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Thrombospondin-1 (TSP-1) and TSP-1-derived Heparin-binding Peptides Induce Promyelocytic Leukemia Cell Differentiation and Apoptosis

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Abstract. *Thrombospondin-1 (TSP-1) is a multifunctional adhesive glycoprotein that is synthesized by several cell types and modulates cell growth and differentiation. In this study, we showed that the amount of TSP-1 secreted by two human leukemia cell lines, HL-60 and NB4, increased markedly during differentiation of these cells by all-trans retinoic acid (ATRA) (10^{-7} M), reaching about 100 ng/10^6 cells after 3 days. Addition of purified TSP-1 alone (10^{-9} – 5 x 10^{-8} M) to HL-60 or NB4 cell cultures dose-dependently inhibited cell growth and differentiation. Differently to ATRA, TSP-1-induced differentiation of HL-60 and NB4 cells occurred independently of Bcl-2 regulation, as shown by immunofluorescence and Western immunoblotting. At day 5, TSP-1 also induced promyelocytic leukemia cell apoptosis. The percentage of apoptotic cells in NB4 cultures was higher with TSP-1 (5 x 10^{-8} M) than with ATRA (10^{-7} M) (46±3% versus 19±7%, $p<0.001$), whereas similar levels of apoptosis (37±7% and 38±6%) were reached with both agents in HL-60 cultures. Studies performed with synthetic peptides derived from the TSP-1 sequence indicated that two heparin-binding peptides, Hep-I and GGWSHW, located within the NH_2-terminal and type 1 repeats respectively, were strong inducers of apoptosis of HL-60 and NB4 cells, suggesting that cell surface heparan sulfate molecules might be involved in the apoptotic effect of TSP-1 on promyelocytic cells.*

Previous studies have shown that the HL-60 myeloid leukemia cell line (M2) can be induced to differentiate into neutrophils and die *in vitro* upon treatment with all-*trans* retinoic acid (ATRA), a vitamin A derivative (1, 2). ATRA has been successfully used to induce remission of a majority of patients with acute promyelocytic leukemia, although ATRA-resistant relapses are still a major problem (3). The effect of ATRA on cells is mediated by nuclear receptors acting as transcriptional factors, which modulate the expression of many genes implicated in cell proliferation, differentiation and survival (4). Among these, extracellular matrix proteins have the ability to transmit signals into cells *via* specific receptors, and act in concert with cytokines to influence cell functions (5, 6). Thrombospondin-1 is a cytoadhesion molecule expressed in bone marrow that modulates the adhesion and proliferation of hematopoietic cells *in vitro* (7, 8). Its synthesis is up-regulated during the differentiation of many cells by ATRA, including neuronal cells, embryonal carcinoma cells and HL-60 cells (9-11).

Thrombospondin-1 (TSP-1) is a large multifunctional homotrimeric glycoprotein (*M*r, 450 000). Each subunit consists of multiple domains which may react with several cell surface receptors, matrix components and growth factors (12, 13). These include an amino-terminal heparin-binding domain, a pro-collagen-like domain, three type I (properdin-like) repeats, three type II (epidermal growth factor-like) repeats, seven type III (calcium-binding) repeats and a carboxy-terminal cell binding domain. In the last decade, TSP-1 and type-1 repeat peptides have been recognized as inhibitors of endothelial cell proliferation and angiogenesis (14-17), whereas the amino-terminal domain produced by proteolysis is pro-angiogenic (18-20). TSP-1 may bind to cell surface-associated proteoglycans and sulfatides, CD36 or 88-kDa transmembrane protein, a yet unidentified 50-kDa receptor, several integrins (α3β1, α4β1, α5β1, αIIbβ3, αvβ3) and CD47 or 50-kDa integrin-associated protein (IAP) (for a review see ref. 13). Among these receptors, CD36 was shown to mediate the anti-angiogenic activity of TSP-1, and type I-derived peptides, on

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capillary endothelial cells, *via* a signaling pathway leading to endothelial cell apoptosis (21-23). On the other hand, two other receptors, the integrin α3β1 and syndecan 4, have been implicated in the pro-angiogenic effect of TSP-1 and its amino-terminal domain (19, 20). Finally, the interaction of TSP-1 with CD47/IAP was shown to induce a caspase-independent death signaling pathway in T-lymphocytes and chronic myeloid leukemia cells (24, 25).

