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TGF β 1 Kills Lymphoma Cells Using Mitochondrial Apoptotic Pathway with the Help of Caspase-8

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Abstract. It is a known paradox that many TGF β 1-producing tumor cells are resistant to this, otherwise, inhibitory cytokine. In a lymphoma of B-cell origin exogenous TGF β 1 was able to induce apoptosis, suggesting that the apoptosis program can be switched on. The apoptosis induction was independent of the death receptors but dependent on mitochondrial pathway and caspase-3. Probably due to the weak starting signal, caspase-3 further activated caspase-8 which, through the Bid cleavage and Bax translocation into the mitochondria, provided an autocatalytic support for the apoptotic program. There is a time-gap between the early activation of Smad-dependent TIEG and the accumulation of ROS, therefore other participants that start the increase in mitochondrial membrane permeability should be identified.

TGF β 1, a multifunctional cytokine, is part of the regulatory network deciding the size of a lymphocyte population, either by inhibiting proliferation or by inducing apoptosis. The TGF β 1 signal is received by its receptors and transferred by Smads and protein-kinases to the nucleus to activate TGF β 1 responding genes (1, 2). Many actively proliferating lymphomas can produce TGF β 1, which has no effect on lymphoma cells but block the proliferation of normal lymphocytes (causing immunosuppression) (3). Previously we showed that the lost capacity of endogenous TGF β 1 to induce apoptosis can be reverted by exogenous TGF β 1 in proliferating HT58 lymphoma cells and that this is a death-receptor independent, but a caspase-3 dependent response (4). Besides death-receptors the other option to activate caspase-3 is the use of the mitochondrial pathway. In hepatocytes the TGF β 1-induced apoptosis was accompanied by an increased level of ROS and decreased expression of

antiapoptotic members of the BCL-2 family (5). Here we show that, in HT58 lymphoma cells, the exogenous TGF β 1 induces apoptosis using mainly the mitochondrial apoptotic pathway with the involvement of TIEG and caspase-8.

Materials and Methods

Cells. Experiments were performed on a human non-Hodgkin lymphoma cell line of B cell origin (6). The cells were cultured in RPMI 1640, with 10% FCS, glutamine and gentamycin, at 37°C, in an atmosphere of 95% air and 5% CO₂. For all experiments cells growing in the exponential phase of the culture were used. The cells were treated in 24-well plates at a density of 2 x 10⁵ cells/ml/well.

Treatment. The cells were treated with 1 ng/ml TGF β 1 (R&D System Minneapolis, MN, USA). Caspase-8 inhibitor [Z-IETD-fmk (Pharmingen Becton-Dickinson Co., San Diego, CA, USA), Z-LETD-fmk (Biorad)] was given in 50 μ M, caspase-9 inhibitor (Z-LEHD-fmk; Pharmingen, Becton Dickinson Co.) in 50-100 μ M and N-acetyl-cysteine (NAC; Fluka Chemie AG Buchs, Switzerland) in 5 mM concentration. The caspase inhibitors and NAC were introduced 0.5 hours before the TGF β 1 treatment.

Apoptosis. Cells for apoptosis detection were prepared according to the method described by Gong *et al* (7). Medium-free cells were suspended in 70% ethanol (-20°C), left at room temperature for 30 minutes, and kept at -20°C until the measurements. Ethanol-fixed cells were pelleted and suspended in 500 μ l buffer (200 mM disodiumphosphate, pH 7.8, adjusted with 200 mM citrate, and containing 100 μ g/ml RNase [Sigma St. Louis, MI, USA]). The samples were incubated on room temperature for 30 minutes and 5 μ l ethidium bromide (Calbiochem Darmstadt, Germany) was given (final concentration: 10 μ g/ml). The cells were kept for another 15 min before flow cytometric measurements to estimate the cell cycle and proportion of sub-G1 (apoptotic) cells.

Flow cytometry. Measurements were made on a FACScan flow cytometer (Becton-Dickinson San Jose, CA, USA), and for each sample data 6-10,000 cells were collected. An argon ion laser was used at 15 mW and 488 nm.

Measurement of intracellular ROS. For measuring intracellular ROS, an oxidation sensitive probe, H₂DCF-DA (Molecular Probes, Eugene, OR, USA), was used. After treatment with TGF β 1 and/or NAC, the cells were incubated with H₂DCF-DA (final conc: 5 μ M) for 30 minutes. Then we measured its fluorescence by flow cytometry.

Alternatively, ROS was detected by confocal microscope using the method described by Telek *et al.* (8). The cells were washed in 0.9%

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Key Words: Lymphoma, apoptosis, TGF β 1, caspase-8, Bax, Bid, TIEG.

