original paper
Haematologica 1997; 82:395-401

HUMAN LEUKEMIA K562 CELLS: INDUCTION TO ERYTHROID DIFFERENTIATION BY GUANINE, GUANOSINE AND GUANINE NUCLEOTIDES

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ABSTRACT

Background and Objective. Human leukemic K562 cells are able to undergo erythroid differentiation *in vitro* when cultured with a variety of inducers, leading to increased expression of embryo-fetal globin genes such as the ζ, ϵ and γ-globin genes. Therefore the K562 cell line has been proposed as a very useful *in vitro* model system for determining the therapeutical potential of new differentiating compounds as well as for studying the molecular mechanism(s) that regulate changes in the expression of embryonic and fetal human globin genes. In this study we explored whether nucleoside triphosphates and related compounds are able to induce differentiation of K562 cells.

Methods. K562 cell differentiation was studied using the benzidine test; hemoglobins were characterized by cellulose acetate gel electrophoresis and mRNA accumulation was investigated by Northern blot analysis.

Results. The main conclusion of this paper is that guanine, guanosine and guanine ribonucleotides are effective inducers of K562 cell differentiation. Expression of both Hb Portland and Hb Gower 1 is increased in GTP-induced K562 cells. This increase is associated with greater γ-globin mRNA accumulation. By contrast, ATP, CTP and UTP are not able to induce erythroid differentiation.

Interpretaton and Conclusions. These findings suggest that guanine, guanosine and guanine ribonucleotides are inducers of erythroid differentiation of K562 cells. This is of some relevance since differentiating compounds have been proposed as antitumor agents. In addition, inducers of erythroid differentiation that stimulate γ-globin synthesis might be considered in the experimental therapy of hematological diseases associated with a failure in the expression of adult β-globin genes.

Key words: K562 cells, erythroid differentiation, globin, GTP, thalassemia

The human K562 cell line was established by Lozzio and Lozzio[1] from a patient with chronic myelogenous leukemia in blast crisis. This cell line is one of the best known *in vitro* experimental systems mimicking erythroid differentiation.[2-9] The K562 cell line exhibits a low proportion of hemoglobin-synthesizing cells under standard cell growth conditions, but erythroid differentiation occurs when K562 cells are treated with a variety of compounds, including hemin,[3,4] cytosine arabinoside,[5] butyric acid[6] and 5-azacytidine.[7]

Following erythroid induction of K562 cells, a sharp increase in Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\epsilon_2$) is observed, accompained by an increase in the expression of human ϵ and γ globin genes.[2,3,8,9] The K562 cell line has thus been proposed as a very useful model system for identifying the therapeutical potential of new differentiating compounds as well as for studying the molecular mechanism(s) regulating the expression of embryonic and fetal human globin genes.[3,9]

These issues are of relevance for the development of anti-tumor drugs that act by inducing the activation of differentiation rather than by displaying cytotoxic and/or cytolytic activities.[3] For instance, cytosine arabinoside is currently exploited as an antitumor agent.[9] In addition, the pharmacologically-mediated regulation human γ-globin gene expression could be of interest, at least in theory, in the search for potential therapeutic agents in hematological disorders, including β°-thalassemia.[10-20] *In vitro* studies demonstrate that hydroxyurea, erythropoietin, butyrates and 5-azacytidine are capable to induce fetal hemoglobin production when administred singularly or in combination.[11,12] With respect to this point butyric acid and 5-azacytidine have been the object of recent reports focused on *in vivo* treatment of β-thalassemia patients.[11,12]

Among possible biological response modifiers, extracellular purine nucleosides and nucleotides could be of interest, since it has been reported that

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Acknowledgements: the financial support of Telethon-Italy (grant no.E.58) is gratefully acknowledged. This work was also supported by AIRC, by CNR Target Project ACRO and by Fondazione Italiana "Leonardo Giambrone" per la Guarigione dalla Thalassemia. F.O. is the recipient of a fellowship from Associazione per la Guarigione del Bambino Thalassemico (Ferrara) and Associazione Veneta per la Lotta alla Thalassemia (Rovigo).
Received February 7, 1997; accepted May 24, 1997.

guanosine, GTP, adenosine and ATP are able to stimulate proliferation of astrocytes and microglia.[21] These compounds are of some interest, as they are active in a variety of experimental systems and are not expected to be toxic to eukaryotic cells, unless administred at high concentrations.[22-28]

In this paper we first explored whether nucleosides triphosphates are able to induce differentiation of K562 cells. The first set of results obtained suggest that, among nucleosides triphosphates, only GTP is capable to efficiently induce differentiation of K562 cells. Accordingly, we analysed the activity of GTP, GDP, GMP, guanosine and guanine, and found that all these compounds are able to induce erythroid differentiation of K562 cells. Erythroid differentiation was associated with (a) an increase of the content of embryo-fetal hemoglobins and (b) an increase of globin mRNA accumulation.

