regulation of magnesium efflux is concerned, our data confirm that the Na-Mg antiporter is present in HL-60 cells [Günther and Vormann, 1990] and provide novel evidence for the importance of receptor-mediated regulation of magnesium efflux during the process of differentiation. In previous papers we demonstrated that receptor-mediated stimuli could stimulate magnesium efflux by a cAMP-dependent signal [Wolf et al., 1996, 1997]. This was shown to occur directly, via an ATP-driven Ca^{2+} rise, or indirectly, via an IFN-α–induced stimulation of PLC or PLD and consequent production of prostaglandins from arachidonate [Wolf et al., 1996, 1997]. Here we have shown that undifferentiated cells express the cAMP-sensitive Na-Mg antiporter but cannot respond to the aforementioned stimuli. Consistent with our findings, earlier studies have shown that extracellular ATP cannot induce arachidonic acid release from HL-60 cells, suggesting that this might be a peculiar characteristic of undifferentiated cells [Xing et al., 1994]. Maturation of HL-60 by DMSO treatment restores sensitivity to ATP, PGE1, and IFN-α, allowing both PLA2 activation and consequent cAMP-driven stimulation of magnesium efflux (cf. Figs. 1, 2). This behavior likely depends on maturation-induced expression of specific surface receptors, in analogy with previous observations on the expression of granulocyte-specific surface antigens such as CD11b [Covacci et al., 1998].

The effect of IFN-α on magnesium efflux from neutrophyl-like HL-60 cells illustrated in Figures 1 and 2 seems of particular interest. It is well known that IFN-α displays differentiative effects on several leukemic malignancies, either per se or in combination with other agents [Hemmi and Breitman, 1987; Gutterman, 1994]. Furthermore, IFN-γ was shown to induce monocitoid maturation in HL-60 cells [Ball et al., 1984]. In a previous investigation we described that IFN-α was capable of triggering magnesium efflux from Ehrlich ascites tumor cells [Wolf et al., 1997]. Even though in the

present study IFN-α was unable to elicit magnesium efflux from undifferentiated HL-60 cells, it is reasonable to correlate its differentiative effect with the induction of magnesium efflux. Therefore, we propose that the differentiating effect of IFN-α may include the stimulation of magnesium efflux which in turn leads to a decrease and redistribution of intracellular magnesium similar to those observed in DMSO-treated HL-60 cells (cf. Figs, 2, 4).

The results obtained by permeabilizing HL-60 cells with A23187 and digitonin may help obtain some information on intracellular magnesium compartmentalization (cf. Figs. 3, 4). In particular, we have attempted to correlate the release of magnesium induced by A23187 with the metabolic state of undifferentiated and neutrophyl-like HL-60 cells. To this end, we have used rotenone to inhibit respiration and thus induce rapid exhaustion of cell ATP content. In these circumstances, A23187-driven magnesium release from HL-60 cells would reflect the release (loss) of magnesium which is no longer bound to ATP. When cells are treated with both rotenone and glucose, ATP content is restored through anaerobic glycolysis [Wolf and Cittadini, 1988]; hence, A23187 cannot promote substantial release of magnesium (Fig. 3). In light of these results, magnesium released by A23187 really reflects the free intracellular Mg^{2+} pool which is readily exchangeable with extracellular cations such as Ca^{2+}. Even though not quantitative, measurements in the presence of A23187 may thus provide a useful approach to the evaluation of $[Mg^{2+}]_i$. These data also suggest that ATP is the major ligand controlling intracellular $[Mg^{2+}]_i$. This is further confirmed by experiments in which membrane permeabilization by digitonin allows the release not only of $[Mg^{2+}]_i$ but also of Mg-ATP (Fig. 4). Inasmuch as the ATP content of undifferentiated cells is the same as in neutrophyl-like HL-60 cells [Covacci et al., 1998], we can conclude that the difference in magnesium content and distribution between the two cell populations pertains exclusively to the pool of bound magnesium.

