Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas apoptosis in Burkitt's lymphomas with loss of multiple pro-apoptotic proteins

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Background and Objectives. Normal B-cells in the germinal center (GC) may be exposed to both tumor necrosis factor-related apoptosis inducing ligand (TRAIL) and Fas-L. Whether abrogation of TRAIL apoptosis is a feature in the genesis of B cell lymphomas of GC-phenotype is not known. We assessed the integrity of the TRAIL pathway in Fas-resistant and Fas-sensitive Burkitt's lymphomas (BLs).

Design and Methods. Expression of TRAIL receptors was determined by flow cytometry and Western blots. The extent of apoptosis following exposure to TRAIL was measured by annexin-V/propidium iodide dual staining. The integrity of the Fas and TRAIL apoptotic pathways was determined by Western blotting to assess cleavage of downstream caspases. Western blot analyses were used to determine the expression of pro- and anti-apoptotic proteins and the profile of expression was correlated with response to TRAIL and CH11.

Results. Our results demonstrate that BL expresses both functional and decoy TRAIL receptors. BLs with a functional Fas pathway retained sensitivity to TRAIL. Frequent and compound loss of expression of pro-apoptotic proteins can be identified in BLs resistant to Fas. However, loss of Bax, Bak and Bcl-Xs did not compromise sensitivity to TRAIL.

Interpretation and Conclusions. Our results indicate that BLs frequently retain sensitivity to the TRAIL pathway. These results underscore the utility of TRAIL-based therapeutic strategies in the treatment of those B-cell lymphomas that may have compromised expression of several pro-apoptotic proteins.

Key words: lymphoma, Fas/CD95, Bcl-Xs, cIAP, mitochondria, caspase.

Haematologica 2003; 88:167-175
http://www.haematologica.org/2003_02/88167.htm

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Induction of cell death can result from signaling via external death receptors such as CD95/Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors[1-4] or it can result from exposure to chemicals, irradiation and serum starvation.[5-7] Signaling through external receptors activates the *extrinsic* pathway[8] headed by the apical caspases, caspase-8 and caspase-10. On the other hand, death resulting from chemotherapeutic agents appears to require exclusively the *intrinsic* pathway[9] involving the mitochondrial release of death activators – cytochrome c and Smac/Diablo, which in turn activate the caspase cascade led by caspase-9 via the apoptosome.[10,11] While both these pathways may operate proximally independently of each other, they converge at the central executioner of the apoptotic process, caspase-3. Crosstalk between the extrinsic and intrinsic pathways does also occur. Signaling by CD95, for example, can also result in the activation of the mitochondrial pathway following caspase-8 cleavage of Bid and translocation of the truncated Bid product to the mitochondria.[12-14]

The Bcl-2 family of proteins plays a key role in the involvement of the mitochondria in the apoptotic process. Bax has recently been reported to be crucial for efficient signaling of TRAIL-mediated cell death,[15] at least for the mitochondrial changes and downstream caspase activation in colon cancer.[16] The importance of these proteins has been highlighted by the death resistance phenotype of Bax/Bak double knockouts.[17] Whether these results can be extended to other cell types is not known.

We and others have previously shown that at least one extrinsic pathway, the CD95 death pathway, is compromised in B-cell neoplasias derived from the germinal center (GC).[18-20] Normal GC B-cells show an impressive ability to undergo apoptosis when cultured *in vitro*.[21] Furthermore, death *in vivo* in the GC is an important mediator of the B-cell repertoire.[22] Therefore, it is not surprising that B-cell neoplasias derived from the GC must be dependent upon the abrogation of apoptotic checkpoints. Indeed, deregulation of, or mutations in cell death regulators such as Bcl-10, cIAP2, Bcl-2, CD95 and Bax are a recurrent theme in lymphomagenesis.[23,24]

In addition to CD95, other apoptotic mechanisms may also operate in the GC, for example apoptosis via CD77 or TRAIL.[25,26] Dendritic cells dispersed throughout the GC dark and light zones express TRAIL.[27] Two alternatively spliced functional TRAIL receptors have been

identified, DR4 and DR5, containing a death domain capable of transmitting a death signal similar to Fas/CD95. Two additional receptors, the decoy receptors DcR1 and DcR2, are devoid of the cytoplasmic tail and consequently incapable of transmitting death signals.[28-31] The signaling of the death pathway through the TRAIL receptors appears to involve preferentially tumor cells rather than their normal counterparts.[3] However, very little is known regarding TRAIL sensitivity or the expression of its receptors in Burkitt's lymphoma (BL), a tumor derived from the GC.

