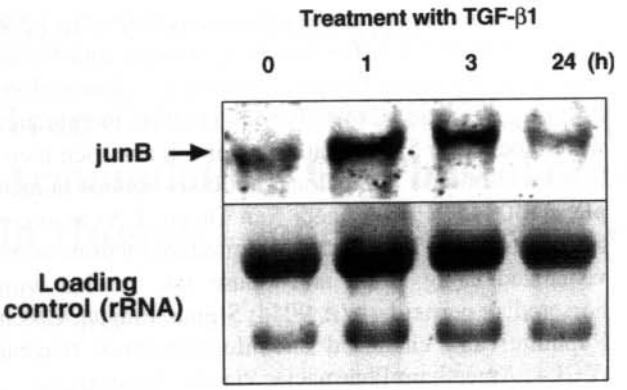

Fig. 1. Accumulation of junB protooncogene in TGF-β1-treated HL-60 cells. Cells were exposed continuously to 200 pM TGF-β1 and the levels of junB mRNA were determined by Northern blot analysis in unstimulated – control cells (0 h) and 1, 3, and 24 h after the beginning of the treatment of cells with TGF-β1. Total RNA visualized by ethidium bromide staining is shown to demonstrate equal loading. The data are representative of at least three independent experiments.

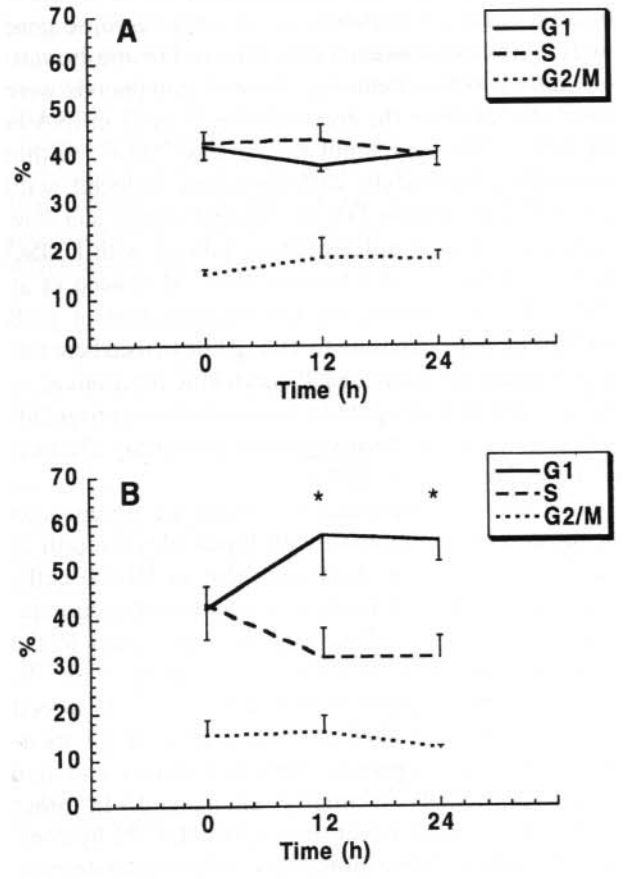

Fig. 2. The effect of TGF-β1 on the progression of the cell cycle in HL-60 cells. Cells were cultured without (A) and with (B) 200 pM TGF-β1 for 12 and 24 h, respectively. Symbol [*] represents the values that are significantly different from nontreated controls, $P < 0.05$.

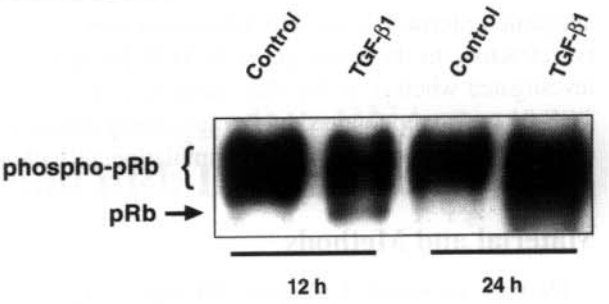

Fig. 3. The changes in the phosphorylation status of pRb in HL-60 cells upon their treatment with TGF-β1. Cells were cultured with and without TGF-β1 for 12 and 24 h, respectively. Cells cultivated for 12 or 24 h, respectively, without TGF-β1 served as a control. Cell lysates were Western-blot analyzed for pRb. The data are representative of at least three independent experiments.