Apoptosis is a highly ordered cell death process characterized by ultrastructural modifications, nuclear alterations and enzyme activations, leading to fragmentation of dying cells into apoptotic bodies that are phagocytosed by neighboring cells or macrophages (26). The life and death of cells must be balanced for the maintenance of normal tissue homeostasis, and cancer is most often associated with a resistance of cells to apoptosis.

The present study was designed to investigate the response of two different promyelocytic cell lines, HL-60 (M2) and NB4 (M3), to TSP-1 in terms of cell growth, differentiation and apoptosis. We were particularly interested to investigate the NB4 cell line, the first established human promyelocytic cell line with the specific t(15:17) chromosomal translocation, as these cells are highly sensitive to differentiation by ATRA, but largely resistant to apoptosis induced by this agent (27-29). Differently to ATRA, we found that TSP-1 was a potent inducer of both differentiation and apoptosis in NB4 cells.

Materials and Methods

Reagents. All-*trans*-retinoic acid (ATRA), bovine serum albumin (BSA), human transferrin and bovine insulin were purchased from Sigma Aldrich Chimie SARL (St Quentin Fallavier, France). ATRA was solubilized in 100% ethanol at 10^{-2} M and stored at 10^{-4} M at -20˚C in aliquots. Thrombospondin-1 (TSP-1) was purified from the supernatant of thrombin-activated platelets and characterized as described (30). The following TSP-1 peptides were purchased: GGWSHW and RFYVVMWK from BACHEM Biochimie SARL (Voisins-le-Bretonneux, France). The ELTGAARKGSGRRLVKGPD (Hep-I) synthesized by Serbio (Gennevilliers, France) was 99% pure. The CSVTCG peptide was a gift from Dr G.P. Tuszynski (Allegheny University, Philadelphia, PA, USA). The monoclonal (5G11) and polyclonal (R1) anti-TSP antibodies used for ELISA have already been reported (31). The mouse monoclonal anti-human Bcl-2 antibody, rabbit polyclonal anti-Bax antibody, goat anti-mouse or anti-rabbit polyclonal antibodies conjugated to peroxidase (for immunoblotting) or to FITC (for immunofluorescence) were from Dako (Glostrup, Denmark).

Cells. The HL-60 acute human myeloid cell line (M2) was obtained from ATCC (Rockville, MD, USA). The NB4 acute promyelocytic leukemia cell line (M3) that contains the translocation t(15:17) (27) was kindly provided by Dr M. Lanotte (U496 INSERM, Hôpital St Louis, Paris). Cells were maintained in suspension culture at 3.5 x 10^5 cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 100 units/ml penicillin G and 100 μg/ml streptomycin (all products from GIBCO BRL-Life Technologies SARL, Cergy-Pontoise, France), at 37˚C with 5% CO_2. For differentiation into neutrophils, cells were grown for up to 5 days in the presence of 10^{-7} M ATRA. For the untreated control, the same final amount of ethanol was added to the culture medium. Cell viability was assessed by the trypan blue exclusion dye test.

Enzyme-linked immunosorbent assay (ELISA). Cells ($3.5x10^5$/ml) were grown in the presence of RPMI 1640 containing 10% of serum plant substitute (Amplicell, BIOMEDIA, Boussens, France) instead of 10% FCS to minimize the presence of an exogenous source of TSP-1 in the ELISA assay. At selected times after the addition of ATRA (10-7 M), the cells were centrifuged and the conditioned medium was stored at -80˚C until the assay. The level of TSP-1 was quantified by ELISA using purified TSP-1 diluted in the same medium as standard, as described previously (31).

