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Interrelationship between Cell Differentiation and Expression of mRNA for Transferrin in HL-60 Leukemia Cell Line

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The interrelationship between the differentiation and expression of mRNA for transferrin in the HL-60 leukemia cell line was studied. Transferrin mRNA was expressed in HL-60 leukemia cells and the amount was 50% of that in the positive control cell line, HepG-2 cells. The expression of T_f mRNA in HL-60 cells was not regulated by IL-1, IL-6 and TNF-α, respectively. The expression of T_f mRNA in the differentiated cells into a granulocyte lineage by DMSO, or *all-trans* RA, was up-regulated (160-170% of control cells); whereas, the expression was not regulated in the differentiated cells into a macrophage lineage by PMA. These results suggest that the differentiation to a granulocyte lineage of HL-60 leukemia cells appear to be related with the upregulation of transferrin mRNA expression.

Keywords: Differentiation, HL-60 leukemia cell line, Transferrin.

Introduction

It is known that transferrin (T_f) is highly expressed in the adult mammalian liver and is secreted by hepatocytes into the serum where it functions as an iron transport protein and growth factor for a variety of cells. The liver T_f expression was reported to be activated by steroid hormones and iron deficiency. T_f has been known to be synthesized to a lower extent by Sertoli cells in the adult testis, as well as by adult brain oligodendrocytes, astrocytes, and epithelial cells of the choroid plexus, where it is involved in the maturation of germinal cells and in central nervous system proliferation and differentiation processes (Hertz *et al.*, 1996). T_f has also been known to act as part of an important autocrine mechanism that permits proliferation of the above cells and also allows tumor cell growth *in vivo* in areas not well vascularized (Vostrejs *et al.*, 1988).

In order to understand the interrelationship between cellular differentiation and the expression of mRNA for T_f in the HL-60 leukemia cell line that is known to be differentiated into granulocyte or macrophage lineages, a reverse transcriptase-polymerase chain reaction (RT-PCR) method was used for quantification of the expressed mRNA.

Materials and Methods

HL-60 and HepG-2 (positive control) cells were maintained in RPMI-1640 plus 10% fetal calf serum and antibiotics for 7 days at 37°C, 5% CO_2.

When the cells were grown to 90% confluency, 1.2% of DMSO (dimethyl sulfoxide), 1 μM of *all-trans* RA (retinoic acid) or 50 nM of PMA (phorbol myristate acetate) was added respectively and cultured for 4 days.

In cases of cytokine treatment, the culture media was replaced by a serum free media and treated with IL-1β (1000 units/ml), IL-6 (200 units/ml) and TNF-α (200 units/ml) two times with a 24 h interval.

RT-PCR was performed as described (Choi *et al.*, 1998). Briefly, total RNAs were isolated from the cells and reverse transcribed into cDNA using random hexamer. Polymerase chain reaction (PCR) assays were performed in order to detect the mRNAs for T_f and β-actin. cDNAs were amplified using primers that are specific to T_f and β-actin in the presence of 5 pmol of 5' and 3' primer, 2.5 mM $MgCl_2$, 1 U of Taq polymerase and 10 μCi of [^{32}P] dCTP for 40-45 cycles: 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C. A 15 μl sample of each PCR reaction mixture was electrophoresed in a 2% agarose gel containing 0.5 μg/ml of ethidium bromide in 0.5 × TBE buffer. Following electrophoresis, the gels were photographed. Appropriate bands were cut from the gels and radioactivities were counted. The cDNAs derived from the samples were normalized to yield equivalent β-actin products.

Oligonucleotide sequences of primers were as follows:

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Transferrin (172 bp)
Sense 5'-GGA AGC CGG TAG ATG AAT ACA AG-3'
Antisense 5'-TGA GGA GAG CTG AAT AGT TGG AA-3'

β-actin (541 bp)
Sense 5'-CTA GCC TGA GGA GCT GCT GCG ACA G-3'
Antisense 5'-GTT CCC TGC TCT CTG TCG GCT CGG CT-3'