Materials and Methods

Cell lines and culture conditions

The human myeloid leukemia K562(S) cells were kindly provided by Dr. Livia Cioè.[6] The cell line was maintained in RPMI 1640 (Flow Laboratories) in 10% fetal bovine serum (FBS, CELBIO), 5% CO_2 supplemented with 50 units/mL penicillin, 50 mg/mL streptomycin.[2,4,7] Cell growth was studied by determining the cell number/mL after different days of *in vitro* cell culture.[2] Semi-solid cell cultures were performed in RPMI 1640 medium, 0.33% agar (DIFCO), 10% FBS.[4] Guanine, guanosine, ATP, CTP, GTP and UTP were purchased from Sigma and were stored at –20°C in acqueous solution at 10 mM (ATP, GTP, CTP, UTP, GMP and GDP), 7 mM (guanosine). 10 mM stock solution of guanine was in 150 mM KOH.

Hemoglobin determination

K562 cells containing heme or hemoglobin were detected by specific reaction with a benzidine/hydrogen peroxide solution as reported elsewhere (3,4). The final concentration of benzidine was 0.2% in 0.5 M glacial acetic acid, 3% H_2O_2.[29]

In order to analyze hemoglobin production by erythroid induced K562 cells total fresh post-mitochondrial cell lysates were electrophoresed on cellulose acetate strips (Chemotron) in Tris-EDTA-borate buffer.[3,29]

After electrophoresis the gels were stained with benzidine/hydrogen peroxide (1% benzidine in 4.3 M acetic acid, 3% H_2O_2) and photographed, and the relative proportion of separated hemoglobins was calculated from the areas of the densitometric peaks.[29]

Northern blotting

RNA was isolated from uninduced and erythroid-induced K562 cells according to the RNAzol Method (BIOTECX).[30-32] Northern blotting was performed using 15 µg of RNA as described elsewhere.[33] RNA was electrophoresed in formaldehyde gels containing 20 mM MOPS (pH 7), 8 mM sodium acetate, 1 mM EDTA, 2.2 M formaldehyde.[33] Hybridization was carried on with a ^{32}P-labelled pJW151 plasmid that specifically recognized γ-globin mRNA sequences.[7] Hybridization buffer consisted of 1 M NaCl, 1% sodium dodecyl sulphate (SDS), 50% formamide, 10% dextran sulphate, 200 µg/mL denatured salmon sperm DNA. After 16 hours of hybridization, filters were washed in 2 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate)(room temperature, 5 min, twice), followed by a washing step in 2 × SSC, 1% SDS (60°C, 5 min, twice); the final washing step was carried out at room temperature for 5 min in the presence of 0.1 x SSC. Autoradiography was performed with X-Omat Kodak films.

Results

Cell growth and differentiation of K562 cells cultured in the presence of nucleoside triphosphates

Figure 1 shows the results from two preliminary experiments performed to determine the effects of nucleoside triphosphates on the proliferation and differentiation of human leukemic K562 cells. In the first experiment the cell number/mL and the proportion of benzidine-positive cells were determined in K562 cells treated for different times with 75 mM ATP, GTP, UTP and CTP (Figure 1A and 1C). In the second experiment determinations of cell growth and differentiation were performed after 7 days of treatment with increasing concentrations (up to 300 µM) of GTP.

Figure 1A shows that nucleoside triphosphates cause only minor changes in the proliferation efficiency of K562 cells, with the exception of GTP, which causes a decrease in the rate of K562 cell growth. This inhibitory effect is dose dependent, as demonstrated by the results in Figure 1B showing that GTP does not alter cell proliferation when added at 18.75 µM, while inhibition of cell proliferation does occur when K562 cells are cultured for 7 days in the presence of 37.5 (60% inhibition), 75 (85% inhibition), 150 (90% inhibition) and 300 (90% inhibition) µM GTP.