Cell growth, differentiation and apoptosis. Cells (3.5 x 10^5/ml) in RPMI 1640 containing 10% of serum plant substitute were seeded in 24-well plates with or without TSP-1 (from 10^{-9} M to 5 x 10^{-8} M) or ATRA (10^{-7} M). Peptides of TSP-1 were used between 10^{-6} and 10^{-4} M. The number of viable cells was evaluated over a 5-day period by direct cell counting in a hemocytometer chamber using the trypan blue dye exclusion method. Viable cells comprise proliferating cells and differentiated cells. Cell differentiation was assessed by the ability of cells to produce oxidative bursts using the nitroblue tetrazolium (NBT; Sigma) dye reduction assay and confirmed by morphological criteria after May-Grünwald-Giemsa coloration. Cell apoptosis was assessed using the APO-BrdU terminal deoxynucleotidyl transferase 9TDT-mediated dUTP-biotin nick end-labelling kit (TUNEL assay), according to the manufacturer's instructions (Immunotech, Marseille, France). The proportion of labelled (apoptotic) cells was determined relative to negative controls (labelled with dUTP without terminal deoxynucleotidyl transferase). DNA fragmentation was also evaluated using a DNA ladder assay as described previously (28).

Immunofluorescent localization of Bcl-2 and Bax. Cells were washed twice with phosphate-buffered saline (PBS), pelleted onto glass slides in a cyto-centrifuge and the slides were quickly air-dried, fixed in acetone and dried as described (28). They were incubated with normal goat anti-serum (2% in PBS) for 15 min to reduce non specific binding, washed and successively incubated for 1 h with the monoclonal mouse anti-Bcl-2 or rabbit polyclonal anti-Bax antibody (1 μg/ml), then with a 1 : 1, 000 and 1 : 5, 000 dilution of goat anti-mouse or anti-rabbit FITC antibody, respectively. The cells were finally stained for 5 min with 5 μg/ml DAPI (4', 6-diamino-2-phenylindole hydrochloride) to label the nuclei, and analyzed under the microscope.

Western immunoblotting. Cells (2 x 10^7 / ml) were solubilized in 50 mM Tris, pH 7.4, 150 mM NaCl, 20 mM EDTA, 20 mM EGTA, 1% Triton X-100, 1% Na-deoxycholate, 0.1% SDS and a cocktail of proteases inhibitors. After centrifugation, protein extracts were boiled in the presence of 5% β-mercaptoethanol and loaded (10 μg protein/lane) on a 12% SDS-polyacrylamide gel. The proteins were separated by electrophoresis and blotted onto nitrocellulose membranes (Amersham, Les Ulis, France), then visualized with Ponceau S (Sigma) to confirm equal loading. The membranes were saturated with 5% non-fat powdered milk in PBS and incubated

overnight in PBS, 1% non-fat milk, 0.1% Tween 20, with the mouse anti-Bcl-2 or rabbit anti-Bax antibody (0.1 μg/ml). After washing, the membranes were incubated with peroxidase-conjugated goat anti-mouse or anti-rabbit antibody for detection with ECL chemiluminescent substrate (Amersham).

Statistical analysis. The results are presented as the mean ± S.D. of data obtained from at least three experiments performed in triplicate. Significance of differences between parameters measured for untreated and treated cells was estimated with the Student's *t*-test for paired samples. Values were considered significantly different when $p<0.05$.

Results

ATRA induces TSP-1 secretion in HL-60 and NB4 cell cultures. The secretion of TSP-1 by HL-60 and NB4 cells during their differentiation by ATRA (10^{-7} M) was assessed by ELISA of conditioned media from cells grown in a synthetic serum substitute from plant, free of TSP-1 (Figure 1). An increase of TSP-1 level was observed at day 1 of treatment and reached a maximal level of 98±7 and 94±2 ng of TSP-1/10^6 HL-60 and NB4 cells, respectively, at day 3. This level was maintained at about 80 ng/10^6 cells after 5 days of culture. In these experiments, we verified that the growth and differentiation of HL-60 and NB4 cells were not affected by the replacement of serum by a plant substitute.

