Interleukin-1 Protects Transformed Keratinocytes from Tumor Necrosis Factor-related Apoptosis-inducing Ligand*

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family. It induces apoptosis primarily of transformed but not of normal cells and may therefore be a promising anti-cancer drug. Studying the role of TRAIL in apoptosis of keratinocytes, we detected TRAIL transcripts and protein in both normal human keratinocytes and transformed keratinocyte cell lines HaCaT and KB. Although normal keratinocytes were resistant to TRAIL, HaCaT and KB cells underwent apoptosis following TRAIL exposure. When HaCaT and KB cells were pretreated with the pro-inflammatory cytokine interleukin-1 (IL-1), cells became resistant to TRAIL-induced apoptosis. IL-1 significantly induced activation of the transcription factor NFκB in transformed keratinocytes. Moreover, the proteasome inhibitor MG132, which inhibits IL-1-induced NFκB activation, completely prevented the protective effect of IL-1. Thus, IL-1 appears to protect transformed keratinocytes from the cytotoxic effect of TRAIL via activation of NFκB. These data suggest that NFκB activation may protect cells from TRAIL-induced apoptosis and indicate a TRAIL receptor-independent pathway, which allows cells to escape the cytotoxic effect of TRAIL. Because IL-1 is secreted by a variety of tumor cells and is also released by inflammatory cells participating in the tumor-host immune response, tumors under these conditions could become resistant to TRAIL.

Although the expression of CD95L appears to be rather limited and restricted to immune privileged organs and tumor cells (3–6), TRAIL is found widely expressed at significant levels in normal tissues (1).

To date, the cDNAs of four TRAIL receptors have been cloned. Although TRAIL binds to all four receptors, only two, called DR4 (death receptor 4)/TRAIL-R1 and DR5 (death receptor 5)/TRAIL-R2/Trick2/KILLER-DR5, respectively, transduce the death signal and induce the apoptosis program (7–12). The third receptor, designated DR1 (decoy receptor 1), TRAIL-R3, or TRID (TRAIL receptor without an intracellular domain), binds TRAIL, but because of the lack of the intracellular death domain does not transduce the apoptotic signal. Thus, it was even proposed that Dr1 acts as an antagonizing decoy receptor inhibiting TRAIL-mediated apoptosis (8, 9, 13, 14). Dr1 transcripts were detected in many normal human tissues but not in most cancer lines examined, it was, therefore, suggested that Dr1 expression may determine whether cells are susceptible or resistant to TRAIL (9, 13). However, the system appears to be more complex because recently a fourth member of the TRAIL receptor family was cloned, designated TRAIL-R4, Dr2, or TRUND (15–17). Although TRAIL-R4 binds TRAIL, it does not transmit the death signal, because it carries a cytoplasmic region lacking most of the death domain, which is crucial for the induction of apoptosis. Overexpression of TRAIL-R4 was even found to confer resistance to TRAIL-mediated apoptosis (15–17). Because of the existence of at least four different receptors with different signaling capacities, the TRAIL receptor family appears to represent a complex system that may contribute to whether a cell reacts to TRAIL with apoptosis or not (18). To date, the biological relevance of the complex TRAIL receptor system is not well understood and conflicting data exist (15). Nevertheless, because of its preferential cytotoxicity for cancer cells, TRAIL is regarded as a promising anti-cancer drug that might be highly effective in vivo with few side effects as it has little or no lethal effect on normal cells.

Apoptosis of keratinocytes has recently been identified as a crucial event during the development of skin cancer (19). Therefore, we were interested in studying whether TRAIL is involved in apoptosis of keratinocytes. Here, we show that both normal human keratinocytes (HNK) and transformed keratinocyte cell lines express TRAIL transcripts and synthesize TRAIL protein. Although normal keratinocytes were resistant to TRAIL, transformed keratinocytes underwent apoptosis following TRAIL exposure. Both HNK and transformed keratinocytes express transcripts for DR4, DR5, and TRAIL-R4, whereas Dr1 transcripts were only detected in normal but not in transformed keratinocytes. Moreover, we demonstrate that TRAIL-induced apoptosis of transformed keratinocytes can be

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IL-1 Protects from TRAIL-induced Apoptosis

Experiment Design

Cells—Long term cultures of foreskin HNKs were prepared according to the method described by Boyce et al. (20). The spontaneously transformed human keratinocyte cell line HaCaT, which was kindly provided by N. Fusenig, Deutsches Krebsforschungszentrum, Heidelberg, Germany (21) and the epithelial carcinoma cell line KB (ATCC, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium and RPMI, respectively, containing 10% heat-inactivated fetal calf serum and 1% glutamine at 37 °C with 5% CO2 in a humidified atmosphere. Irradiation of cells with ultraviolet (UV) light was performed as described recently (22).