We compared TRAIL signaling in Fas sensitive and resistant BLs.[19] We demonstrated that BLs with an intact Fas signaling pathway are also sensitive to TRAIL. Two of the three Fas-resistant BLs were also resistant to TRAIL. However, one Fas-resistant cell line (CA46) was, unexpectedly, sensitive to TRAIL suggesting that different mechanisms can promote these death pathways in BL. Expression analyses of caspase, inhibitor of apoptosis proteins (IAP) and Bcl-2 families involved in both Fas-L and TRAIL pathways demonstrated that all the Fas-resistant BLs are compromised in the expression of multiple pro-apoptotic genes including the Bcl-2 multidomain proteins, caspase-3 or DAP-kinase. Surprisingly, loss of expression of Bax, Bak and Bcl-Xs alone was not sufficient to block TRAIL-mediated cell death in the Fas-resistant TRAIL-sensitive CA46. These data suggest that mitochondrial gateway proteins may not always be necessary in the induction of TRAIL-mediated cell death in BL.

Design and Methods

Cell lines

The cell lines used in this study have already been described[19] and include 3 Epstein-Barr virus (EBV)-positive BLs (AS283A, KK124 and PA682PB) and 3 EBV-negative BLs (CA46, BML895 and LW878). Two of these cell lines were derived from AIDS-associated BL (AS283A, PA682PB). To characterize the influence of the presence of EBV on surface expression of the TRAIL receptors, we also used isogenic cell lines BL30 and the EBV-infected counterparts BL30/B95.8 and BL30/P3HR1.

Western blotting

Cell pellets were obtained by centrifugation, washed twice with cold PBS and finally suspended in extraction buffer containing 6% SDS, 500mM LiCl, 100mM DTT, 2mM EDTA, 20 mM Tris-HCl, pH 7.2 supplemented with protease inhibitors. Total cell extracts were obtained by first heating the suspension in a 70°C water bath for 5 min, frequent pulse vortexing and then freezing the samples at –80°C. Cell protein extracts were subjected to electrophoresis in either 10 or 15% highly porous SDS-polyacrylamide 5.5 cm minigels (SDS-PAGE).[32] Proteins were electrophoretically transferred to Immobilon-P (Millipore, Bedford, MA, USA) membranes as described by Towbin *et al.*[33] with the exception that methanol was omitted from the transfer buffer.

Table 1. Antibodies used, manufacturer and dilutions for immunoblots.

Antibody	*Manufacturer*	*Catalog no.*	*Dilution*
Actin	Abcam, Cambridge, England	6476-100	1:5000
DR4	Alexis Corp., San Diego CA, USA	PSC-1139	1:1000
DR5	Caymen Chemical. Ann Arbor, MI, USA	160770	1:1000
DcR1	Alexis Corp., San Diego CA, USA	PSC-2179	1:1000
DcR2	Alexis Corp., San Diego CA, USA	PSC-2021	1:1000
Caspase-3	BD Pharmingen, San Diego CA, USA	610323	1:1000
Caspase-9	Calbiochem, San Diego CA, USA	218794	1:1000
Bid	Cell Signaling Tech. Beverl, MA, USA	2002	1:1500
Cleaved Caspase-3	Cell Signaling Tech. Beverly, MA, USA	ASP175	1:1000
Bak	Dako. Carpinteria, CA, USA	A3538	1:1000
Bcl-2	Dako. Carpinteria, CA, USA	M0887	1:2000
Caspase-8	R&D systems, Minneapolis, MN, USA	AF832	1:1000
cIAP-1	R&D systems, Minneapolis, MN, USA	AF818	1:1000
cIAP-2	R&D systems, Minneapolis, MN, USA	AF817	1:1000
Survivin	R&D systems, Minneapolis, MN, USA	AF886	1:1000
XIAP	R&D systems, Minneapolis, MN, USA	AF822	1:1000
Bax	Santa Cruz Biotechnology, Santa Cruz CA, USA	SC493	1:2000
Bcl-Xl/s	Zymed Lab. San Francisco, CA, USA	33-6300	1:500
PARP	Zymed Lab. San Francisco, CA, USA	33-3100	1:500

The relevant information regarding the primary antibodies, their concentration, and the manufacturers is given in Table 1. For immunostaining, primary antibodies were used at concentrations recommended by the vendors in 2% or 5% milk in TBST and the membranes were exposed overnight at 4°C or for 1 hr at room temperature. Immunoreactive bands were assessed using appropriate secondary antibodies (1/2000 dilution) from Amersham Pharmacia Biotech (Arlington Heights, IL, USA) or Jackson ImmunoResearch Laboratories (West Grove, PA, USA) and developed using the enhanced chemiluminescence kit (ECL) as recommended by the manufacturer (Amersham Pharmacia Biotech). Equal amounts of proteins were loaded and β-actin was used as a control in the determination of relative expression levels.

FACS analysis of receptors

Goat monoclonal antibodies against TRAIL R1, R2, R3 and R4 (R&D Laboratories, Minneapolis, MN, USA) were used to stain 1×10^6 viable cells at the concentration of 1mg in 100μL of PBS with 3% BSA followed by FITC-conjugated anti-goat IgG (Santa Cruz Technology, Santa Cruz, CA, USA). Irrelevant isotype-matched immunoglobulin was used as a control. Stained cells were analyzed using

Table 2. Flow cytometric analysis of TRAIL receptors in Burkitt's lymphoma cell lines.