Biosynthetic labeling, immunoprecipitation, SDS-PAGE and autoradiography were carried out in order to confirm the validity of the RT-PCR. Cells were pulse-labeled at 37°C for 5 h with [^{35}S]-methionine, 50 μCi/ml RPMI-1640 lacking L-methionine. Monolayers of the cells were rinsed three times with PBS, solubilized in a lysis buffer that contained 1% Triton X-100, 0.5% deoxycholic acid, 10 mM EDTA, and a protease inhibitor cocktail in PBS. It was then subjected to three freeze-thaw cycles. Supernatants and lysates were clarified by centrifugation for 15 min at 10,000 × g. The supernatants were incubated with an anti-T_f antibody overnight at 4°C. Immune complexes were then precipitated with excess protein G-agarose at 4°C for 5 h, washed three times with a washing buffer containing 1% Triton X-100, 5 mM EDTA and a protease inhibitor cocktail in PBS. It was then washed two times with the washing buffer containing 1 M NaCl, released by boiling in a Laemmli sample buffer, and

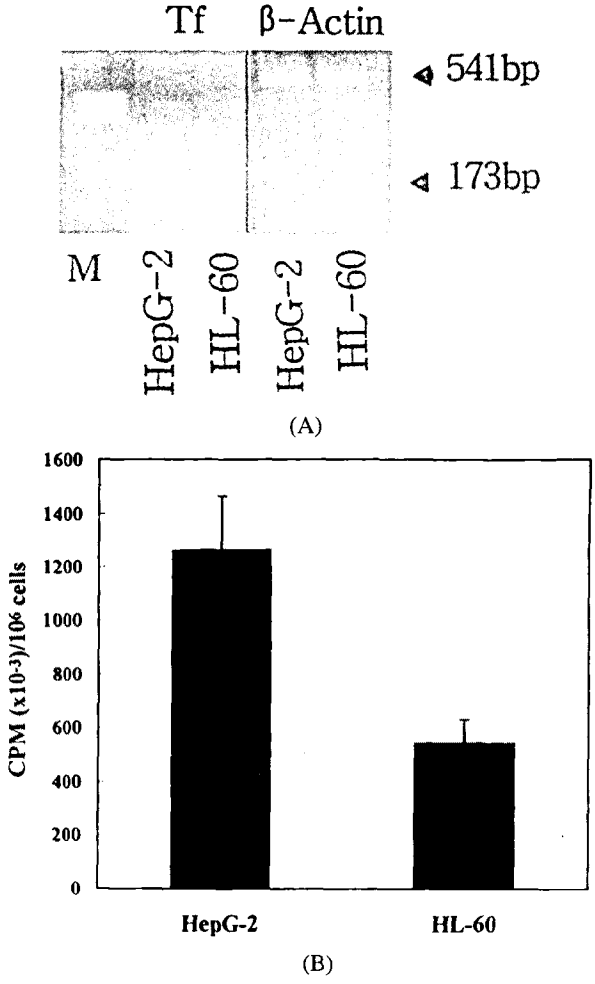

Fig. 1. Expression of T_f mRNA in HepG-2 and HL-60 cells. HL-60 and HepG-2 (positive control) cells were maintained in RPMI-1640 plus 10% fetal calf serum and antibiotics for 7 days at 37°C, 5% CO_2. The cDNAs were amplified using primers for T_f and β-actin for 45 cycles. (A) Ethidium bromide staining of T_f PCR products; M, fragment of Hae III digest of pBR322; 173 bp and 541 bp are the length of amplified fragments (base pairs) of T_f and β-actin. (B) Quantification of T_f PCR products. Following electrophoresis, appropriate bands were cut from the gels and radioactivities were counted. The cDNAs derived from the samples were normalized to yield equivalent β-actin products.