Determination of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ and of their movements in undifferentiated vs. neutrophyl-like HL-60 cells offers additional points of discussion. We have reported previously [Covacci et al., 1998] and herein confirmed (cf. Fig. 4) that differentiation of HL-60 cells causes a 20% reduction of cell total magnesium with a concurrent increase of $[Mg^{2+}]_i$. Taking into account that $[Mg^{2+}]_i$ is less than 10% of total cell magnesium and that cell volume is reduced during differentiation, (16.6 vs. 10.4 mg H_2O/mg protein, as reported in Covacci et al., 1998), a substantial portion of the decrease of cell total magnesium must therefore involve a modification of the bound cation pool. As a consequence, crucial magnesium-dependent reactions, such those involved in the maintenance of cell proliferation, may undergo regulation by magnesium availability during differentiation.

Measurements of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ have shown that activation of a Na-Mg antiporter by receptor-mediated stimuli is followed by modulation of $[Mg^{2+}]_i$ which is totally independent from $[Ca^{2+}]_i$ (cf. Figs. 5, 6). Few data available in the literature were able to correlate stimulation of magnesium efflux with modulation of intracellular ionized cation, and results were often contradictory [Günther and Vormann, 1992; Dai and Quamme, 1992; Matsuura et al., 1993]. The increase of $[Mg^{2+}]_i$ herein described, rather than reflecting an increase in uptake, probably reflects a release of magnesium from the bound cation pool, since under *0-trans* conditions the concentration gradient of extracellular vs. intracellular magnesium is unfavorable. Intracellular free magnesium, therefore, is subject to modulation by cAMP level, which is in turn under the control of various receptor-mediated stimuli differently expressed in undifferentiated and in neutrophyl-like HL-60 cells. It is interesting to underline that some of the stimuli which induce magnesium release have differentiative or antiproliferative effects (i.e., IFN-α and -γ, some cytokines, extracellular ATP) [Gutterman, 1994; Hemmi and Breitman, 1987; Ball et al., 1984; Chahwala and Cantley, 1984; Lasso De La Vega et al., 1994], once again supporting the importance of intracellular magnesium distribution in the maturation process.

In conclusion, we have shown that intracellular magnesium homeostasis is correlated to the differentiative state of HL-60 cells. Undifferentiated cells are less sensitive to receptor-mediated stimuli (such as IFN-α, ATP, and PGE1) which stimulate magnesium release in mature cells. Therefore, undifferentiated cells display a high content of total cell magnesium with a large portion of bound cation pool. Upon differentiation, HL-60 cells recover the capacity to actively extrude magnesium upon exposure to specific stimuli and concurrently increase their

$[Mg^{2+}]_i$. These changes result in a reduction of the bound magnesium pool and probably in a modulation of Mg-dependent reactions which are essential for the maintenance of the high proliferative rate that characterizes undifferentiated phenotypes. We propose that the differentiation process induced by various agents, besides inducing well-established signal transduction mechanisms and transcription of specific genes, can be associated with critical modifications of magnesium homeostasis.

ACKNOWLEDGMENTS

This work was supported by MURST 40% and by the Italian Association for Cancer Research (AIRC Milano, Italy) and the Italian National Council (Convenzione CNR-Consorzio Mario Negri Sud).

REFERENCES

Ball ED, Guyre PM, Shen L, Glynn JM, Maliszewski CR, Baker PE, Fanger MW (1984): Gamma interferon induces monocytoid differentiation in the HL-60 cell line. J Clin Invest 73:1072–1077.

Bizzarri C, Corda D (1994): Norepinephrine, unlike ATP, induces all-or-none increase in cytosolic calcium in thyroid cells. The role of inositol-triphosphate-sensitive stores and calcium channels. Eur J Biochem 219:837–844.

Brocard JB, Rajdev S, Reynold IJ (1993): Glutamate-induced increase in intracellular free Mg^{2+} in cultured cortical neurones. Neuron 11:751–757.

Chahwala SB, Cantley LC (1984): Extracellular ATP induces ion fluxes and inhibits growth of Friend erythroleukemia cells. J Biol Chem 259:13717–13722.

Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC (1978): Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compound. Proc Natl Acad Sci U S A 75:2458–2462.

Covacci V, Bruzzese N, Sgambato A, Di Francesco A, Russo MA, Wolf FI, Cittadini A (1998): Magnesium restriction induces granulocytic differentiation and expression of $p27^{Kip1}$ in human leukemic HL-60 cells. J Cell Biochem 70:313–322.

Cowan JA (1995): "The Biological Chemistry of Magnesium." New York: VCH Publisher Inc.

Dai LJ, Quamme GA (1992): Cyclic nucleotides alter intracellular free Mg^{2+} in renal epithelial cells. Am J Physiol 262:F1100–1104.