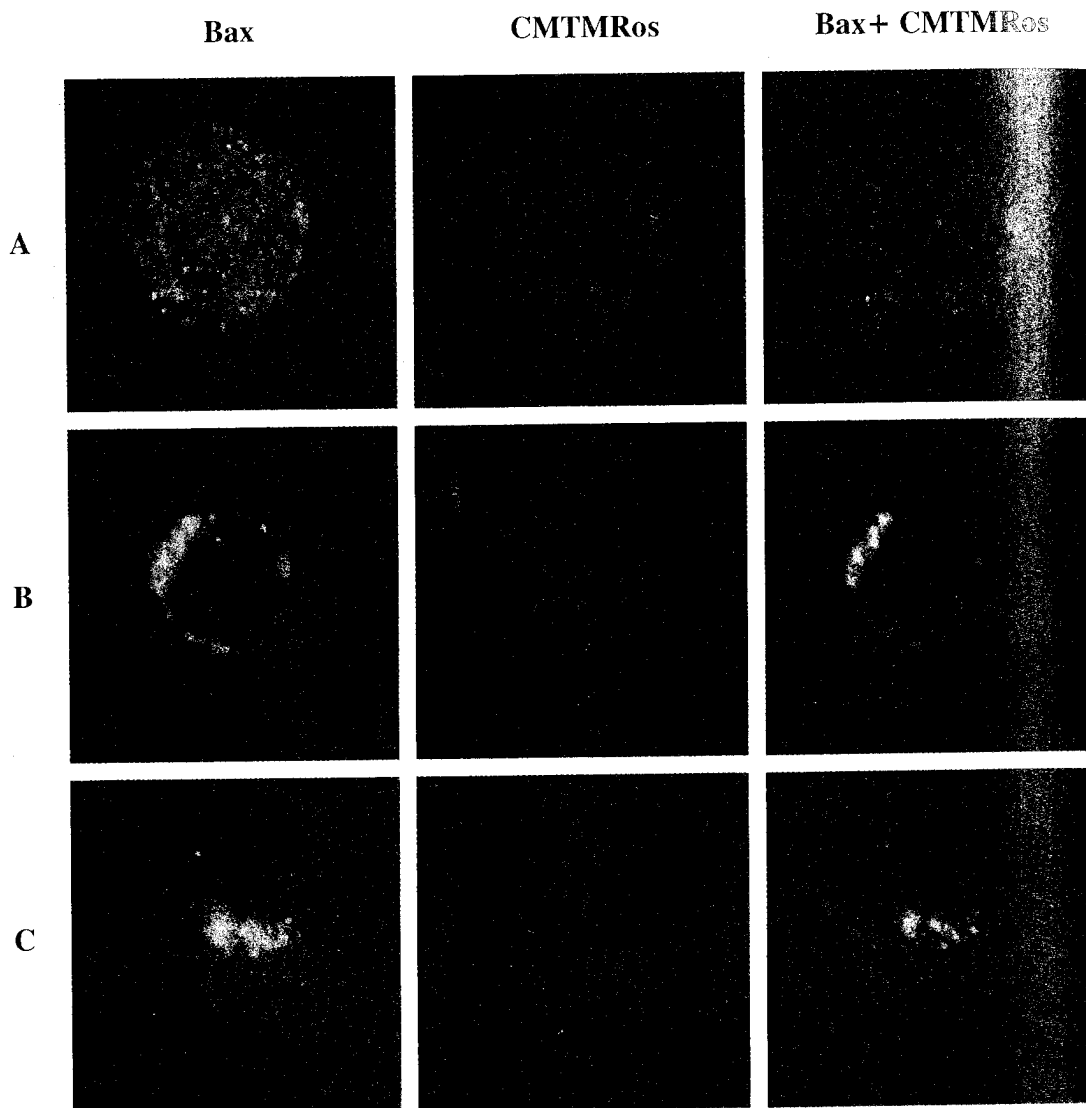


Figure 1. Translocation of Bax. Proapoptotic signals induce the polymerisation and translocation of Bax from the cytosol to the mitochondria. (A) In control cells Bax is dispersed in the cytosol. (B) After 24 hours of TGF β 1 treatment (1 ng/ml), Bax is translocated in the mitochondria. (C) After 48 hours of TGF β 1 treatment, Bax is in the mitochondria which is depolarized. We used CMTMRos (50 nM) staining to label the mitochondria and immunostaining to detect Bax (DAKO).