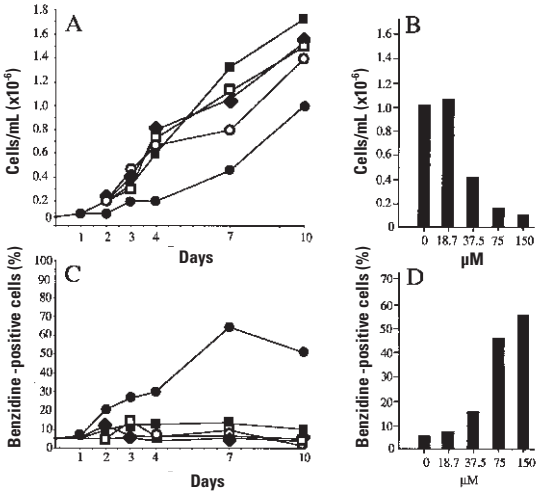

Figure 1. Effects of ATP, GTP, CTP and UTP on cell proliferation (A,B) and erythroid differentiation (C,D) of K562 cells.
A, C. Cells were cultured for the indicated length of time in the absence [○] or in the presence of 75 µM ATP [■], GTP [●], CTP [□] and UTP [◆]. B, D. Effects of increasing amounts of GTP on K562 cell growth (B) and erythroid differentiation (D). In this experiment K562 cells were cultured as indicated for 7 days.

These data clearly indicate that among nucleosides triphosphates GTP exerts the highest antiproliferative activity.

Figure 1C demonstrates that GTP is the only nucleoside triphosphate able to induce a significant increase in the proportion of benzidine-positive (hemoglobin-containing) K562 cells after 7-10 days of induction. In Figure 1D reports data showing that the GTP-mediated induction of K562 cells to erythroid differentiation is dose dependent. In this experiment, the proportion of benzidine-positive cells was determined after 7 days of cell culture.

Erythroid differentiation induced by GTP is a fairly reproducible phenomenon, as suggested by data shown in Figure 2. In these experiments the kinetics of the increase in the proportion of benzidine-positive K562 cells was determined after 1, 3, 5, 6, 7 and 8 days of K562 cell culture in the presence of 150 mM GTP. The results shown in Figure 2 are drawn from eleven independent induction experiments. Similar experiments performed with 75 mM and 300 mM GTP confirmed that the treatment of K562 cells with this compound consistently causes an increase in the proportion of benzidine-positive cells (data not shown).

Taken together, these results show that GTP could be considered an inducer of erythroid differentiation in K562 cells.

Differentiation of K562 cells cultured with guanine, guanosine, and guanine nucleotides

Figure 3A and 3C show two independent experiments demonstrating that guanine, guanosine and guanine nucleotides (GMP, GDP and GTP) are all differentiating compounds of the human leukemic K562 cell line.

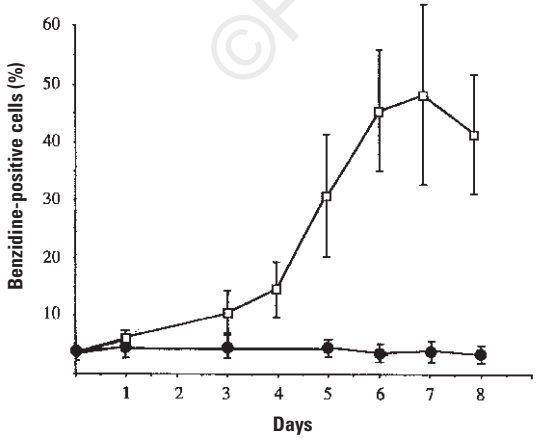

Figure 2. Kinetics of erythroid induction IN K562 cells by 150 µM GTP (□). ● = uninduced control K562 cells. Results represent the mean ± S.D. determined from eleven independent experiments.

In addition, our results indicate that all these compounds, when administered at concentrations retaining inducing capabilities, are inhibitors of K562 cell growth (Figure 3B and data not shown).