Reagents—Recombinant human TRAIL protein was obtained from Immunex Corp., Seattle WA. This is a leucine zipper form of TRAIL that requires no further cross-linking for induction of maximal cytotoxicity (10). A monoclonal antibody directed against human TRAIL (M180) and monoclonal antibodies directed against DR4 (M273) and DR5 (M311), respectively, were obtained from Immunex Corp. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG was purchased from Sigma. Antibodies directed against caspase 3 (CPP32) and anti-poly(ADP-ribose) polymerase (PARP), respectively, were obtained from Enzyme Systems Products, Dublin, CA. Recombinant human IL-1 was purchased from Boehringer Mannheim, the proteasome inhibitor MG132 from Calbiochem, San Diego, CA. Cycloheximide was obtained from Sigma. Antibody directed against IC8 was obtained from Upstate Biotechnology, Lake Placid, NY.

Detection of Cell Death—For the detection of DNA fragmentation, a cell death detection ELISA (Cell Death Detection ELISA Plus, Boehringer Mannheim) was used. The enrichment of mono- and oligonucleosomes released into the cytoplasm was calculated using the formula, absorbance of sample/absorbance of control. Enrichment factor was used as a parameter of apoptosis and shown on the y-axis as mean + S.D. of triplicates. Quantitation of apoptosis by annexin V binding was performed by using a commercially available kit (Bender Corp., Vienna, Austria). Briefly, cells were washed and resuspended in annexin V binding buffer. Fluorescein isothiocyanate-conjugated annexin V was added, and the samples were analyzed by flow cytometry (Epics XL, Coulter, Miami, FL). Cell permeability was determined by staining with propidium iodide.

Flow Cytometry—Aliquots of 2 x 10⁶ cells were incubated with antibodies directed against DR4 and DR5, respectively, for 45 min on ice. Purified mouse IgG was used as an isotype control. After washing, cells were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG. Propidium iodide was added; cells were washed twice and subsequently analyzed in a flow cytometer (Epics XL).

Western Blot Analysis—Cells were harvested and lysed in RIPA buffer (10 mM Tris, pH 8, 150 mM NaCl, 1 mM Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 4 μg/ml aprotinin, 1 mM sodium orthovanadate) for 15 min on ice. After centrifugation, supernatants were collected, and the protein content was measured by Bio-Rad protein assay kit (Bio-Rad). Protein samples were subjected to SDS-polyacrylamide gel electrophoresis, blotted onto nitrocellulose membranes, and incubated with antibodies of interest. To monitor equal loading of proteins, membranes were incubated with an antibody directed against β-actin (Pharmingen). Specific protein binding was detected by use of an ECL® kit (Amersham Pharmacia Biotech).