NaCl solution then resuspended. CeCl₃ solution was added to the cells (10 mM final conc.) for 15 minutes. The mitochondria were stained by DiOC₆ (20 nM; Sigma) and the nucleus by propidium iodide (10 μ g/ml; Sigma). After incubation we examined the cerium perhydroxide precipitates in the samples by confocal microscope in reflectance mode (MRC1024 Biorad, Labs, Hercules, CA, USA).

Bid Western-blot. 10⁶ cells were treated with the TGF β 1. The treated cells (1 μ g/ml/10⁶ cells) were harvested at the indicated time-points and were washed in 0.9% NaCl solution twice. The cells were pelleted (1300 rev/min, 5 minutes, Sigma 3K10 Laborcentrifuge) then 130 μ l lysis buffer (50 mM Tris-HCl, pH = 7.5, 150 mM NaCl, 1% NP40, 1mM PMSF, 10 mM NaF, 0.5 mM sodium-vanadate, 10 μ l/mg leupeptin, 10% glycerol) was added and the samples were incubated for 10 minutes on ice. After incubation samples were centrifuged (20 minutes, 15000g, 4°C) and the

protein content of the supernatants was measured by the Bradford assay. Twenty μ g of total protein was placed for Western-blot on a 15% polyacrylamide gel. We used a monoclonal antibody (1:1500) specific for Bid (Transduction Laboratories, Lexington, KS, USA). Developing was performed by the Vectastain ABC Kit and ECL+Plus chemiluminescence detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Detection of Bax translocation. The TGF β 1-treated cells were incubated with CMTMRos (Molecular Probes) (50 nM) for 15 minutes. After washing in PBS, they were fixed in ice-cold 80% methanol. We used a polyclonal antibody (1:20) specific for Bax (DAKO, Glostrup, Denmark). Developing was performed by the Vectastain ABC Kit and Streptavidin-FITC (DAKO). Samples were studied by confocal microscope (Biorad, MRC1024).