This could be compatible with a cell-cycle effect of these inducers, leading to the inhibition of cell growth efficiency of erythroid-differentiating cells. This is also suggested by the decrease in the proportion of benzidine-positive cells when cell cultures are analyzed for periods of induction longer than 8 days (Figure 1C, Figure 2A and data not shown). The decrease in the percentage of benzi-

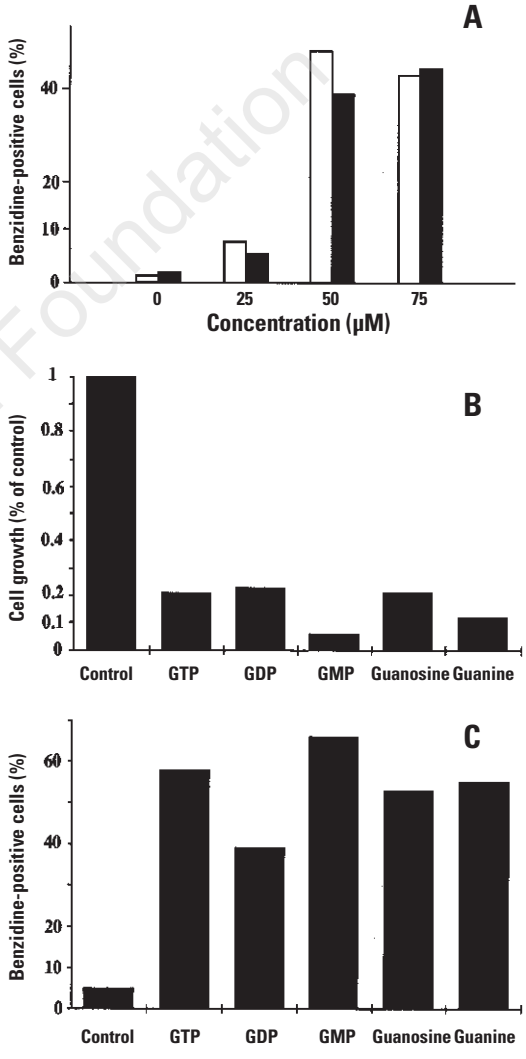

Figure 3. A. Effects of GTP and guanosine on erythroid differentiation of K562 cells. Cells were cultured in the absence (0) or in the presence of different concentrations (25-75 µM) of GTP (■) and guanosine (□). After 7 days the % of benzidine-positive cells was determined. B, C. Effects of GTP, GDP, GMP, guanosine and guanine on growth (B) and differentiation (C) of K562 cells. Determinations were performed after 7 days of cell culture. Control = uninduced K562 cells.