	Functional		Decoy	
Cell line	*DR4*	*DR5*	*DcR1*	*DcR2*
PA682PB	8	8	8	3
KK124	1	5	16	8
AS283A	5	6	5	3
CA46	2	10	44	22
LW878	8	10	1	2
BML895	6	10	16	8

The values shown reflect typical fluorescence values of each TRAIL receptor relative to non-specific, goat IgG antibody. Most of the cell lines express low to moderate levels except CA46 (high decoy receptor levels) as determined in two independent experiments.

Table 3. Percentage of apoptosis in Fas-sensitive and Fas-resistant Burkitt's lymphoma cell lines following treatment with TRAIL in the presence or absence of cycloheximide (CHX).

Cell line	*rTRAIL*	*rTRAIL + CHX*
FAS-sensitive		
PA682PB	84 (±11)	96 (±2)
KK124	39 (±15)	62 (±12)
AS283A	50 (±9)	89 (±1)
FAS-resistant		
CA46	43 (±7)	81 (±3)
LW878	8 (±5)	16 (±10)
BML895	1 (±0.4)	9 (±3)

1×10^6 cells were treated with 200 ng/mL TRAIL either in the presence or absence of 1 μg/mL CHX. Apoptosis was measured after 24 hrs by annexin V/PI dual staining. Averages ± standard deviation from 2 to 4 experiments are shown.

FACScan flow cytometry equipped with a Cell Quest data analysis program (Beckon Dickinson, San Diego, CA, USA). For each sample, a minimum of 10,000 events were collected.

Cytotoxicity studies

Exponentially growing cells were plated at 1×10^6 cells/mL in the presence or absence of 200 ng/mL of recombinant human TRAIL[34,35] or 100 ng/mL of anti-Fas (clone CH-11) (MBL, Watertown, MA, USA) as indicated. These concentrations were chosen on the basis of results from preliminary titration experiments. Treatment with TRAIL or CH11 was carried out with or without a pretreatment with cycloheximide (CHX) (1 mg/mL for 24 hours). Cells were harvested and apoptosis was measured using the annexin-V kit (Molecular probes, Eugene, OR, USA) using the protocol recommended by the manufacturer. Cell lines were considered sensitive to TRAIL or CH11 when exposure to either of the ligands resulted in apoptosis in more than 25% of cells. Two to four independent experiments were performed for each data point and the average of these was used to reflect percentage of apoptosis.

Reverse transcriptase polymerase chain reaction (RT-PCR) for detection of DAP-kinase transcripts

Total RNA was extracted from all the cell lines using Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA) and reverse transcribed into cDNA using random hexamers. DAP-kinase was PCR-amplified with primers GATAGAAATGTCCCCAAACCTCG and TCTTCTTGGATCCTTGACCAGAA for 35 cycles (58°C annealing temperature). The 343 bp PCR product was electrophoresed on an ethidium bromide stained 4% agarose gel and visualized under UV light. Amplification of GAPDH transcripts was used as a control for the integrity of the cDNA.

Results

Expression of TRAIL receptors in BL

Surface expression of TRAIL receptors by FACS analysis suggested considerable variation in expression (Table 2, Figure 1A). However, unlike the modulation of Fas receptors by EBV,[18,19] surface expression of functional or decoy TRAIL receptors was independent of EBV. Indeed, FACS analysis failed to indicate notable differences in the levels of TRAIL receptors in the EBV-negative cell line BL30 and the isogenic EBV-infected counterparts BL30/B95.8 and BL30/P3HR1 (*data not shown*).

To assess whether these differences in the surface level of individual TRAIL receptors reflected actual differences in expression, we determined the levels of TRAIL receptors by Western blot analyses (Figure 1B). All cell lines, including those with low surface expression, demonstrated easily detectable DR4, DR5, DcR1 and DcR2.

TRAIL and CH-11 induced apoptosis are not always concordant in BL

We hypothesized that if common pathways were involved in transducing both TRAIL and Fas-L signals, Fas-sensitive and Fas-resistant BLs should also respond similarly to TRAIL. In order to test this hypothesis, we pursued functional studies on BL cell lines previously shown to be sensitive (N=3) or resistant (N=3) to cell death mediated by anti-Fas monoclonal antibody (CH11).[19] We considered a cell line to be sensitive if either CH11 or TRAIL induced more than 25% cell death. The apoptotic nature of this cell death was confirmed by annexin-V staining.

Exposure to 200 ng/mL recombinant TRAIL induced apoptosis in 4 of the 6 cell lines (range, 39-84%). As shown in Table 3, all Fas-sensitive cell lines were sensitive to TRAIL (PA682PB, KK124, AS283A) and 2 of the 3 Fas-resistant cell lines were resistant to TRAIL (1–8%) (LW878 and BML895).