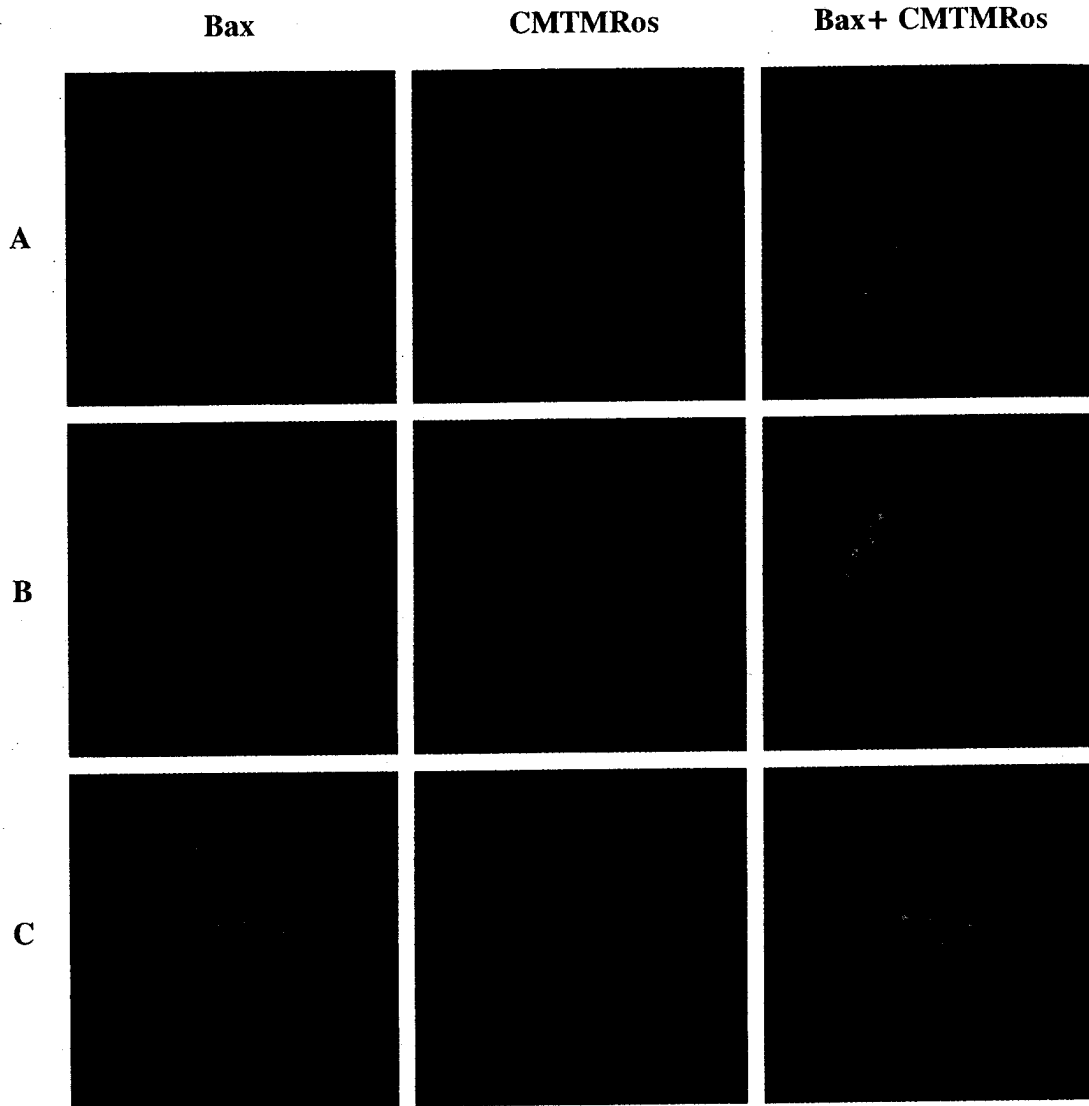


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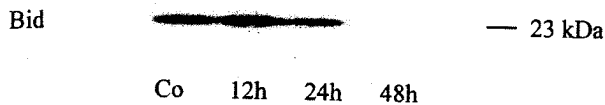


Figure 2. Cleavage of Bid. The protein level of Bid was decreased after 24 hours of TGFβ1 (1 ng/ml) treatment. There was no detectable Bid protein at 48 hours after TGFβ1 treatment. The cleavage site of Bid by caspase-8 is upstream from the immunization site, therefore the cleavage of Bid decreases the amount of immunolabelled protein.

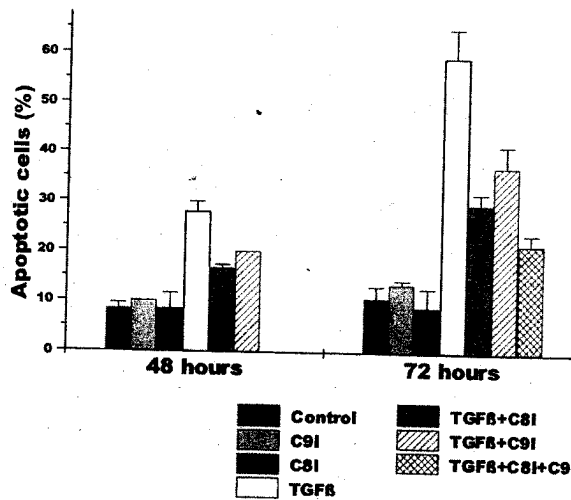


Figure 3. Effect of inhibition of caspase-8 and -9. Specific caspase-8 inhibitors (zIETD-fmk, zLETD-fmk 50 μM) and specific caspase-9 inhibitor (zLEHD-fmk 100 μM) decreased the level of apoptosis induced by TGFβ1 (1 ng/ml). C81: caspase-8 inhibitor; C91: caspase-9 inhibitor.

TGFβ1-induced apoptosis in spite of the previously observed independency from death-ligands and death-receptors.

Activation of caspases. Bid can be cleaved mainly by caspase-8. Here we studied the effect of the inhibition of caspase-8 activity on TGFβ1-induced apoptosis using inhibitors such as zIETD-fmk and zLETD-fmk. These inhibitors decreased the level of apoptosis, but not completely (Figure 3). This suggests that in TGFβ1-induced apoptosis caspase-8 is activated by caspase-3, and there is no need for death receptors. On the other hand, caspase-3 could be activated by caspase-9. Again, an inhibitor, zLEHD-fmk, was used to prove the participation of caspase-9: the inhibitor lowered the number of apoptotic cells. The inhibitory affect was cumulative when caspase-8 and -9 inhibitors were added together, but they could not decrease the apoptosis to the control level (Figure 3). This means that a fraction of caspase-3 activation is independent from these initiator caspases.

Role of reactive oxygen species (ROS). If we suggest that caspase-3 is activated *via* the mitochondrial pathway, then to change the mitochondrial permeability an activator should be found. One of the candidates is ROS (oxygen-containing reactive substances), which can be overproduced in increased quantity by different cellular stresses. In hepatocytes TGFβ1 induced an increase in ROS level (5). In HT58 cells, the elevation in ROS level caused by TGFβ1 treatment was detected by H2DCF-DA staining in flow cytometry (Figure 4) and confocal microscopy (not shown). TIEG (a Smad-dependent transcription factor; TGFβ1-induced early gene) could be a link between TGFβ1 action and ROS production. TIEG can increase the expression of some pro-oxidative enzymes: *e.g.* NADH-oxidase (11) and decrease some anti-

RT-PCR. RNA was isolated from the cells with the RNeasy Total RNA kit (Qiagen, Hilden Germany) and checked by photometry (quantity) and gel electrophoresis (quality). The reverse transcription reaction was started on 100 ng RNA with random primers at 42 °C for 1 hour in 20 μl.