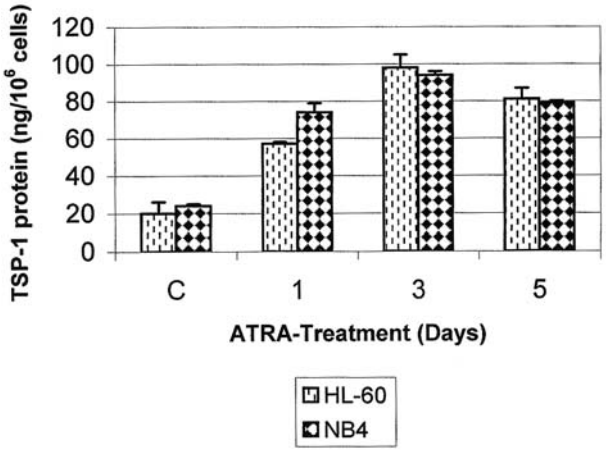

Figure 1. *Quantification of TSP-1 levels in ATRA-treated HL-60 and NB4 cell cultures. Cells were suspended in RPMI 1640 medium containing 10% serum-free plant substitute, 0.1% BSA, antibiotics and L-glutamine (serum-free medium), and exposed to ATRA (10^{-7} M) for up to 5 days. The level of TSP-1 in the culture medium was quantified by ELISA using purified TSP-1 as standard. Data are mean values ± S.D. of three independent experiments performed in triplicate.*

TSP-1 inhibits growth and induces cell differentiation of HL-60 and NB4 cells. To evaluate whether TSP-1 *per se* could influence the behavior of promyelocytic cells, we added increasing concentrations of purified TSP-1 to NB4 and HL-60 cells, in the range of TSP-1 concentrations present in the blood under normal and pathological situations (32). In these experiments, ATRA (10^{-7} M)-treated cells were used as a positive control in separate samples.

The addition of purified TSP-1 to NB4 and HL-60 cultures dose-dependently reduced the number of viable cells in the culture medium, starting at day 3 (Figure 2), with a maximal effect observed at 5 x 10^{-8} M TSP-1. Morphological observations together with the detection of NBT-positive cells indicated that TSP-1-treated cells, as ATRA-treated cells, were being differentiated over this time. After 5 days, the percentage of differentiated HL-60 cells was similar in TSP-1 (5 x 10^{-8} M)- and ATRA (10^{-7} M)-treated cultures (30±5% *versus* 27±7%) (Table I). In contrast, the percentage of differentiated NB4 cells was lower in TSP-1- than in ATRA-treated cultures (24±5% *versus* 56±5%, $p<0.01$). This was in relation to a higher number of apoptotic cells in the TSP-1-treated cultures (see below).

TSP-1 induces apoptosis of HL-60 and NB4 cells. Using the TUNEL assay which detects DNA strand breaks, we measured 37±7% apoptotic HL-60 cells after 5 days of exposure to TSP-1 (5 x 10^{-8} M) *versus* 38±6% after exposure to ATRA (10^{-7} M), as compared to only 2±1% in controls (Table I). The effect of TSP-1 on NB4 cells resulted in the detection of a much higher number of apoptotic cells than with ATRA (46±3% *versus* 19±7%, $p<0.001$). Thus, TSP-1 was more pro-apoptotic than ATRA on NB4 cells.

Detection of Bcl-2 and Bax proteins in TSP-1-treated HL-60 and NB4 cells by immunofluorescence and Western blot analysis. Cell differentiation induced by ATRA in promyelocytic cells is regulated by changes in gene expression of many proteins, among which Bcl-2 plays a major role (29, 33). Bcl-2 may also function in an anti-apoptotic pathway to prevent cell death; this effect is antagonized by several Bcl-2-associated proteins, especially Bax protein which induces cell apoptosis (34). However, in promyelocytic cells, the down-regulation of Bcl-2 is probably not related to apoptosis but rather linked to differentiation, as a decrease of Bcl-2 in NB4 cells is correlated with a high number of differentiated cells and low number of apoptotic cells (28, 29). We used indirect immunofluorescence and Western immunoblotting with specific antibodies to evaluate the expression of Bcl-2 and Bax proteins in HL-60 and NB4 cells before and after 5 days of treatment with TSP-1 (5 x 10^{-8} M), as compared to treatment with ATRA (10^{-7} M). The untreated NB4 or HL-60 cells displayed the typical normal cytoplasmic staining of Bcl-2 and Bax. There were no visible changes in the staining pattern of Bcl-2 and Bax in NB4 (Figure 3) or HL-60 cells (not shown) upon TSP-1 treatment,