Taken together, our results demonstrate for the first time that also in myeloid leukemia cells, here represented by the HL-60 cell line, TGF-β1 is capable to induce the molecular changes that are typically associated with entry into differentiation of blood cell lineages, including 1) accumulation of the early response gene junB, 2) accumulation of cells in G1 phase of the cell cycle, and 3) dephosphorylation of pRb. Based on these findings, we conclude that TGF-β1 signal transduction in myeloid cells is probably realized by a similar way as in other TGF-β1-responding cells. Thus, the insufficiency of TGF-β1 to induce differentiation in the myeloid cell lineage reported previously must be due to the requirement of this cell type for signals also from TGF-β1-independent regulatory pathways. As is obvious from the data mentioned above, the pathways regulated by TNF-α or vitamin D3 might be the potential candidates.

Acknowledgements

We thank Dr. Martina Kovaříková from our laboratory for critically reading the manuscript. We also thank Dr. Annie Valette, Laboratoire de Pharmacologie et de Toxicologie Fondamentales, C.N.R.S, Toulouse, France, for kindly providing us with junB cDNA.

References

Collins, S. J. (1987) The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. *Blood* **70,** 1233-1244.

Kamijo, R., Takeda, K., Nagumo, M., Konno, K. (1990) Effects of combination of transforming growth factor-β1 and tumor necrosis factor-α on induction of differentiation of human myelogenous leukemic cell lines. *J. Immunol.* **144,** 1311-1316.

Kozubík, A., Hofmanová, J., Dušek, L., Musilová, E. (1997) 5-lipoxygenase inhibitors potentiate effects of TGF-β1 on the differentiation of human leukemia HL-60 cells. *J. Leukocyte Biol.* **62,** 240-248.

Massagué, J. (1998) TGF-beta signal transduction. *Annu. Rev. Biochem.* **67,** 753-791.

Massagué, J., WeisGarcia, F. (1996) Serine/threonine kinase receptors: mediators of transforming growth factor beta family signals. *Cancer Surveys* **27,** 41-64.

Mollinedo, F., Naranjo, J. R. (1991) Uncoupled changes in the expression of the jun family members during myeloid cell differentiation. *Eur. J. Biochem.* **200,** 483-486.

Mollinedo, F., Gajate, C., Tugores, A., Flores, I., Naranjo, J.R. (1993) Differences in expression of transcription factor AP-1 in human promyelocytic HL-60 cells during differentiation towards macrophages versus granulocytes. *Biochem. J.* **294,** 137-144.

Reynisdóttir, I., Polyak, K., Iavarone, A., Massagué, J. (1995) Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev.* **9,** 1831-1845.

Testa, U., Masciulli, R., Tritarelli, E., Pustorino, R., Mariani, G., Martucci, R., Barberi, T., Camagna, A., Valtieri, M., Peschle, C. (1993) Transforming growth factor-β potentiates vitamin D3-induced terminal monocytic differentiation of human leukemic cell lines. *J. Immunol.* **150,** 2418-2430.

Turley, J. M., Falk, L. A., Ruscetti, F. W., Kasper, J. J., Francomano, T., Fu, T., Bang, O. S., Birchenall-Roberts, M. C. (1996) Transforming growth factor beta 1 functions in monocytic differentiation of hematopoietic cells through autocrine and paracrine mechanisms. *Cell Growth Differ.* **7,** 1535-1544.

Vindelov, L. L. (1977) Microfluorometric analysis of nuclear DNA in cells from tumors and cell suspension. *Virchows Arch. Cell. Pathol.* **24,** 227-242.

Xie, W. Q., Rothblum, L. I. (1991) Rapid, small-scale RNA isolation from tissue culture cells. *BioTechniques* **324,** 326-327.