In PCR, 2.5-3.0 μl reverse mix (~0.075 μg total RNA) was used as the template. The cDNA was amplified with red-Taq polymerase from Sigma. In semiquantitative RT-PCR we checked the equal quantity of cDNA by β-actin amplification in the control and TGFβ-treated samples. The primers used were:

TIEG	F	ACA-GGA-GAA-AAG-CCT-TTC-AGC	
	R	TTT-TAC-ATC-ACC-ACT-GGC-TCC	size: 328 bp
SOD	F	AGG-GCA-TCA-TCA-ATT-TCG-AG	
	R	TCT-TCA-TTT-CCA-CCT-TTG-CC	size: 355 bp
Catalase	F	CCT-GAC-TAT-GGC-ATC-CGG	
	R	TAG-TTG-GCC-ACT-CGA-GCA-C	size: 386 bp
β-actin	F	GTG-GGG-CGC-CCC-AGG-CAC-CA	
	R	CTC-CTT-ATT-GTC-ACG-CAC-GAT-TTC	size: 538 bp

DNA (PCR fragments) were size-separated by agarose gelelectrophoresis (1.0-1.5% agarose and 1 μg/ml ethidium bromide). The gels were evaluated with Eagle-Eye Videodensitometer (Stratagene, Heidelberg, Germany).

Results

TGFβ1 induces depolarization of mitochondria. We already shown that activated exogenous TGFβ1 induces the depolarization of the mitochondrial membrane. The universal caspase inhibitor zVAD-fmk rescued the cells partially from mitochondrial depolarization, suggesting that not only the caspases are involved in depolarization or that the inhibition is incomplete (4). Nevertheless the mechanism of depolarization remained to be clarified. One common possibility in mitochondrial depolarization is the activation of proapoptotic and/or inhibition of antiapoptotic members of Bcl-2 family (9). Measuring the expression (mRNA, protein) of some Bcl-2 family members (Bcl-2, Bcl-X_L, Bax) we found no change in TGFβ1-treated HT58 lymphoma cells (4). Another possibility to modify the pro- versus anti-apoptotic balance is the translocation of Bax from the cytosol to the mitochondrial membrane. In fact, this happened in HT58 lymphoma: in non-treated cells Bax was dispersed in the cytosol, and was translocated into the mitochondria at 24 hours after TGFβ1 treatment (Figure 1). Such translocation is promoted by signals like cleavage of Bid (10). Therefore, we studied the cleavage of Bid by Westernblot: the detectable fragment of Bid decreased at 24 hours after TGFβ1 treatment (Figure 2). This result indicates the possible involvement of caspase-8 in

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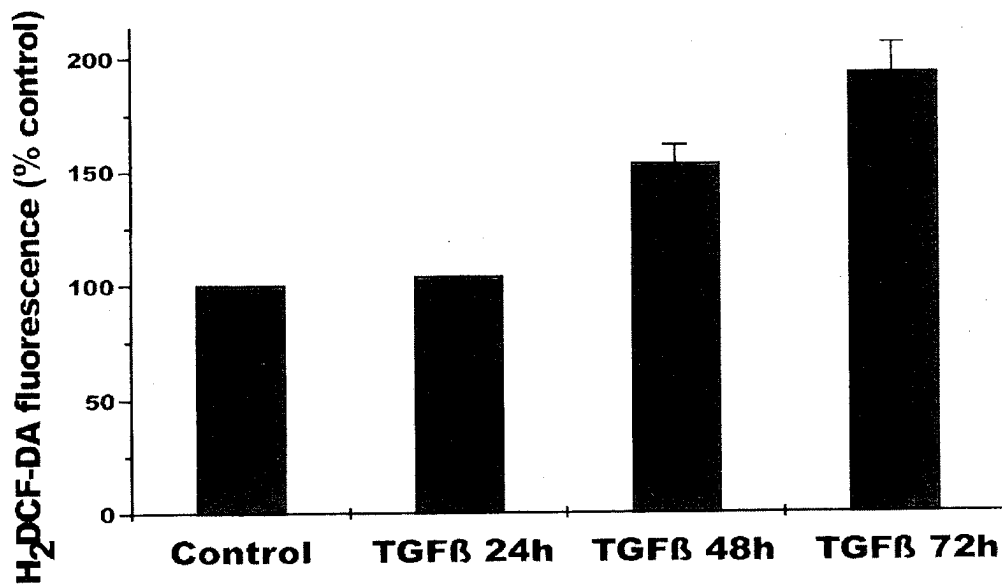