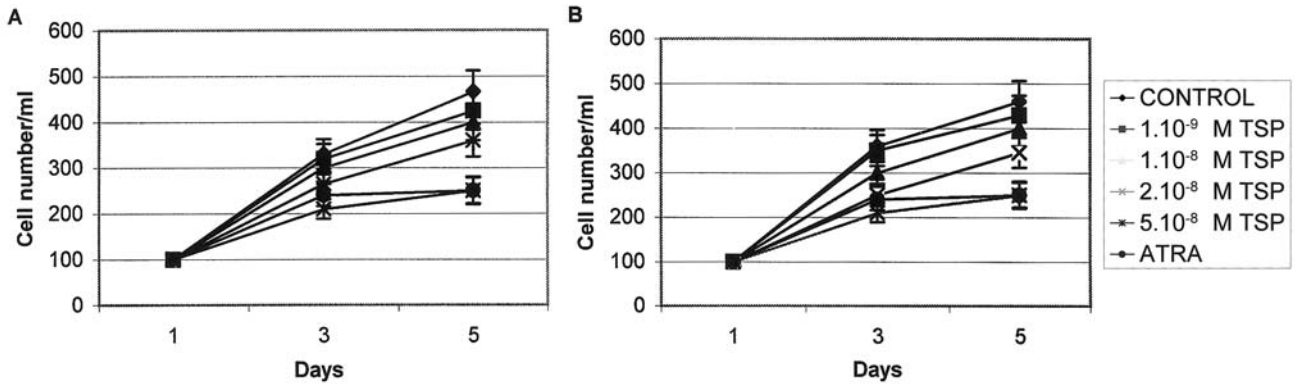

Figure 2. *Effects of TSP-1 and ATRA on the growth of HL-60 and NB4 cells. HL-60 (A) or NB4 cells (B) were treated with or without TSP-1 (10^{-9} to 5 x 10^{-8} M) or ATRA (10^{-7} M) in serum-free medium, and the number of viable cells was determined each day up to 5 days. Each point represents the mean value ± S.D. of three independent experiments performed in triplicate.*

Table I. *Induction of differentiation and apoptosis in HL-60 and NB4 cells exposed to ATRA or TSP-1. HL-60 or NB4 cells were treated with or without TSP-1 (5 x 10^{-8} M) or ATRA (10^{-7} M) in serum-free medium for 5 days, and the number of differentiated (NBT dye reduction assay) and apoptotic (TUNEL assay) cells was determined as described in Materials and Methods. The results are expressed as percentages of the total number of cells (viable and apoptotic) in the culture. Data are the mean ± S.D. of three independent experiments performed in triplicate.*

	HL-60		NB4	
	Apoptotic cells (%)	Differentiated cells (%)	Apoptotic cells (%)	Differentiated cells (%)
CONTROL	2±1	5±2	6±1	10±5
ATRA (10^{-7} M)	38±6	27±7	19±7	56±5
TSP-1 (5.10^{-8} M)	37±7	30±5	46±3	24±5
ATRA/TSP-1	NS	NS	$p<0.001$	$p<0.01$

Table II. *Induction of apoptosis in HL-60 and NB4 cells exposed to synthetic peptides of TSP-1. HL-60 or NB4 cells were incubated with or without different peptides of TSP-1 (10^{-4} M) in serum-free medium for 5 days and the number of differentiated and apoptotic cells was determined. The data are the mean ± S.D. of three experiments performed in triplicate.*

	Apoptotic cells (%)	
	HL-60	NB4
CONTROL	2±1	6±1
Hep I	42±2	53±9
GGWSHW	42±2	36±2
RFYVVMWK	9±4	25±4
CSVTCG	5±3	ND

compared to an apparent absence of Bcl-2 protein upon ATRA treatment. We confirmed the results of Bcl-2 protein by immunoblot analysis, showing only a slight reduction in Bcl-2 staining in TSP-1-treated cells when the protein was strongly reduced in ATRA-treated HL-60 and NB4 cells (Figure 4). Thus, TSP-1-induced differentiation and apoptosis of HL-60 and NB4 cells did not occur in parallel with significant modifications of Bcl-2 or Bax expression.