Polymerase Chain Reaction—Total RNA was extracted according to the method described by Chomczynski and Sacchi (23). 1 μg of total RNA was reverse transcribed with SuperScript RNase H reverse transcriptase (Life Technologies, Inc.). For PCR amplification (35 cycles), a 50-μl reaction containing 1 μg of cDNA, 200 μM dNTPs (each), 20 pmol of each primer, the standard buffer supplemented with Taq polymerase (2.5 units/reaction, Promega, Madison, WI) and 1.5 mM MgCl₂ was used. Nucleotide sequences of PCR primers (MWG Biotech, Ebersberg, Germany) were as follows: TRD, 5′-CCG AAC AAA ACG GAA GGT GTG ACC TCGAC-3′ (sense), 5′-TGACG GAC ACG GGA GAG CAG TGT GCT GCA TAC-3′ (antisense); DR5, 5′-ATG GAA GAA CGG GGA CAG AAC-3′ (sense), 5′-TGA GCA GAC AGA GTC GCT TGC ATT AC-3′ (antisense); TRAIL specific transcripts were detected in both normal keratinocytes and transformed keratinocyte cell lines express TRAIL. Northern blot analysis was performed. TRAIL specific transcripts were detected in both normal keratinocytes and transformed keratinocyte cell lines. Although the epidermoid carcinoma cell line KB expressed TRAIL transcripts in amounts comparable with those found in normal keratinocytes, much higher amounts of TRAIL mRNA were detected in transformed keratinocyte cell lines express TRAIL. Northern blot analysis was performed according to the method described recently in detail (24). Briefly, total cellular RNA was separated by gel electrophoresis and blotted onto nylon membranes. After prehybridization, hybridization carried out using [α-32P]ATP-labeled cDNA probes encoding human TRAIL and β-actin, respectively. After washing, membranes were exposed to x-ray films at −70 °C.

Electrophoretic Mobility Shift Assays—After stimulation, nuclear proteins were extracted as described previously in detail (25). Binding reactions were carried out by addition of 2 μg of poly(dI-dC) (Boehringer Mannheim) and 10⁷ cpm of [32P]labeled double-stranded oligonucleotide to the nuclear protein extracts for 20 min at 22 °C. Reaction samples were separated electrophoretically on native agarose gels at 150 V for 1.5 h and detected by autoradiography. NFκB oligonucleotide had the following sequence: 5′-AGT TGA GGC GAT TCC GGG C-3′. Competition analysis was performed by adding 40 molar excess amounts of unlabeled oligonucleotides.

RESULTS

TRAIL Kills Transformed but not Normal Keratinocytes—To determine whether normal keratinocytes and keratinocyte cell lines express TRAIL, Northern blot analysis was performed. TRAIL specific transcripts were detected in both normal keratinocytes and transformed keratinocyte cell lines. Although the epidermoid carcinoma cell line KB expressed TRAIL transcripts in amounts comparable with those found in normal keratinocytes, much higher amounts of TRAIL mRNA were detected in the spontaneously transformed keratinocyte cell line HaCaT (Fig. 1a). Western blot analysis using an antibody directed against TRAIL revealed higher amounts of TRAIL protein in extracts from HaCaT cells compared with those of HNK and KB cells (Fig. 1b).

To evaluate the responsiveness of keratinocytes to TRAIL, HNK, HaCaT and KB cells were exposed to recombinant TRAIL, and 16 h later apoptosis was determined using an apoptosis detection ELISA kit. Although TRAIL had no significant effect on the viability of HNK, both HaCaT and KB cells underwent apoptosis following TRAIL treatment (Fig. 2). TRAIL induced apoptosis of KB and HaCaT cells in a dose-de-
pendent manner, maximum apoptosis rate was observed after addition of 20 ng/ml TRAIL (data not shown). TRAIL-mediated killing of HaCaT and KB cells could be prevented by a neutralizing antibody directed against TRAIL (Fig. 2).

Because IL-1β-converting enzyme-related caspases were found to be involved in TRAIL-induced apoptosis of myeloma and leukemia cells (26), we investigated whether TRAIL treatment results in activation of caspase 3 (CPP32) in keratinocytes. Therefore, HaCaT cells were exposed to TRAIL or to UV light, which recently was described to induce apoptosis in HaCaT cells via activation of caspase 3 (22). 16 h later, lysates were prepared, and Western blot analysis was performed using an anti-caspase 3 antibody. This antibody is directed against a domain of the caspase 3 proform and does not recognize the processed 17-kDa form, thus resulting in loss of the immunoreactive band in samples in which caspase 3 has been activated. Similar to UV treatment, exposure of HaCaT cells to TRAIL caused cleavage of caspase 3 (Fig. 3), which was prevented by the anti-TRAIL antibody. The same observations were obtained with KB cells (data not shown). Activated caspase 3 cleaves the death substrate PARP (27). Accordingly, PARP was found cleaved from its intact 116-kDa form into its inactive 85-kDa product in samples obtained from HaCaT cells exposed either to TRAIL or UV light (Fig. 3). Addition of zVAD, a broad spectrum inhibitor of the IL-1β-converting enzyme family proteases, significantly reduced TRAIL-induced caspase 3 and PARP cleavage (Fig. 3), indicating the crucial role of caspases in TRAIL-mediated apoptosis. No cleavage of caspase 3 and PARP was observed in HNK upon TRAIL treatment (data not shown), thus confirming the resistance of normal keratinocytes to TRAIL.