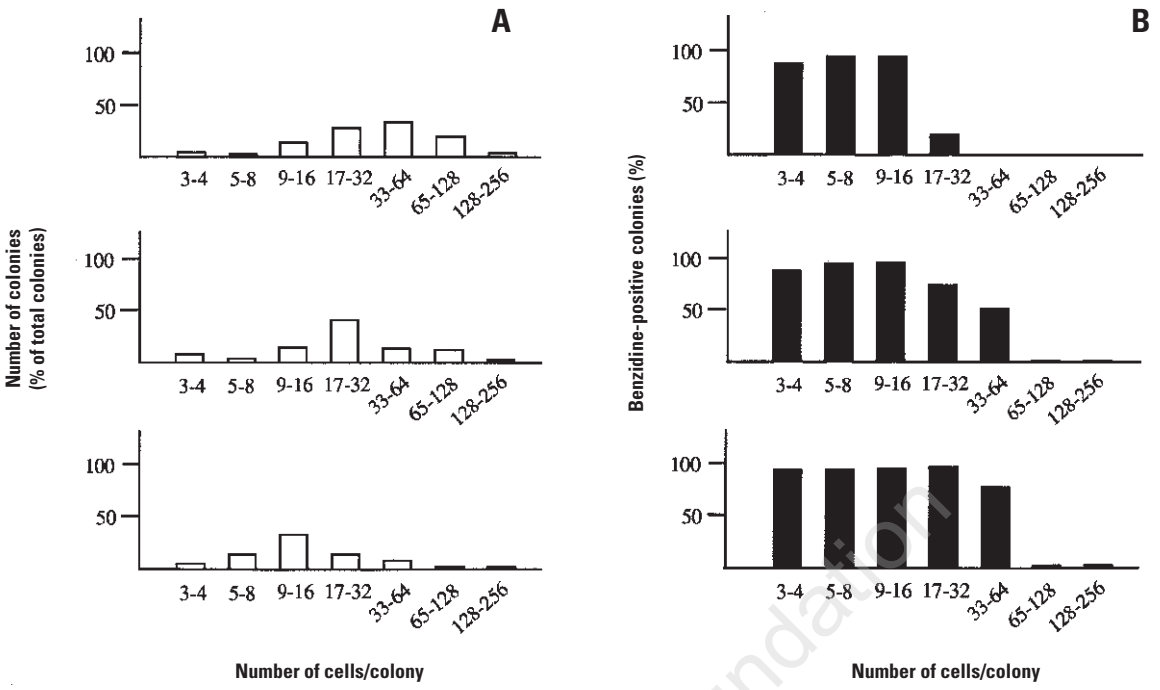

Figure 4. Effects of GTP on semisolid cell cultures. Cells were cultured in the absence (upper) or in the presence of 150 (middle) and 300 (lower) μM GTP for 7 days in semisolid medium.
A. Distribution of colonies containing the indicated number of cells (3-4, 5-8, 9-16 ...).
B. Inverse relationship between number of cells/colony and differentiation state. The data represent the proportion of benzidine-positive colonies (%) within different classes of colonies originating from semisolid cultures of K562 cells.

dine-positive cells can be tentatively explained by the presence of at least two cell populations in induced cell cultures. The first is an uninduced K562 cell population exhibiting high cell growth; the other is the erythroid-induced cell population, whose rate of cell growth declines following erythroid induction. In order to verify this hypothesis, induction experiments were performed in semisolid culture conditions.

GTP-induced differentiation of K562 cells cultured in semisolid medium

When uninduced K562 cells are cultured in semisolid medium, the majority of the colonies are large and negative to the benzidine stain, but a small proportion of colonies are small and positive for this assay. This highly reproducible phenomenon (Figure 4, upper side of the panels) is well known[2-9] and is due to K562 cells that spontaneously undergoing terminal erythroid differentiation.[8] For uninduced cells, the data obtained and shown in Figure 4 are compatible with a theoretical value of 6.2% benzidine-positive cells.

Figure 4 clearly demonstrates that after 7 days induction in semisolid medium the erythroid-induced colonies of GTP-cultured K562 cells also contain fewer cells than benzidine-negative colonies. For instance, in K562 cell cultures treated with 300 mM GTP, colonies containing 17-32 cells are nearly 100% positive for benzidine. By sharp contrast, not more than 2-3% of the colonies containing 65-128 cells are positive for the benzidine stain (Figure 4B, lower panel). In conclusion, the data obtained by the experiments shown in Figures 1, 3 and 4 are consistent with inhibition of cell growth of erythroid-induced K562 cells cultured in the presence of GTP. For cells cultured with 300 μM GTP, the data obtained and shown in Figure 4 are compatible with a theoretical value of about 76.3% benzidine-positive cells. The inverse relationship between colony size and proportion of benzidine-positive colonies was confirmed in three independent experiments (data not shown). Moreover, in complete agreement with the data shown in Figure 4, when the benzidine assay was performed after 10, 11 and 12 days of culture with GTP-treated cells, in eleven independent experiments we always found a decrease in the proportion of benzidine-positive cells (data not shown).

Hemoglobin accumulation in GTP-induced K562 cells

The level of hemoglobin production (assessed as μg of Hb/μg of cytoplasmic proteins) was preliminarly analyzed using a benzidine/H_2O_2 reaction performed in post-mitochondrial cell lysates, followed by densitometric analysis at 602 nm. These

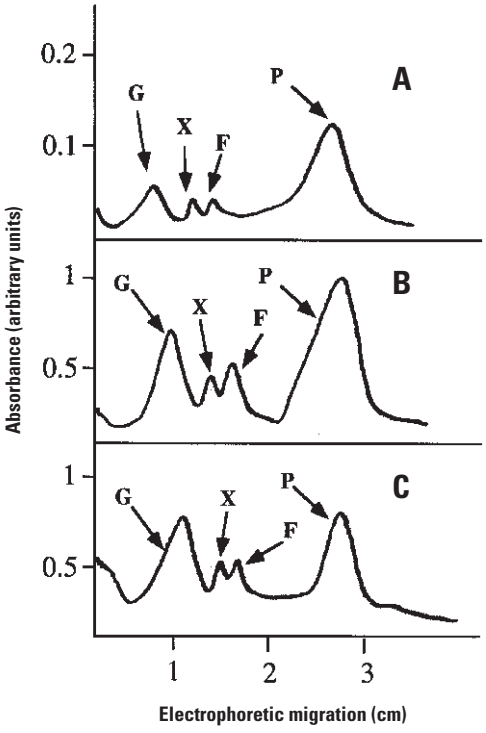

Figure 5. Cellulose acetate gel electrophoresis of hemoglobin produced by K562 cells. Post mitochondrial cell lysates from uninduced (A) K562 cells or from cells induced for 7 days with 2 μM cytosine arabinoside (B) or 100 μM GTP (C) were layered on cellulose acetate strips, electrophoresed and stained with benzidine. Results report the pattern obtained after densitometric analysis of the gels.
P = Hb Portland; G = Hb Gower 1; X = Hb X; F = Hb F.