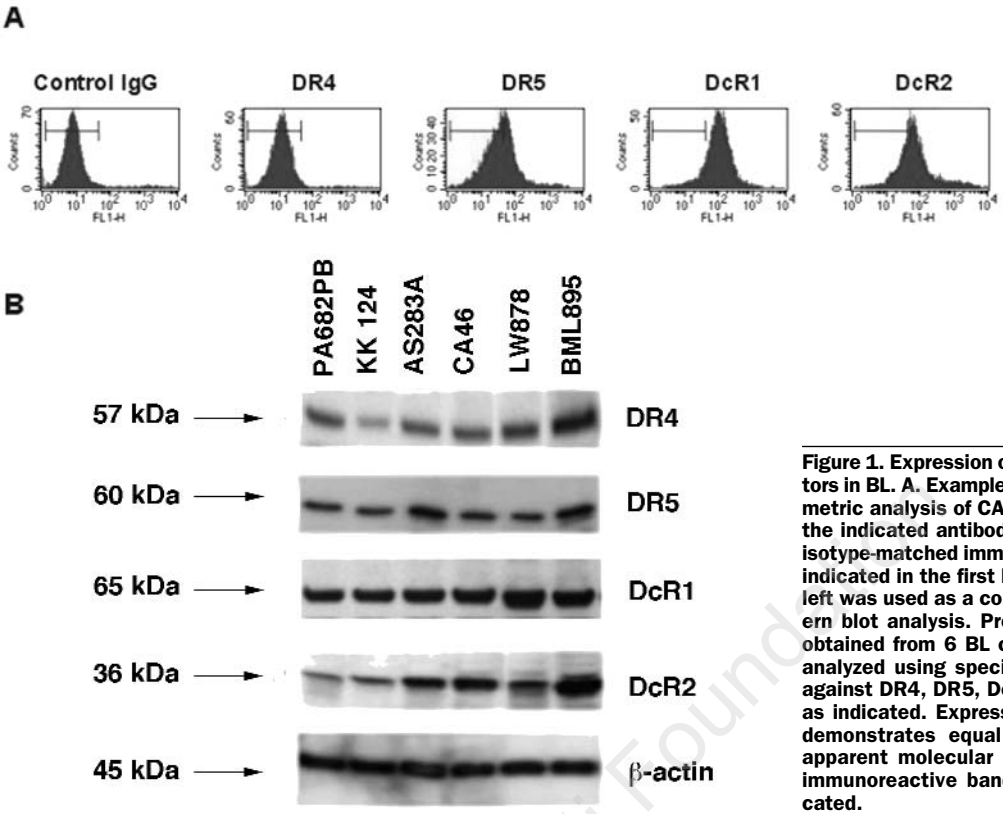

Figure 1. Expression of TRAIL receptors in BL. A. Example of a flow cytometric analysis of CA46 cells using the indicated antibodies. Irrelevant isotype-matched immunoglobulin as indicated in the first block from the left was used as a control. B. Western blot analysis. Protein extracts obtained from 6 BL cell lines were analyzed using specific antibodies against DR4, DR5, DcR1 and DcR2 as indicated. Expression of β-actin demonstrates equal loading. The apparent molecular mass of each immunoreactive band is also indicated.

Previous reports have indicated that inhibition of protein synthesis with cycloheximide (CHX) results in the enhancement of apoptosis induced by CH11 and TRAIL.[19,35] We, therefore, examined the ability of CHX (1μg/mL) to modulate TRAIL-apoptosis in these BL cell lines. Treatment with CHX increased apoptosis in PA682PB, KK124, AS283A and CA46 (range, 62-96%), but failed to sensitize LW878 and BML895 (9-16% apoptotic cells).

Figure 2 shows annexin-V/PI staining from one representative experiment for a cross-sensitive cell line (PA682PB), a cross-resistant cell line (BML895) and CA46, which clearly demonstrates sensitivity to TRAIL but resistance to CH11.

Bax, Bak and Bcl-Xs are not expressed in TRAIL-sensitive and Fas-resistant BL

We used Western blot analyses to determine whether differences in the level of expression of proteins from the Bcl-2, IAP and caspase families, which participate in the Fas and TRAIL pathways, correlated with responsiveness and RT-PCR to define the expression of DAP-kinase. The pro-apoptotic Bcl-Xs was undetectable in the cross-resistant cell lines BML895 and LW878 as well as in the TRAIL-sensitive, Fas-resistant CA46 (Figure 3). Bax was expressed in all BLs except CA46 and BML895, which carry hemi/homozygous frameshift mutations.[19] In LW878, which carries a similar but heterozygous mutation, Bax is expressed only from one allele. Interestingly, loss of Bak protein occurred specifically in cell lines with compromised Bax (CA46, LW878 and BML895). Expression of the inhibitor of apoptosis family of proteins indicated diminished or no expression of c-IAP1 in the cross-sensitive cell lines. Reduced levels of caspase-3 were observed in BML895 and LW878. RT-PCR analysis demonstrated loss of DAP-kinase expression in BML895, LW878 and AS283A.