Effect of synthetic peptides of TSP-1 on HL-60 and NB4 apoptosis. Because promyelocytic cells express several receptors for TSP-1 that could be involved in cell apoptosis, such as CD36 or CD47 (35), we treated HL-60 and NB4 cells with synthetic peptides of TSP-1 derived from the amino-terminal domain, Hep-I (amino acids 17-35), type 1 repeats domain, GGWSHW (amino acids 418-423) and CSVTCG (amino acids 429-434 and 486-491), and carboxy-terminal domain, RFYVVMWK (amino acids 1016-1023), respectively. These peptides have been shown to react with heparin and heparan sulfate proteoglycans (Hep-1 and GGWSHW) (36, 37), CD36 (CSVTCG) (38) or CD47/IAP (RFYVVMWK) (39). The peptides Hep-I and GGWSHW, used at 10^{-4} M, induced a high level of apoptosis in HL-60 and NB4 cell cultures (Table II). Specifically, Hep-I induced 42±2% and 53±9% apoptotic cells in HL-60 and NB4 cultures at day 5. The peptide RFYVVMWK (10^{-4} M) caused a lower but still significant increase in the number of apoptotic HL-60 (9±4%, $p<0.05$) and NB4 (25±4%, $p<0.001$) cells as compared to controls. The peptide CSVTCG (10^{-4} M), tested only on HL-60 cells, did not induce cell apoptosis.