Figure 4. Elevation of ROS level. After TGFβ1 (1 ng/ml) treatment the elevation of the ROS level in living cells was detected by H₂DCF-DA (5 μM) at 24 hours using flow cytometry. The increase was more pronounced at 48 and 72 hours.

oxidatives such as catalase and superoxide dismutase (12). TIEG is activated in HT58 cells as early as 0.5 hours after TGFβ1 treatment (Figure 5), however there was no change either in the mRNA-expression of catalase or SOD (not shown). When we used N-acetyl-cystein (NAC), as an antioxidant to decrease ROS, an antiapoptotic effect was achieved (Figure 6).

Discussion

A concept of cancer therapy is to induce apoptosis in cancer cells by cytotoxic agents. An alternative could be the use of physiological regulators which induce apoptosis in certain cell types, as TGFβ1 does in many cells including lymphocytes. The paradox is that in many cancers, including lymphomas, where the normal counterpart is TGFβ1-sensitive, the tumor cells lose the sensitivity to the endogenously produced TGFβ1. To resensitize the lymphoma cells towards physiological apoptotic signals it is necessary to understand the signaling pathway.

The first question is whether apoptosis is still inducible or not. It turned out, surprisingly, that in an EBV-negative, TGFβ1 producing lymphoma line of B-cell origin (HT58) the exogenous TGFβ1-was able to induce apoptosis (although it did not influence cell proliferation, as we described previously, 13). This slowly developing process (the apoptotic ratio gradually increased from 24 hours after treatment) proved to be independent from the death ligand/- receptor pathway (4).

Another known pathway, besides death-ligands and -receptors, to induce apoptosis is the induction of channels or

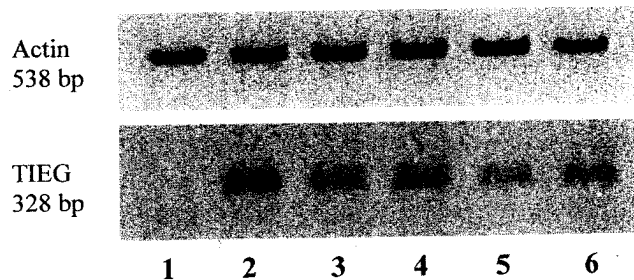


Figure 5. Expression of TIEG. The expression of TIEG mRNA increased after TGFβ1 (1 ng/ml) treatment.

pores in the mitochondrial membranes (14, 15) to let cytochrome c and other substances be released into the cytosol. One of the important consequences is the formation of apoptosome, and activation of caspase-9. In some studies the decreased expression of the antiapoptotic members of the Bcl-2 family has been observed after TGFβ1 treatment in prostate epithelial cells and also in multipotent hematopoietic cells (16, 17), however in HT58 cells the quantity of Bcl-2 family proteins did not change. There are many other triggers which can lead to increased permeability of the mitochondrial membrane, e.g. translocation of Bax and the increased level of ROS. Bax is a cytosolic protein and could be translocated into the mitochondrial membrane by a proapoptotic signal, e.g. by a cleaved fragment of BID (tBID) (18). The cleavage of BID is performed mainly by caspase-8.