The TRAIL signal is transduced efficiently in a cell line deficient for Bax, Bak and Bcl-Xs

To determine whether there are differences in the TRAIL and Fas-L death signaling pathways in CA46, we analyzed the activation of the caspase cascade following exposure to each inducer. We compared these results with those obtained from the cross-resistant BML895, lacking Bax, Bak and Bcl-Xs. Efficient transduction of the TRAIL signal through caspase-8 was indicated by a notable reduction in the intensity of the pro-caspase band (Figure 4). In contrast, activation of caspase-8 following exposure of CA46 cells to CH11 was not evident. Although some reduction in the intensity

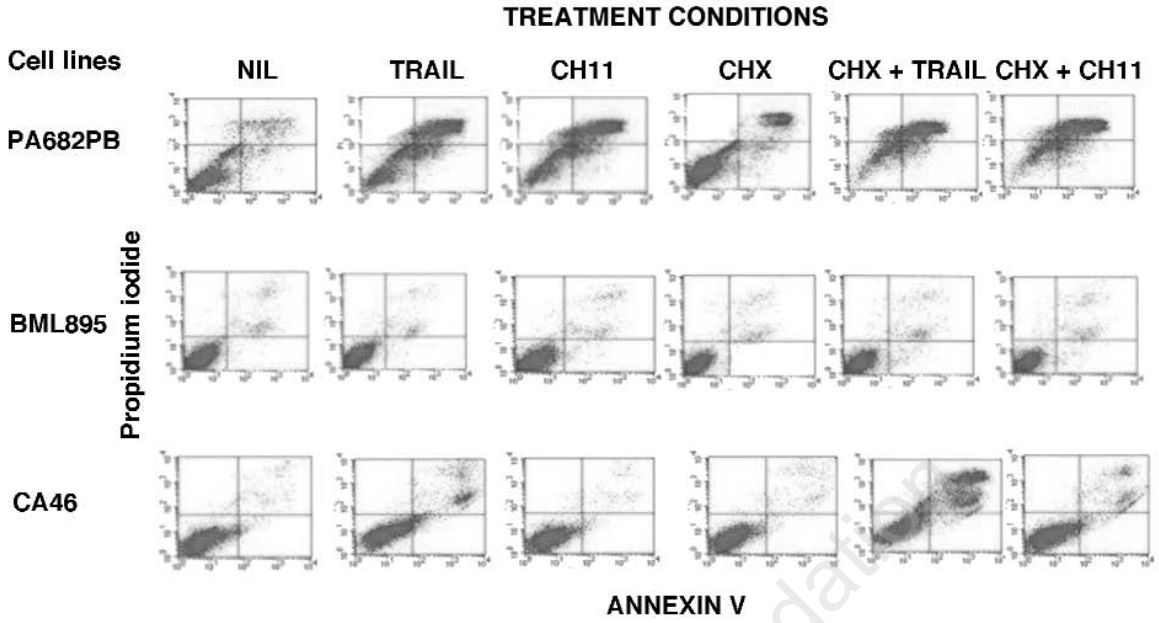

Figure 2. Annexin V/PI staining following TRAIL and CH11 treatment. Cells were treated with 200 ng/mL rTRAIL or 100 ng/mL CH11 for 24 hrs in the presence or absence of 1 μg/mL CHX and analyzed by FACS. Left lower quadrant represents viable cells that have not been labeled with either annexin V or PI. Upper right quadrant indicates apoptotic cells stained with both annexin V and PI.

of the pro-caspase-8 signal was noted in BML895 (especially after exposure to TRAIL or CH11 plus CHX), it was much less pronounced than that observed in CA46. The efficiency of cleavage of caspase-8 in TRAIL-treated CA46 was also supported by a resultant and efficient activation of Bid and caspase-3. Surprisingly, in this Bax, Bak and Bcl-Xs negative BL, a clear activation of caspase-9 was also observed. No activation of downstream caspases was, however, detected in BML895.

Discussion

The CD95/Fas death pathway is abrogated in several BL cell lines, mostly in those with compromised integrity of their Bax gene.[19,24] Mutations in Bax are reported in cell lines derived from lymphoid tumors.[36] However, compromised Bax is not the mere result of *in vitro* selection, since the tumor from which BML895 was derived carried the same mutation as the cell line. We have also demonstrated lack of Bax expression resulting from similar mutational events in several primary BL biopsies.[37] Therefore, loss of Bax expression may influence Burkitt's lymphomagenesis.

Another death signaling pathway involves a family of related functional and decoy TRAIL receptors that are differentially distributed in normal and tumor cells.[3] With the potential promise of TRAIL-based therapeutic approaches,[38] it is also necessary to address the question of the type of cancers that will be sensitive to such therapy. Consequently, it will be relevant to identify those genetic lesions that would confer resistance to TRAIL. In this report we analyzed the TRAIL signaling pathway in BL and the expression profile of proteins involved in order to dissect the TRAIL response and the potential crosstalk with the CD95 response.