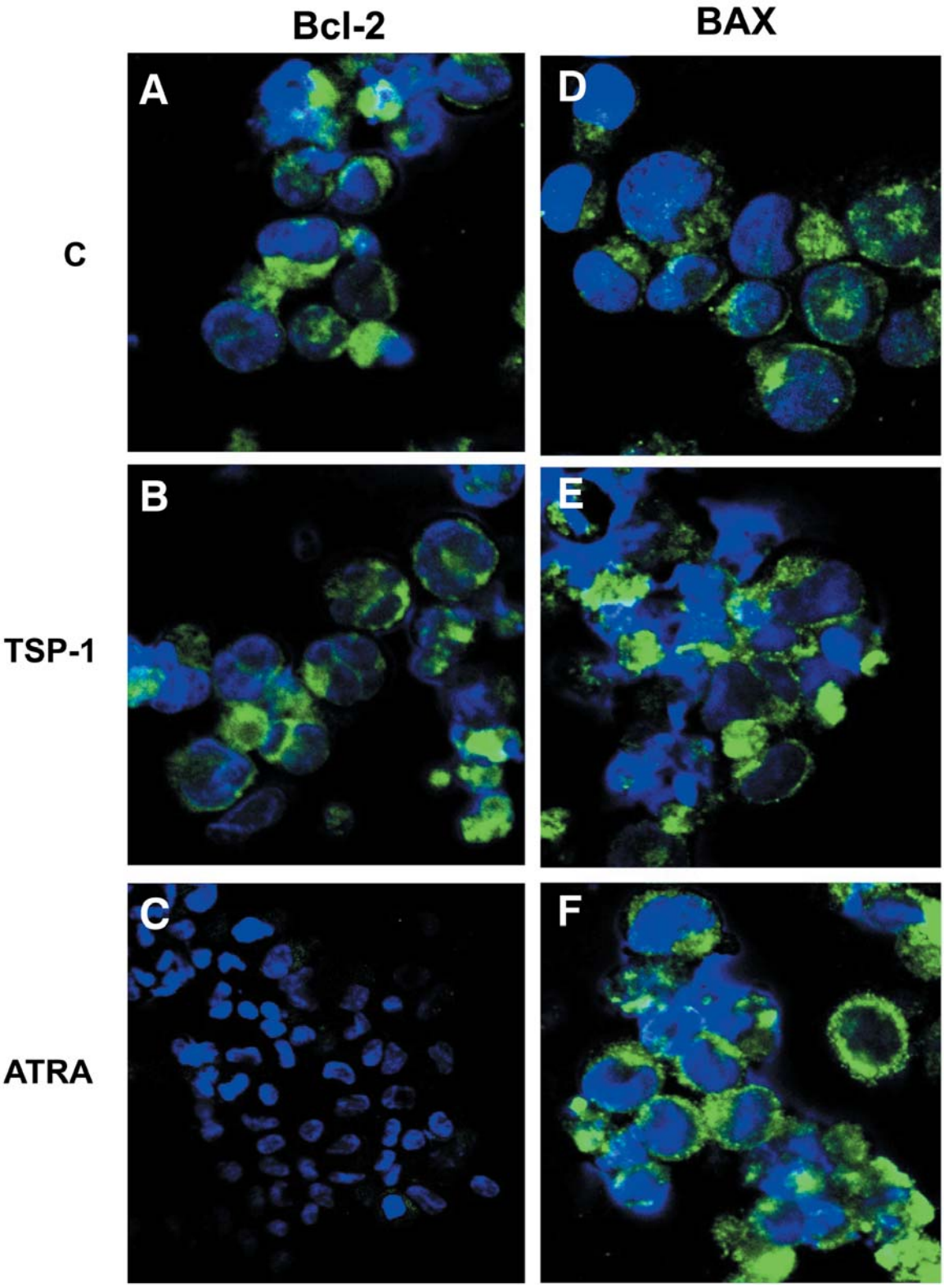

Figure 3. *Immunofluorescence analysis of Bcl-2 and Bax proteins in NB4 cells. Untreated NB4 cells (control) or cells exposed to TSP-1 (5 x 10^{-8} M) or ATRA (10^{-7} M) for 5 days were analyzed for the presence of Bcl-2 or Bax protein. Bright green fluorescence corresponds to Bcl-2 or Bax protein. Blue fluorescence labels the nuclei. The pictures are representative of three independent experiments.*

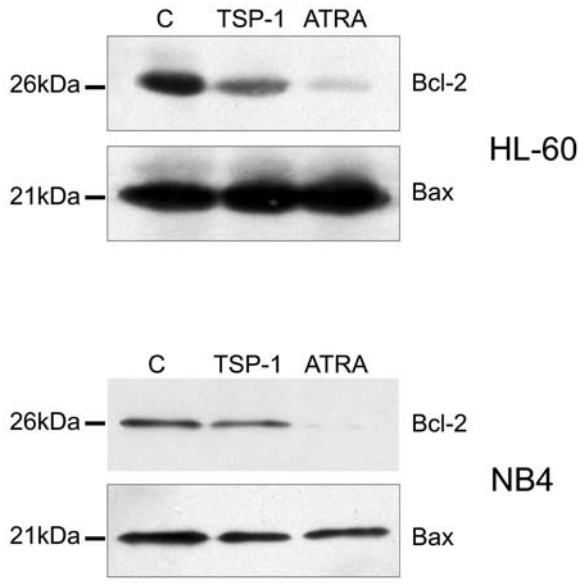

Figure 4. *Western immunoblot analysis of Bcl-2 and Bax expression in untreated HL-60 and NB4 cells or after exposure to TSP-1 (5 x 10^{-8} M) or ATRA (10^{-7} M) for 5 days. HL-60 or NB4 cells were treated with or without TSP-1 or ATRA in serum-free medium for 5 days. Cell extracts were prepared and tested for the content of Bcl-2 and Bax by immunoblot analysis as described in Materials and Methods. Each lane, 10 μg of proteins. The data are representative of two separate experiments.*

Discussion

The HL-60 and NB4 cell lines, initially established from the blood of myeloid leukemia patients, display many of the characteristics of promyelocytic cells during differentiation by ATRA *in vitro*, and constitute interesting models for assessing biological events associated with cell differentiation. In particular, the NB4 cells that express the specific t(15:17) chromosomal translocation characteristic of a subtype of myeloid leukemia (M3) are particularly sensitive to maturation by ATRA (27-29). During this process, the cells undergo dramatic changes in morphology, gene expression and adhesion to extracellular matrix components such as fibronectin and thrombospondin-1 (35).