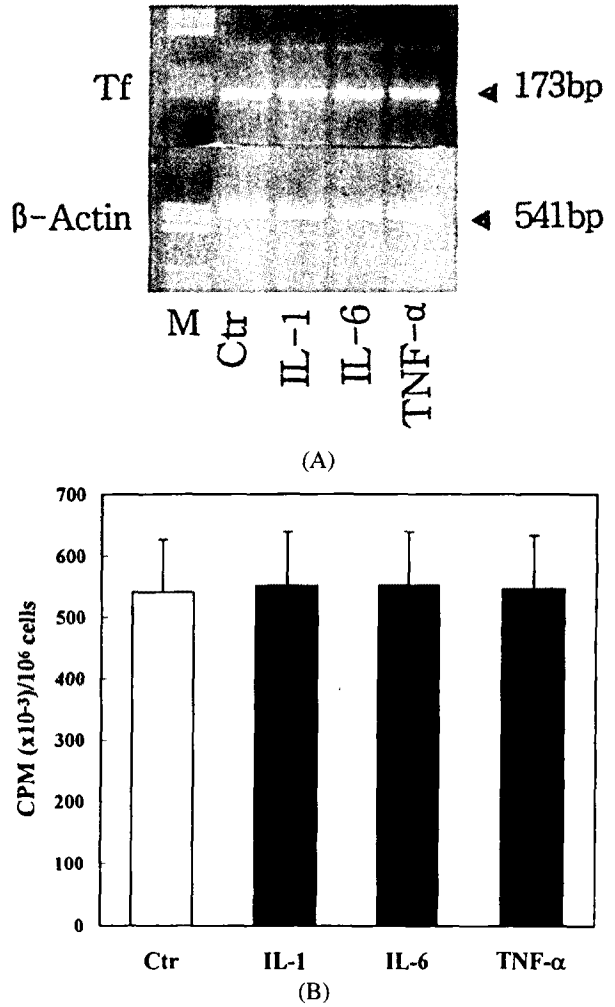

Fig. 2. Effects of cytokines on the expression of T_f mRNA in HL-60 cells. When the cells were grown to 90% confluency, the culture media was replaced by a serum free media and treated with IL-1β (1000 units/ml), IL-6 (200 units/ml) and TNF-α (200 units/ml) two times with a 24 h interval. cDNAs were amplified using primers for T_f and β-actin for 45 cycles. (A) Ethidium bromide staining of T_f PCR products; M, fragment of Hae III digest of pBR322; 173 bp and 541 bp are the length of amplified fragments (base pairs) of T_f and β-actin. (B) Quantification of T_f PCR products.

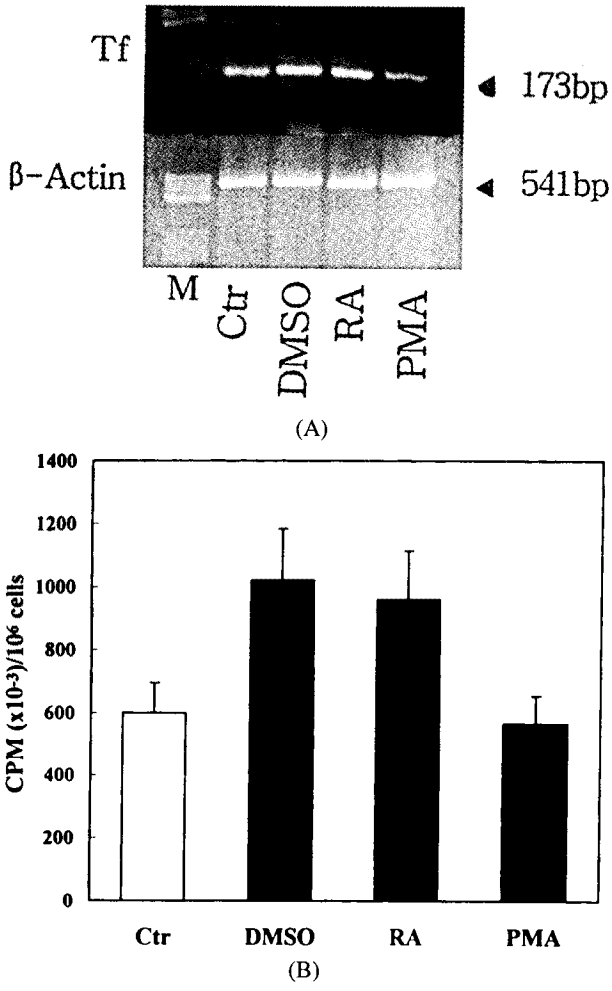

Fig. 3. Expression of T_f mRNA in differentiated HL-60 cells into granulocytes by DMSO or RA, and macrophage by PMA. When the cells were grown to 90% confluency, 1.2% of DMSO (dimethyl sulfoxide), 1 μM of *all-trans* RA (retinoic acid) or 50 nM of PMA (phorbol myristate acetate) were added respectively, and cultured for 4 days. The cDNAs were amplified using primers for T_f and β-actin for 45 cycles. (A) Ethidium bromide staining of T_f PCR products; M, fragment of Hae III digest of pBR322; 173 bp and 541 bp are the length of amplified fragments (base pairs) of T_f and β-actin. (B) Quantification of T_f PCR products.

applied to SDS-PAGE (12% gel) under reducing condition. After electrophoresis, the gels were stained with Coomassie brilliant blue, destained, dried, and subjected to autoradiography on Kodak SB-5X X-ray film for 24 h at RT.