BL cell lines express both functional and decoy TRAIL receptors. However, the expression of decoy receptors does not lend these cells to TRAIL resistance (Tables 2 and 3). Indeed, the cell line with the highest expression of decoy receptors, CA46, is sensitive to TRAIL-mediated apoptosis. Although variable expression of the receptors was noted by flow cytometry (Table 2, Figure 1), generally similar intracellular levels were detected by Western blot (Figure 1). Previous reports have suggested that common post-translational regulatory mechanisms may be operative in the redistribution of these receptors from the cytoplasm.[39,40]

Treatment with 200ng/mL of TRAIL resulted in over 25% of cells undergoing apoptosis in 4 of the 6 cell lines (Table 3 and Figure 2). If both Fas-L and TRAIL signals follow the same extrinsic/intrinsic pathways, a similar response would be expected. Indeed, it was clear that all BLs that were sensitive to anti-Fas (CH11) were also sensitive to TRAIL

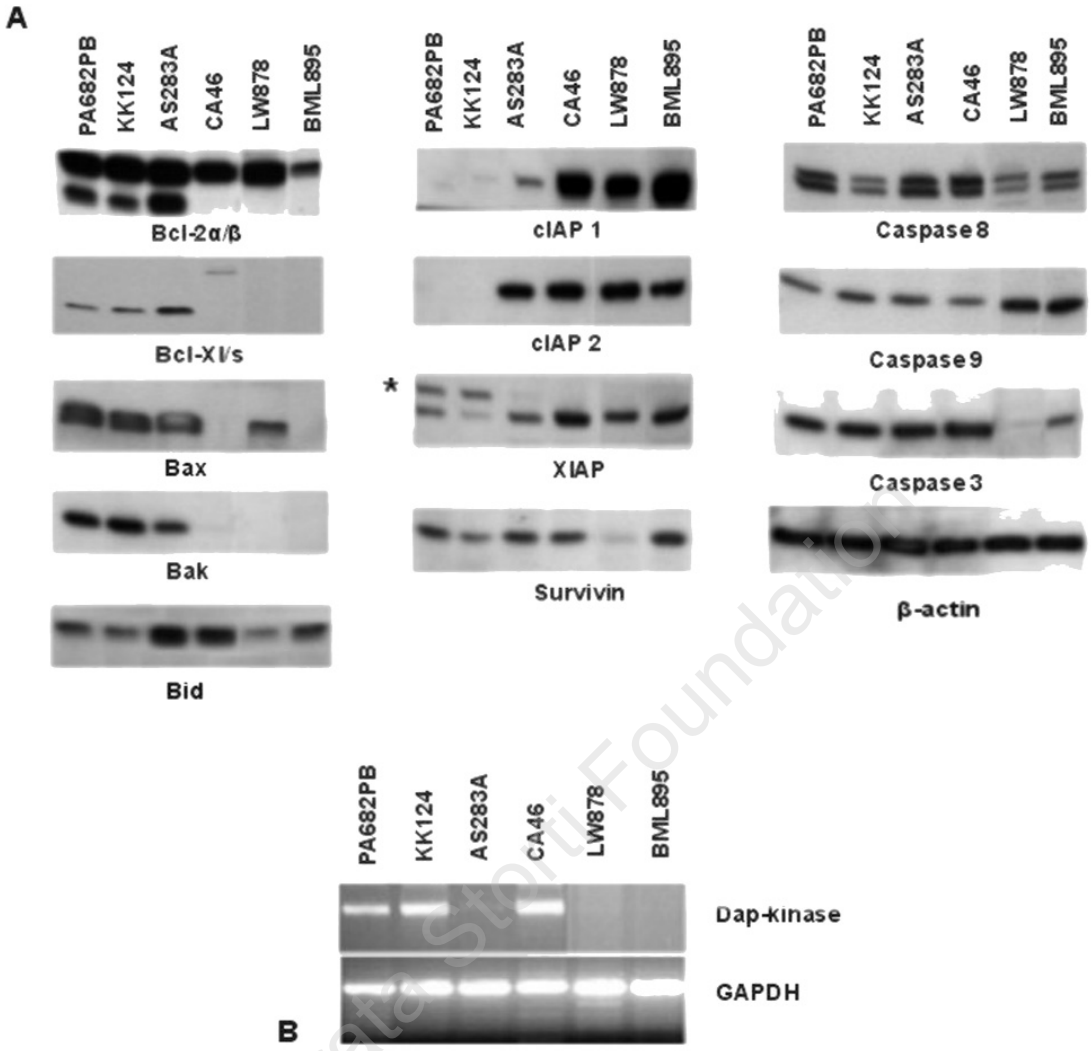

Figure 3. Expression of pro- and anti-apoptotic genes in BL. A. Western blot analyses using the antibodies described in *Design and Methods*. Proteins of the Bcl-2, IAP and caspase families are shown. β-actin indicates equal loading. B: RT-PCR detection of DAP-kinase transcripts. Detection of GAPDH was used as a control. Note: *denotes non-specific binding.