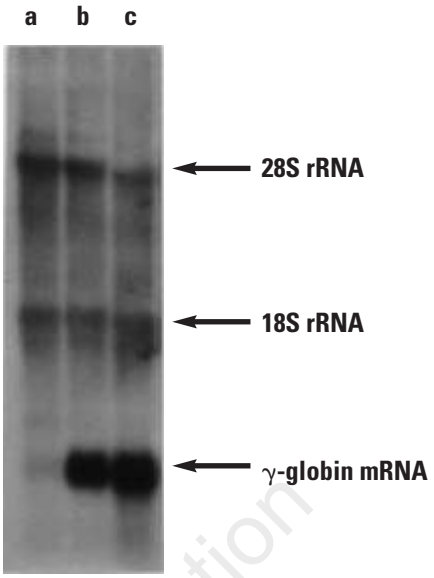

Figure 6. Northern blotting analysis. Total RNA was isolated from uninduced K562 (a) and erythroid-induced K562 cells (b,c). Induction was carried out for 6 days in the presence of (b) 100 μM GTP and (b) 2 μM araC. Fifteen μg of total RNA were electrophoresed. Northern blotting was conducted and hybridization was performed with a ^{32}P-labelled pJW151 plasmid.

results suggest that treatment for 7 days with 150 μM GTP induces at least a 10-fold increase in Hb production. The level of Hb in GTP-treated cells in three independent experiments was always found to be higher than 8 pg of Hb/cell, while uninduced K562 usually accumulate less than 0.7 pg of Hb/cell.

Furthermore, cellogel electrophoresis of post-mitochondrial lysates demonstrated that, following treatment of K562 cells with 100 μM GTP, both Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\epsilon_2$) are abundantly expressed, suggesting that GTP-mediated erythroid differentiation is accompanied by an increase in the expression of embryo-fetal globin genes (the β-like ε and γ, and the α-like ζ) (Figure 5 and data not shown). In the experiment shown in Figure 5 the relative proportions of hemoglobins accumulated in K562 cells treated with 150 μM GTP (% of Hb/total hemoglobins) were 40.1% for Hb Gower 1, 6.7% for Hb X ($\epsilon_2\gamma_2$), 9.3% for Hb F ($\alpha_2\gamma_2$) and 43.9% for Hb Portland.

Northern blotting analysis

Total RNA was isolated from uninduced and erythroid-induced K562 cells. Induction was carried out for 6 days in the presence of (i) 100 μM GTP and (ii) 2 μM cytosine arabinoside. In order to analyze globin genes expression, Northern blotting was performed and hybridization was conducted with a ^{32}P-labelled pJW151 plasmid that specifically recognized γ-globin mRNA sequences.[7] The results are shown in Figure 6 and clearly demonstrate that GTP treatment induced a sharp increase in the accumulation of γ-globin mRNA (compare lane a to lane b). The increased accumulation of γ-globin mRNA in GTP-treated K562 cells (lane b) is similar to that found in cytosine arabinoside-treated cells (lane c).

Discussion

The main conclusion of this paper is that guanine, guanosine and guanosine ribonucleotides are effective inducers of K562 cell differentiation. Production of both Hb Gower 1 and Hb Portland is increased following GTP-mediated erythroid induction of K562 cells. Control experiments suggest that GTP and related compounds induce differentiation of K562 cells at levels similar to those of the best

known K562 inducers, such as butyric acid, cytosine arabinoside and hemin.[3,6,9]

Of interest is the fact that GTP and related molecules are physiological compounds active in a variety of experimental systems and are not expected to be toxic to eukaryotic cells unless administered at high concentrations.[21-28]

Our data do not explain the induction of erythroid differentiation at the biochemical level. It is likely that the active compounds are guanine and guanosine, since in tissue culture conditions ribonucleoside triphosphates are rapidly converted to ribonucleosides (Matteuzzi, unpublished observations). With respect to the molecular basis of erythroid induction, Northern blotting analysis demonstrates that the accumulation of γ-globin mRNA sharply increases following GTP-treatment of K562 cells.