Feray JC, Garay R (1988): Demonstration of Na:Mg exchange in human red cells by its sensitivity to tricyclic antidepressant drugs. Naunyn-Schmiedebergs Arch Pharmacol 338:332–337.

Flatman PW (1991): Mechanisms of magnesium transport. Annu Rev Physiol 53:259–271.

Grubbs RD (1991): Effect of epidermal growth factor on magnesium homeostasis in BC_3H1 myocytes. Am J Physiol 260:C1158–C1164.

Grubbs RD, Collins SD, Maguire ME (1984): Differential compartmentation of magnesium and calcium in murine S49 lymphoma cells. J Biol Chem 259:12184–12192.

Günther T, Vormann J (1990): Na^+-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat thymocytes and HL-60 cells. Magnes Trace Elem 9:279–282.

Günther T, Vormann J (1992): Activation of Na^+/Mg^{2+} antiport in thymocytes by cAMP. FEBS Lett 297:132–134.

Gutterman JU (1994): Cytochine therapeutics: Lessons from interferon α. Proc Natl Acad Sci U S A 91:1198–1205.

Hemmi H, Breitman TR (1987): Combinations of recombinant human interferons and retinoic acid synergistically induce differentiation of the human promyelocytic leukemia cell line HL-60. Blood 69:501–507.

Hurley TW, Ryan MP, Brinck RW (1992): Changes of cytosolic Ca^{2+} interfere with measurements of cytosolic Mg^{2+} using mag-fura2. Am J Physiol 263:C300–C307.

Lasso De La Vega MC, Terradez P, Obrador E, Navarro J, Pellicer JA, Estrela JM (1994): Inhibition of cancer growth and selective glutathione depletion in Ehrlich tumor cells in vivo by extracellular ATP. Biochem J 298:99–105.

Maguire ME (1988): Magnesium and cell proliferation. Ann N Y Acad Sci 551:201–215.

Matsuura T, Kanayama Y, Inoue T, Takeda T, Morishima I (1993): c-AMP induced changes of intracellular free Mg^{2+} levels in human erythrocytes. Biochim Biophys Acta 1220: 31–36.

McKeehan WL, Ham RG (1978): Calcium and magnesium ions and the regulation of multiplication of normal and transformed cells. Nature 275:756–758.

Romani A, Scarpa A (1992): Regulation of cell magnesium. Arch Biochem Biophys 298:1–12.

Romani A, Scarpa A (1995): Regulation of cytosolic magnesium ion in the heart. In Cowan JA (ed): "The Biological Chemistry of Magnesium." New York: VCH Publisher Inc., pp 235–249.

Romani A, Marfella C, Scarpa A (1993): Cell magnesium transport and homeostasis: Role of intracellular compartments. Miner Electrolyte Metab 19:282–289.

Rubin H (1981): Growth regulation, reverse transformation and adaptability of 3T3 cells in decreased Mg^{2+} concentration. Proc Natl Acad Sci U S A 78:328–332.

Rubin H (1982): Effect of magnesium content on density-dependent regulation of the onset of DNA synthesis in transformed 3T3. Cancer Res 42:1761–1768.

Rutter GA, Osbaldeston NJ, McCormack JC, Denton RM (1990): Measurement of matrix free Mg^{2+} concentration in rat heart mitochondria by using entrapped fluorescent probes. Biochem J 271:627–634.

Vormann J, Günther T (1993): Magnesium transport mechanisms. In: Birch NJ (ed): "Magnesium and the Cell." London: Academic Press, pp 137–155.

Wolf FI, Cittadini A (1988): Fructose 2,6-bisphosphate and glycolysis in ascites tumor cells. Ann N Y Acad Sci 551: 259–260.

Wolf FI, Di Francesco A, Covacci V, Corda D, Cittadini A (1996): Regulation of intracellular magnesium in ascites cells: Involvement of different regulatory pathways. Arch Biochem Biophys 331:194–200.

Wolf FI, Di Francesco A, Covacci V, Cittadini A (1997): Regulation of magnesium from rat spleen lymphocytes. Arch Biochem Biophys 344:397–403.

Xing M, Wilkins PL, McConnell BK, Mattera R (1994): Regulation of phospholipase A2 activity in undifferentiated and neutrophyl-like HL-60 cells. J Biol Chem 269: 3117–3124.