(PA682PB, KK124 and AS283A), suggesting that in a fraction of BLs both death pathways are intact. Similarly, 2 of the 3 BLs resistant to CH11 (LW878 and BML895) retained resistance to TRAIL. However, one BL (CA46) responded to TRAIL but not to CH11, suggesting that different pathways may also operate (Figure 2). The differences between CA46, LW878 and BML895 in their response to TRAIL cannot simply be explained by differences in the level of receptors (Table 2).

The ultimate response to death-inducing signals is likely to be modulated by an overall balance of expression of several pro- and anti-apoptotic proteins. We, therefore, analyzed the protein expression profile of genes involved in receptor-mediated apoptosis (Figure 3). As expected, the cross-sensitive cell lines had incurred the least disruption in the pro-apoptotic proteins. The most striking difference between the Fas-sensitive and Fas-resistant cell lines was the low or undetectable levels of the anti-apoptotic protein cIAP-1 in the Fas-sensitive cells.

The other remarkable finding was the compromised expression of multiple pro-apoptotic proteins in several BLs. LW878 was significantly compromised in the expression of Bak, Bcl-Xs, caspase-3 and DAP-kinase. BML895 did not express DAP-kinase, Bax, Bak and Bcl-Xs while CA46 failed to express Bax, Bak and Bcl-Xs (Figure 3). To our knowledge, this is the first report of tumor cell lines

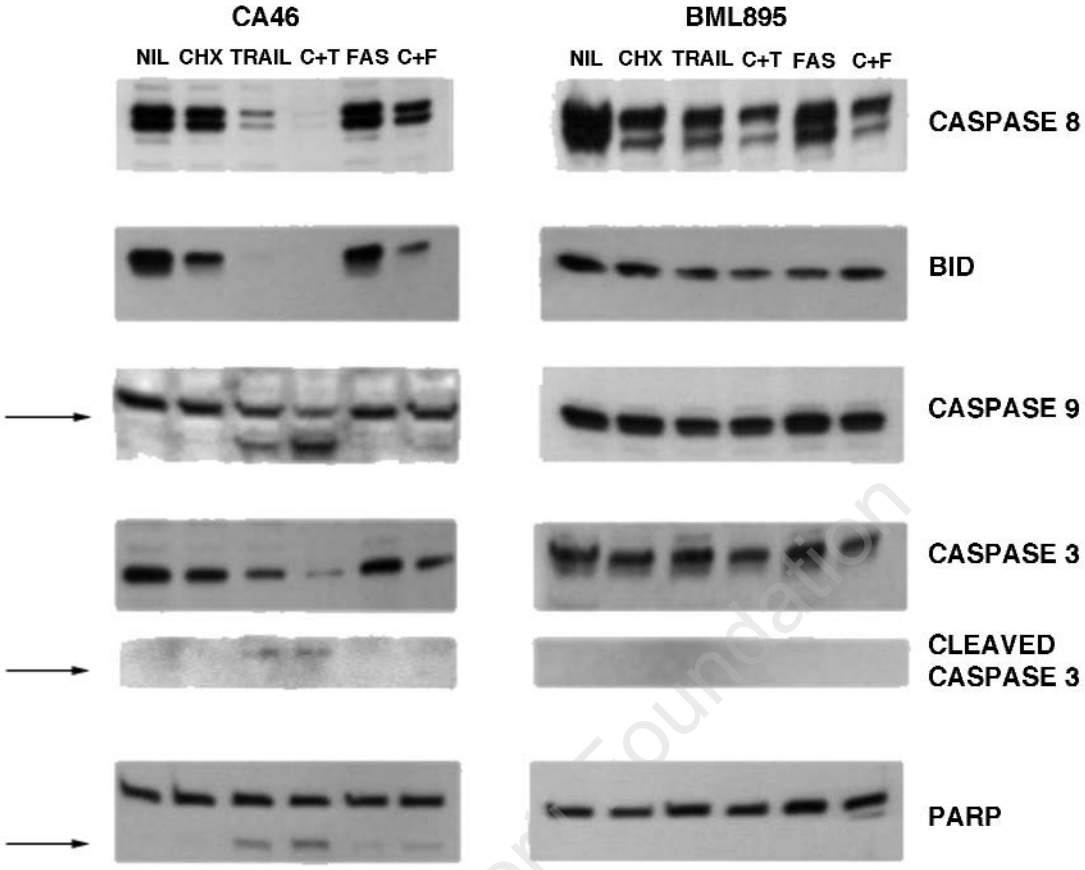

Figure 4. Dissection of the TRAIL and Fas apoptotic pathways. TRAIL-sensitive Fas-resistant CA46 and cross-resistant BML895 cells were treated with either 200ng/mL rTRAIL or 100ng/mL CH11 in the presence or absence of 1 μg/mL CHX. Protein extracts were made after 24 hrs of incubation. The specific antibodies are indicated. Lanes are labeled as follows: Nil = untreated control, CHX = treated with cycloheximide, TRAIL = treated with TRAIL, C+T = treated with CHX and TRAIL, Fas = treated with CH11, C+F = treated with CHX and CH11. Note: arrows denote cleaved bands.

with compromised expression of so many pro-apoptotic proteins.