These findings could be due to increased transcriptional activity of γ-globin genes in GTP-treated K562 cells, as well as to an increase in the processing rate of γ-globin mRNA from the nucleus to the cytoplasm. Finally, our data do not exclude the possibility of an increased half-life of the γ-globin mRNA molecules in erythroid-induced cells. Despite the fact that a change in γ-globin gene transcription could occur in GTP-treated cells, other changes, including globin mRNA processing, should not be ruled out in studies on the modulation of the hemoglobin phenotype, as recently suggested.[34] Further experiments will be required to evaluate these hypotheses or to determine whether a combination of them represent the molecular basis for the increased accumulation of γ-globin mRNA in GTP-induced cells.

With respect to this specific point, we wish to mention that a study of the molecular mechanisms underlying the switch between ε and γ-globin genes is crucial for experiments aimed at the induction of γ-globin gene expression in adults.[19] Pharmacologically-mediated regulation of the expression of human γ-globin genes could be of interest in the search for potential therapeutic agents in hematological disorders, including β°-thalassemia.[15-20] Recently published observations demonstrate that hormones, cytotoxic agents, hemopoietic cytokines and short fatty acids are capable of augmenting fetal hemoglobin levels in humans.[19] For instance, hydroxyurea, erythropoietin, butyrates and 5-azacytidine were found to induce *in vitro* fetal hemoglobin production when administered singly or in combination.[11-20] Erythropoietin has also been successfully employed in individual patients with thalassemia intermedia, particularly in individuals with evidence of defective endogenous production.[35,36] Butyric acid and 5-azacytidine have also been the object of recent reports focusing on *in vivo* treatment of β-thalassemia patients.[11,12] This is a major issue in this field since it is well established that an increase of as little as 30% in HbF production leads to a significant improvement in clinical status.

Therefore it would be interesting to determine whether treatment of human bone marrow cells with GTP and related compound does produce an increase in HbF.

References

1. Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell-line with positive Philadelphia-chromosome. Blood 1975; 45:321-34.
2. Gambari R, Barbieri R, Buzzoni D, et al. Human leukemic K562 cells: suppression of hemoglobin accumulation by a monoclonal antibody to human transferrin receptor. Biochim Biophys Acta 1986; 886:203-13.
3. Rutherford TR, Clegg JB, Weatherall DJ. K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin. Nature 1979; 280:164-5.
4. Gambari R, Raschellà G, Biagini R, et al. Predominant expression of z and e globin genes in human leukemia K-562(S6) variant cell line. Experientia 1983; 39:415-6.
5. Bianchi Scarrà GL, Romani M, Coviello DA. Terminal erythroid differentiation in the K-562 cell line by 1-β-arabinofuranosylcytosine by c-myc messenger RNA decrease. Cancer Res 1986; 46:6327-32.
6. Cioè L, McNab A, Hubbell HR, Meo P, Curtis P, Rovera G. Differential expression of the globin genes in human leukemia K562(S) cells induced to differentiate by hemin or butyric acid. Cancer Res 1981; 41:237-43.
7. Gambari R, del Senno L, Barbieri R, et al. Human leukemia K- 562 cells: induction of erythoid differentiation by 5-azacytidine. Cell Differ 1984; 14:87-97.
8. Gambari R, Amelotti F, Piva R. Efficient cell proliferation and predominant accumulation of ε-globin mRNA in human leukemic K562 cells which produce mostly Hb Gower 1. Experientia 1985; 41:673-5.
9. Bianchi Scarrà G, Fiorentini P, Gambari R, et al. Isolation and characterization of a K562 cell line resistant to 1-β-D arabinofuranosylcytosine-mediated erythroid induction. Exp Hematol 1989; 17:859-64.
10. Maslak PG, Weiss MA, Berman E, et al. Granulocyte colony-stimulating factor following chemotherapy in elderly patients with newly diagnosed acute myelogenous leukemia. Leukemia 1996; 10:32-9.
11. DeSimone J, Heller P, Hall L, Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. Proc Natl Acad Sci USA 1982; 79:4428-31.
12. Lowrey CH, Nienhuis AW. Brief report: treatment with azacytidine of patients with end stage β-thalassemia. N Engl J Med 1993; 329:845-8.
13. Al-Khatti A, Papayannopoulou T, Knitter G, Fritsch EF, Stamatoyannopoulos G. Cooperative enhancement of F-cell formation in baboons treated with erythropoietin and hydroxyurea. Blood 1988; 72:817-9.
14. Rodgers GP, Dover GJ, Uyesaka N, Noguchi CT, Schechter AN, Nienhuis AW. Augmentation by erythropoietin of the fetal-hemoglobin response to hydroxyurea in sickle cells disease. N Engl J Med 1993; 328:73-80.
15. Perrine SP, Ginder GD, Faller DV, et al. A short-term trial of butyrate to stimulate fetal-globin-gene expression in the β-globin disorders. N Engl J Med 1993; 328:81-6.
16. Fibach E, Prasanna P, Rodgers GP, Samid D. Enhanced fetal hemoglobin production by phenylacetate and 4-phenylbutyrate in erythroid precursors derived from normal donors and patients with sickle cell anemia and β-thalassemia. Blood 1993; 82:2203-9.
17. Dover GJ, Brusilow S, Samid D. Increased fetal hemoglobin in patients receiving sodium 4-phenylbutyrate. N Engl J Med 1992; 327:569-70.
18. Torkelson S, White B, Faller DV, Phipps K, Pantazis C, Perrine SP. Erythroid progenitor proliferation is stimulated by phenoxyacetic and phenylalkyl acids. Blood Cells Mol Dis 1996; 22:150-8.
19. Rodgers GP, Rachmilewitz EA. Novel treatment options in the severe β-globin disorder. Br J Haematol 1995; 91:263-8.
20. Rachmilewitz EA, Aker M, Perry D, Dover G. Sustained increase in hemoglobin and RBC following long-term administration of recombinant human erythropoietin to patients with homozygous β-thalassemia. Br J Haematol 1995; 90:341-5.
21. Middlemiss PJ, Gysberg JW, Rathbone MP. AIT-082, a unique purine derivative, enhances nerve growth factor mediated neurite outgrowth from PC12 cells. Brain Res 1995; 677:152-6.
22. Chahwala SB, Cantley LC. Extracellular ATP induces ion fluxes and inhibits growth of Friend erythroleukemia cells. J Biol Chem 1984; 259:13717-22.
23. Rapaport E. Treatment of human tumor cells with ADP or ATP yields arrest of growth in the S phase of the cell cycle. J Cell Physiol 1983; 114:279-83.
24. Gregory SH, Kern M. Adenosine and adenine nucleotides are mito-