Analysis of TRAIL Receptor Expression on Keratinocytes—Because it was postulated that differential expression of TRAIL receptors may contribute to whether a cell is susceptible or resistant to TRAIL (9, 13, 18), PCR analysis was performed. Transcripts for the death-inducing receptors DR4 and DR5 were found in both HNK and transformed keratinocytes, whereas transcripts for the antagonizing decoy receptor DcR1 were detected only in normal keratinocytes (Fig. 4a). In contrast, transcripts for TRAIL-R4 were found in normal keratinocytes as well as in HaCaT and KB cells (Fig. 4b). But semiquantitative PCR revealed that HNK expressed 5 times higher levels of TRAIL-R4 transcripts than transformed keratinocytes (data not shown).

To analyze the surface expression of TRAIL receptors, keratinocytes were incubated with antibodies directed against DR4 and DR5, respectively, and subjected to flow cytometry analysis. In accordance with the PCR data, both HNK and transformed keratinocytes expressed DR4 and DR5 on the surface (Fig. 5). Surface expression of DcR1 and TRAIL-R4 could not be determined by this method, because specific antibodies against DcR1 and TRAIL-R4 are not yet available.

Interleukin-1 Protects from TRAIL-mediated Killing—Because activation of the transcription factor NF-κB recently was found to prevent TNF-induced apoptosis (28–30), we addressed whether transformed keratinocytes, which are susceptible to TRAIL, can escape TRAIL-induced apoptosis through the same pathway. Therefore, KB cells were treated with IL-1, which is...
a potent activator of NFκB (30). After 15 min, TRAIL was added and 16 h later the apoptosis rate was evaluated by annexin V staining followed by flow cytometry analysis. Although KB cells exposed to TRAIL alone became apoptotic (Fig. 6c), KB cells pretreated with IL-1 became resistant to the cytotoxic effect of TRAIL (Fig. 6d). Similar findings were obtained with HaCaT cells and also when recombinant human IL-1 was used (data not shown). Kinetic studies showed that IL-1 did not only protect KB cells from TRAIL-induced apoptosis upon preincubation, but also when IL-1 was added 1 h after TRAIL exposure (Fig. 7). Addition of IL-1 at later time points resulted in a gradual loss of the protective effect. Protection was significantly reduced when IL-1 was added 2 or 4 h after TRAIL exposure and almost completely gone when IL-1 was given after 6 or 8 h. The protective effect of IL-1 was also confirmed by analyzing activation of caspase 3 (Fig. 8). Western blot analysis revealed a complete loss of the immunoreactive band in TRAIL-treated KB cells (lane 2), whereas pretreatment with IL-1β for 15 min prevented TRAIL-induced caspase 3 activation (lane 3).

To show that IL-1 activates NFκB in KB cells, nuclear proteins were obtained from KB cells that were left untreated or stimulated with IL-1β. EMSA revealed binding-active NFκB in IL-1β-treated KB cells (Fig. 9a, lane 2) but not in untreated cells (Fig. 9a, lane 1). Activation of NFκB is associated with degradation of the inhibitory protein IκB by the proteasome pathway. Upon activation, IκB becomes phosphorylated at two serine residues (Ser32 and Ser36), which acts as a signal for ubiquination followed by degradation of IκB by the 26 S proteasome (31, 32). Although high amounts of unphosphorylated IκB were detected in untreated KB cells (Fig. 9b, lane 1), extracts of IL-1β-stimulated cells only showed a fading phosphorylated IκB band (lane 2).