BML895 and LW878, previously characterized to be resistant to Fas-L, also proved resistant to TRAIL-induced apoptosis. This concordance suggests that some common mediators of the Fas and TRAIL pathways may be compromised. Indeed, loss of caspase-3 in LW878 may account for its cross-resistant phenotype. Other candidates are loss of expression of Bax, Bak and Bcl-Xs, as observed in BML895 (Figure 3). However CA46, which does not express any of those three proteins, is resistant to Fas-L but surprisingly sensitive to the TRAIL (Figure 2) suggesting that Bax, Bak and Bcl-Xs are redundant for the TRAIL pathway and that TRAIL resistance is not coupled to Fas resistance. These observations also suggest that it is unlikely that the mitochondria are involved in mediating TRAIL-induced apoptosis in CA46. Dissection of the caspase cascade strongly supports the hypothesis that CA46 can successfully transduce TRAIL signals. While the cross-resistant cell line BML895 does not activate caspases following CH11 and TRAIL signals, CA46 effectively activates downstream proteins after TRAIL induction (Figure 4). In the absence of Bax and Bak, cleavage of caspase-9 in CA46 is surprising and unexpected. Either this activation is independent of the apoptosome, as described by Chauhan[41] or occurs as a positive feedback mechanism through the activation of caspase 3.[42]

Bax participation in apoptosis through death receptors is still not clear. A previous report has suggested that Bax^{-}/Bak^{-} cells (but not loss of either protein singly) have a death-resistant phenotype.[17] Similar to these observations, we also demonstrate that cell lines with compound loss of the mitochondrial pro-apoptotic proteins are resis-

tant to Fas-induced cell death. Deng *et al.*[15] demonstrated that Bax is necessary for TRAIL-mediated apoptosis, even though such cell death occurs without cytochrome c-mediated caspase activation. More recently, LeBlanc *et al.*[16] reported that in colon carcinoma cell lines Bax is crucial for TRAIL-mediated cell death, which is executed via the mitochondrion. Our observations would, however, indicate that loss of Bax and Bak in some B-cell neoplasms might be insufficient to protect cells from TRAIL-induced cell death, since TRAIL signals may bypass the mitochondria (extrinsic pathway). Whether the implication of these results also extends to additional cell types remains to be determined. It is, however, interesting that TRAIL can induce apoptosis in these B-cell tumors that are resistant to Fas-mediated apoptosis.

Whatever the mechanism, this observation has a direct implication for the utility of TRAIL-based therapies in the treatment of B-cell neoplasia.

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Pre-publication Report & Outcomes of Peer Review

Contributions

AH: execution of experiments, analysis and interpretation, revision and final approval of manuscript; J-PD: execution of experiments, analysis and interpretation, revision and final approval of manuscript; MIG: design, analysis, interpretation of data and drafting the article and final approval of manuscript; MA: design, analysis, interpretation of data and drafting the article and final approval of manuscript; KA-H: design, analysis, interpretation of data and contributions to drafting and manuscript approval; DC: design, analysis, interpretation of data and contributions to drafting and manuscript approval; GG: design, analysis, interpretation of data and contributions to drafting and manuscript approval; KB: contribution to the concept, design and analysis of data, drafting and revision of the manuscript, and approval. AH and J-PD contributed equally to this work. The authors would like to thank Dr. Konrad Famulski for expert help and advice in the purification of TRAIL, Per-Anders Bertilsson and Pulicat S Manogaran for their technical assistance with flow cytometric analyses and Maha Al-Rasheed for technical assistance. Author taking primary responsibility for the paper: KB; Figures 1-4: AH; Table 1: J-PD; Table 2: AH; Table 3: MIG.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Klas G. Wiman, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Wiman and the Editors. Manuscript received September 18, 2002; accepted November 27, 2002.

In the following paragraphs, Prof. Wiman summarizes the peer-review process and its outcomes.

What is already known on this topic

Normal B-cells in the germinal center may be exposed to both TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand. Whether abrogation of TRAIL apoptosis is a feature in the genesis of B-cell lymphomas of germinal center-phenotype is not known.

What this study adds

Findings of this study indicate that B-cell lymphomas frequently retain sensitivity to the TRAIL pathway, excluding the hypothesis that abrogation of TRAIL apoptosis is a feature in the genesis of B-cell lymphomas of germinal center.