Results and Discussion

This study demonstrated: (1) The amount of expressed mRNA for T_f in HL-60 cells was 50% of that in the positive control cell line, HepG-2 cells (Fig. 1). (2) The expression of T_f mRNA in HL-60 cells was not regulated by IL-1, IL-6 and TNF-α, respectively (Fig. 2). (3) The expression of T_f mRNA

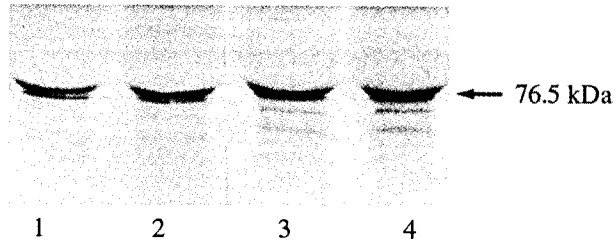

Fig. 4. Effect of cytokines on synthesis of T_f in HL-60 cells. After reaching confluence the cells were incubated overnight at 37°C in serum-free medium (control; Lane 1), serum free medium supplemented with IL-1 (Lane 2), IL-6 (Lane 3) and TNF-α (Lane 4). The cells were labeled at 37°C for 5 h with [^{35}S] methionine, 50 μCi/ml RPMI-1640 medium lacking methionine. The cells were then homogenized and cell lysates were prepared for sequential immunoprecipitation with an antibody to T_f. Immunoprecipitates were analyzed by SDS-PAGE/autoradiography as described under Materials and Methods. The arrow indicates a molecular weight of T_f.

in the differentiated cells into a granulocyte lineage by DMSO or *all-trans* RA, was up-regulated, 160-170% of control cells; whereas, the expression was not regulated in the differentiated cells into macrophage lineage by PMA (Fig. 3). (4) The synthesized T_f levels when treated with IL-1, IL-6 and TNF-α were parallel to the T_f mRNA levels using the RT-PCR method (Fig. 4).

It is recognized that T_f is synthesized mainly in hepatocytes and at a lower extent in Sertoli cells, oligodendrocytes, astrocytes and epithelial cells of the choroid plexus (Hertz *et al.*, 1996). This study is the first to demonstrate a high expression of mRNA for T_f in HL-60 leukemia cell line.

T_f has been known to act as part of an important autocrine mechanism that permits proliferation of the Sertoli cells, oligodendrocytes, astrocytes and epithelial cells of the choroid plexus (Vostrejs *et al.*, 1988). Molmenti *et al.* (1993) reported that the synthesis of T_f in differentiated Caco2 cells was significantly decreased when treated with IL-1β and IL-6. No change, however, was observed when treated with TNF-α. This confirms our results.

Chitambar *et al.* (1983) reported that the T_f receptor expressed human leukemic cells during proliferation and induction of differentiation. In this study the expression of T_f mRNA in the differentiated cells into a granulocyte lineage by DMSO or *all-trans* RA was significantly upregulated. However, the expression was unchanged in the differentiated cells into macrophage lineage by PMA. This indicates that the regulation of T_f mRNA expression might be dependent on the differentiation lineage in the HL-60 leukemia cell line. Molmenti *et al.* (1993) also demonstrated that the differentiated Caco2 cells grown on collagen-coated nitrocellulose filters enhanced the synthesis of acute phase proteins. Thus, the upregulation of T_f mRNA expression in the differentiated granulocyte lineage by DMSO or *all-trans* RA in the present study, appeared to be the result, but not the cause of the differentiation. In this study

in order to confirm the validity of RT-PCR, T_f synthesis was studied. The synthesized T_f levels were parallel to the T_f mRNA levels using the RT-PCR method. It has been suggested that T_f is one of the growth factors for normal and malignant cells because T_f was demonstrated as one of the essential constituents in DNA synthesis and cell growth (Chitambar *et al.*, 1986).

In conclusion, T_f mRNA was expressed in HL-60 leukemia cells and the expression was upregulated and differentiated into a granulocyte lineage. This suggests that T_f appears to function for cellular proliferation in the myelocytic leukemia cells.

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