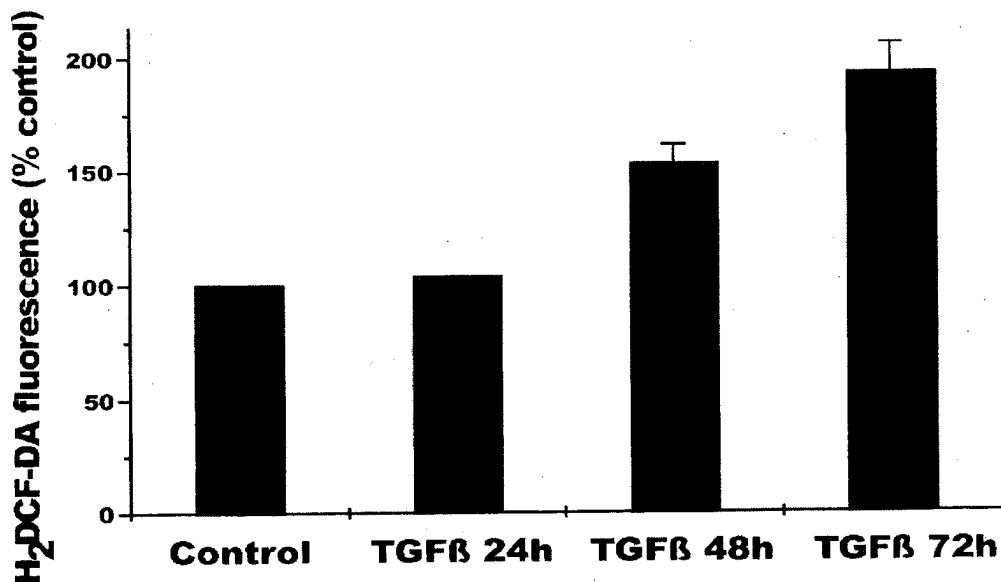


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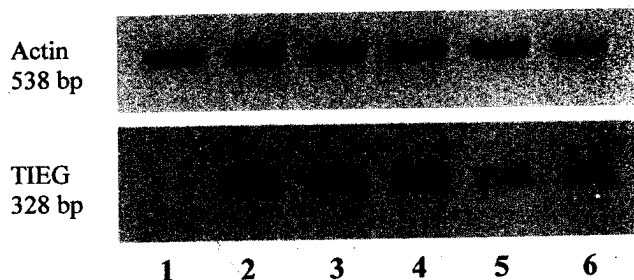


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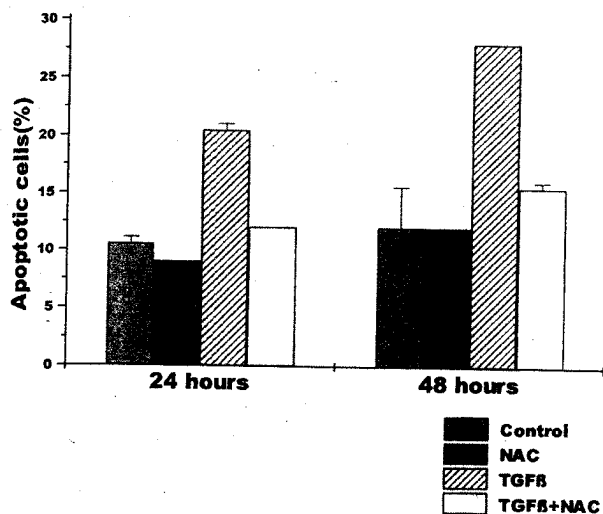


Figure 6. Effect of N-acetyl cysteine. N-acetyl cysteine (NAC) (5 mM) decreased the apoptosis by its antioxidant effect after 24 and 48 hours of TGFβ1 (1 ng/ml) treatment.

It has been described that, at least in the case of the death-receptor pathway, there are two types of cells: those which have sufficient activated caspase-8 to induce apoptosis (type I cells), and those which have a weaker caspase-8 signal so the "help" of the mitochondrial pathway (essentially by cleaving Bid) (type II cells) is needed (19). Similarly, we suggest that in lymphoma cells following the first wave of caspase-3 activation, procaspase-8 is a target for caspase-3. Caspase-8 can enhance further activation of caspase-3 and can switch on the Bid-Bax sequence to support the mitochondrial pathway. Essentially, this seems to be the opposite than was described for type II cells in the death receptor pathway, although the end result, apoptosis induction, is the same. In type II cells the mitochondrial pathway helps caspase-8, while here caspase-8 is the helper.

The missing link between TGFβ1 action and pore formation in the mitochondrial membrane could be the increased expression of Smad-dependent TIEG, which elevated the intracellular ROS formation (20), probably through the inhibition of certain antioxidants. The role of antioxidants was underlined by the use of NAC (an antioxidant) which behaved as an antiapoptotic agent. However, in our case the activation of TIEG was "too early" compared to the accumulation of ROS. Therefore other mitochondrial membrane attacking agents could be present as well.

It is clear, that the effect of TGFβ1 changes from one cell type to another. Also, it can influence many signaling pathways. Here we described that the Smad-pathway is inducible and the result is apoptosis even in otherwise TGFβ1-resistant cells. Probably, the apoptotic pathway is engaged by the inhibition of a survival signal maintained by

protein kinases (21). This is a clear indication that facets of TGFβ1 action should still be recognized.