Thrombospondin–1 (TSP-1) is an ubiquitous adhesive glycoprotein whose synthesis is regulated by hormones and growth factors (40). In this study, we showed that NB4 cells are induced to secrete increasing levels of TSP-1 upon treatment with ATRA, as also shown for the HL-60 cells which belong to the M2 subtype of myeloid leukemia (11). The relatively low levels of TSP-1 produced by the promyelocytic cells (0.02-0.1 μg/10^6 cells) could be related to the culture methodology. The use of a plant-derived substitute for FCS, although not affecting cell growth and differentiation by ATRA, might not provide optimal conditions for the synthesis/secretion or stability of TSP-1. To explore whether soluble TSP-1 could influence the behavior of promyelocytic cells, we added increasing concentrations of purified TSP-1 to NB4 and HL-60 cells. We found that TSP-1 induced a dose- and time-dependent reduction in cell growth, together with induction of cell differentiation. TSP-1 also induced promyelocytic cell apoptosis, and the NB4 cells, which are largely resistant to apoptosis induced by ATRA (28, 29 and this study), were shown here to be very sensitive to apoptosis induced by TSP-1.

The TSP-1 concentrations used in this study (10^{-9}-5.10^{-8} M) are low compared to the large amounts of TSP-1 measured in whole marrow cell lysates (7), and in the range of those measured in the blood of normal individuals or cancer patients (32). Thus, promyelocytic cells might be exposed to TSP-1 at different stages of the disease.

How might TSP-1 influence leukemia cell growth and induce their differentiation and apoptosis? TSP-1 contains several functional domains which may interact with one of several potential cell surface receptors leading to specific intracellular signals and distinct biological responses (13). For instance, in endothelial cells, heparin-binding sequences located in the NH_2-terminal domain and type I repeats of the molecule inhibit cell proliferation (41), whereas other sequences interacting with the CD36 receptor mediate apoptosis and inhibition of angiogenesis (15-17). In melanoma cells, TSP-1 promotes cell spreading *via* its cooperative interactions with heparan sulfate proteoglycans, αvβ3 integrins and CD47, thereby transmitting signal(s) leading to focal adhesion kinase phosphorylation (42). Recently, CD47 ligation was shown to induce apoptosis in T lymphocytes and in chronic lymphocytic leukemia cells (24, 25). Thus, differential expression or activation of cell surface receptors for TSP-1 may dictate the specific responses of each cell type to TSP-1.

We previously reported the presence of several receptors for TSP-1 on NB4 cells, including CD47, β1 and β3 integrins (34), and others have described the presence of heparin-like receptors on HL-60 cells (43). In this study, we found that two peptides, ELTGAARKGSGRRLVKGPD (Hep-1) and GGWSHW, located in the amino-terminal domain and type I repeats respectively, with high affinity for heparin (35, 36), induced a high level of apoptosis in HL-60 and NB4 cultures. This suggests that the receptors recognized by these peptides, most probably heparan sulfate proteoglycans, may transmit intracellular signal(s) that leads to apoptosis in myeloid leukemia cells. Interestingly, in Jurkat T lymphoma cells, a heparin-binding peptide containing the GGWSHW sequence was shown to induce activation of the transcriptional factors, Elk-1 and AP-1, through binding to cell surface proteoglycans and activation of the Ras/MEK/ERK MAP kinase pathway (44). The participation of the transmembrane heparan sulfate proteoglycan, syndecan-1, in the regulation of active cell

death in HT58 lymphoma cells was also reported recently (45). The possibility of such a mechanism operating in promyelocytic cells will be investigated in further studies.

In addition to the heparin-binding sequences, the RFYVVMKVK peptide that interacts with CD47 (38) induced mild but significant apoptosis in HL-60 and NB4 cells, suggesting that CD47 may also participate in cell death. Thus, the delineation of the mechanism of action of different TSP-1 functional peptides in myeloid leukemia cells will help in the understanding of how TSP-1 acts on cell differentiation and apoptosis in these cells.

In conclusion, we have shown that TSP-1, an important adhesive glycoprotein in bone marrow and blood, may influence several promyelocytic cell functions such as cell growth, differentiation and apoptosis. The finding that TSP-1, and some TSP-1-derived heparin-binding peptides, are more efficient than ATRA in inducing apoptosis of the NB4 cells may potentially be of therapeutic interest, in particular in those leukemias resistant to apoptosis under ATRA treatment.

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