genic for mouse thymocytes. Biochem Biophys Res Comm 1978; 83:1111-6.
25. Huang N, Wang D, Heppel LA. Extracellular ATP is a mitogen for 3T3, 3T6 and A431 cells and acts synergistically with other growth factors. Proc Natl Acad Sci USA 1989; 86:7904-8.
26. Tokumitsu Y, Yanagawa Y, Nomura Y. Stimulation of DNA synthesis in Jurkat cells by synergistic action between adenine and guanine nucleotides. FEBS Lett 1991; 288:81-5.
27. Murgia M, Hanau S, Pizzo P, Rippa M, Di Virgilio F. Oxidized ATP. J Biol Chem 1993; 268:8199-203.
28. Peter ME, She J, Huber LA, Terhorst C. Labeling of adenine and guanine nucleotide-binding proteins in permeabilzed cells with in situ periodate-oxidized nucleotides. Anal Biochem 1993; 210:77-85.
29. Weatherall DJ, Clegg JB. The thalassemias syndromes. Oxford: Blackwell Sci. Publ. Co., 1981.
30. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156-9.
31. Cox RA. The use of guanidinium chloride in the isolation of nucleic acids. In: Grossman L, Moldave K, eds. Methods in Enzymology. London: Academic Press, 1968. p. 120-9.
32. Chirgwin JM, Przybyla AE, McDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979; 18:5294-9.
33. Sambrook J, Fritsch EF, Maniatis T. Extraction, purification and analysis of messenger RNA from eukaryotic cells. In: [anonymous] Molecular cloning, 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1981. p. 43-5.
34. Kollia P, Fibach E, Najjar SM, Schechter AN, Noguchi CT. Modification of RNA processing modulate the expression of hemoglobin genes. Proc Natl Acad Sci USA 1996; 93:5693-8.
35. Camaschella C, Cappellini MD. Thalassemia intermedia. Haematologica 1995; 80:58-68.
36. Dore F, Bonfigli S, Gaviano E, Pardini S, Longinotti M. Serum transferrin receptor levels in patients with thalassemia intermedia during rHuEPO administration. Haematologica 1996; 81:37-9.