Acknowledgements

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References

- 1 Attisano L, Silvestri C, Izzi L and Labbe E: The transcriptional role of Smads and FAST (FoxH1) in TGFβ and activin signalling. *Mol Cell Endocrinol* 180: 3-11, 2001.
- 2 Derynck R, Akhurst RJ and Balmain A: TGFβ signalling in tumor suppression and cancer progression. *Nature Genet* 29: 117-129, 2001.
- 3 Lotz M, Ranheim E and Kipps TJ: Transforming growth factor beta as endogenous growth inhibitor of chronic lymphocytic leukemia B cells. *J Exp Med* 179: 999-1004, 1994.
- 4 Tóth A, Sebestyén A, Barna G, Nagy K, Göndör A, Bocsi J, Mihalik R, Petak I, Houghton J and Kopper L: TGFβ1 induces caspase-dependent but death-receptor independent apoptosis in lymphoid cells. *Anticancer Res* 21: 1207-1212, 2001.
- 5 Herrera B, Alvarez AM, Sanchez A, Fernandez M, Roncero C, Benito M and Fabregat I: Reactive oxygen species (ROS) mediates the mitochondrial dependent apoptosis induced by transforming growth factor β in fetal hepatocytes. *FASEB J* 15: 741-751, 2001.
- 6 Kopper L, Bánkfalvi Á, Mihalik R, Nagy P, Fülöp Cs and Sármay G: New *in vitro* line from human (B) non-Hodgkin lymphoma. *Anticancer Res* 11: 1645-1651, 1991.
- 7 Gong J, Traganos F and Darzynkiewicz Z: A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Anal Biochem* 218: 314-319, 1994.
- 8 Telek G, Scoazec JY, Chariot J, Ducroc R, Feldmann G and Roz C: Cerium-based histochemical demonstration of oxidative stress in taurocholate-induced acute pancreatitis in rats. A confocal laser scanning microscopic study. *J Histochem Cytochem* 47: 1201-1212, 1999.
- 9 Lee RH, Song JM, Park MY, Kang SK, Kim YK and Jung JS: Cisplatin-induced apoptosis by translocation of endogenous Bax in mouse collecting duct cells. *Biochem Pharmacol* 62: 1013-1023, 2001.
- 10 Eskes R, Desagher S, Antonssai B and Martinou JC: Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Mol Cell Biol* 20: 929-935, 2000.
- 11 Thannickal VJ and Fanburg BL: Activation of an H₂O₂-generating NADH oxidase in human lung fibroblasts by transforming growth factor beta 1. *J Biol Chem* 270: 334-338, 1996.
- 12 Kayanoki Y, Fujii J, Suzuki K, Kawata S, Matsuzawa Y and Taniguchi N: Suppression of antioxidative enzyme expression by transforming growth factor-beta 1 in rat hepatocytes. *J Biol Chem* 269: 15488-15492, 1994.
- 13 Nagy P, Török N, Ladányi A and Kopper L: Expression of transforming growth factor-beta in human non-Hodgkin's lymphoma xenografts. *J Natl Cancer Inst* 83: 1174-1175, 1991.
- 14 Kroemer G: Mitochondrial control of apoptosis: an overview. *Biochem Soc Symp* 66: 1-15, 1999.
- 15 Chao DT and Korsmeyer J: BCL-2 family: regulators of cell death *J Annu Rev Immunol* 16: 395-419, 1998.
- 16 Chipuk JE, Bhat M, Hsing AY, Ma J and Danielpour D: Bcl-X_L blocks transforming growth factor-beta-1 induced apoptosis by inhibiting cytochrome c release and not by directly antagonizing Apaf-1-dependent caspase activation in prostate epithelial cells. *J Biol Chem* 276: 26614-26621, 2001.
- 17 Francis JM, Heyworth CM, Spooncer E, Pierce A, Dexter TM and

- Whetton AD: Transforming growth factor-beta 1 induces apoptosis independently of p53 and selectively reduces expression of Bcl-2 in multipotent hematopoietic cells. *J Biol Chem* 275: 39137-39145, 2000.
- 18 Motyl T, Gajkowska B, Ploszaj T, Wareski P, Skierski J and Zimowska W: Expression and subcellular redistribution of Bax during TGF-beta1-induced programmed cell death of HC11 mouse mammary epithelial cells. *Cell Mol Biol* 46: 175-185, 2000.
- 19 Krammer PH: CD95's deadly mission in the immune system. *Nature* 407: 789-795, 2000.
- 20 Ribeiro A, Bronk SF, Roberts PJ, Urrutia R and Gores GJ: The transforming growth factor beta(1)-inducible transcription TIEG1, mediates apoptosis through oxidative stress. *Hepatology* 30: 1490-1497, 1999.
- 21 Sebestyen A, Barna G, Nagy K, Jánosi J, Paku S, Bocsi J, Berczi L, Mihalik R and Kopper L: A classical and alternative signaling pathways in TGFβ1 induced apoptosis of lymphoma cells. *Int J Cancer* (submitted for publication).

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