Because IκB becomes degraded by the 26 S proteasome, NFκB activation can be blocked by proteasome inhibitors (32). Thus, we used this approach to prove whether the protective effect of IL-1 is causally linked to NFκB activation. Addition of the proteasome inhibitor MG132 effectively blocked IL-1-induced NFκB activation, which was revealed by EMSA analysis (Fig. 9a, lane 3). In addition, MG132 prevented IκB degradation, indicated by a strong double band of unphosphorylated and phosphorylated IκB in Western blot analysis (Fig. 9b, lane 3). After having shown that MG132 suppresses NFκB activation in KB cells, we next determined whether MG132 inhibits the protective effect of IL-1 on TRAIL-mediated apoptosis. Therefore, cells were treated with the combination of IL-1 and TRAIL in the absence or presence of MG132, and the rate of apoptosis was determined by annexin V staining (Fig. 6). Exposure of KB cells to MG132 alone (Fig. 6e) or to the combination of IL-1β plus MG132 (Fig. 6f) only minimally enhanced the rate of apoptosis. In contrast, the preventive effect of IL-1β on TRAIL-induced apoptosis was completely lost in the presence of MG132 (Fig. 6h). MG132 inhibited the protective effect of IL-1 against TRAIL-induced cytotoxicity in a dose-dependent way (Fig. 10). To evaluate the effect of MG132 in another apoptosis read-out system, the effect on IL-1-mediated inhibition of TRAIL-induced caspase 3 activation was analyzed. In accordance with the annexin V data, MG132 completely prevented the inhibition of TRAIL-induced caspase 3 activation by IL-1 (Fig. 8, lane 4). Taken together, these data clearly demonstrate that IL-1 causes resistance to TRAIL-induced apoptosis and suggest that this effect may be mediated via activation of NFκB.

**DISCUSSION**

Although a variety of tissues have been screened and were found to be positive for TRAIL expression (1), no data exist as to whether TRAIL is expressed in skin. Here, we show that both HNK and transformed keratinocyte cell lines express TRAIL at the mRNA and the protein levels. However, in their response to TRAIL, HNK and transformed keratinocytes differ significantly. In contrast to HNK, both KB and HaCaT cells undergo apoptosis following exposure to TRAIL. The reason why HNK do not respond to TRAIL remains to be determined. Detection of transcripts of the apoptosis preventing decoy receptor DcR1 in HNK but not in transformed keratinocytes might imply that the expression of DcR1 is responsible for the TRAIL resistance of HNK. Furthermore, transcripts of the antagonizing receptor TRAIL-R4 were found in HNK as well as in transformed keratinocytes, but the levels expressed were much higher in HNK as determined by semiquantitative PCR. Thus, it is tempting to speculate that TRAIL resistance of HNK is because of expression of the antagonizing receptors DcR1 and TRAIL-R4. However, one has to be cautious when predicting cell surface expression of receptor proteins from message levels (15). At this point, it is only possible to detect surface expression of DR4 and DR5, because specific antibodies against.
DcR1 and TRAIL-R4, to the best of our knowledge, are not yet available. In addition, it seems that several cell lines expressing DcR1 appear to be susceptible to TRAIL (15, 33).

To gain further insights into whether DcR1 is responsible for the TRAIL resistance of HNK, we treated HNK with phospholipase C. Phospholipase C, which cleaves phosphatidylinositol anchors, should remove DcR1 from the cell surface and render HNK susceptible to TRAIL if DcR1 is critical for TRAIL resistance, because DcR1 is a GPI-linked surface molecule. When evaluated in an apoptosis ELISA, both untreated and phospholipase C-pretreated HNK were equally resistant to TRAIL (data not shown). Thus, one could conclude that DcR1 is not important for the TRAIL resistance of HNK. However, these data must be interpreted with caution; we cannot formally prove if all DcR1 molecules were cleaved because of the lack of specific antibodies. Moreover, under these conditions, TRAIL-R4 could still protect HNK from TRAIL-induced cytotoxicity. In addition, we observed that inhibition of protein synthesis by cycloheximide renders HNK susceptible to TRAIL (data not shown). This might suggest that the production of anti-apoptotic molecules like Bcl-2, Bcl-xL or FLIP (34–36) could be responsible for TRAIL resistance. The importance of protein synthesis for TRAIL resistance has also been found in other cell lines (33). However, this does not necessarily imply that TRAIL resistance is TRAIL receptor independent in general, as one cannot exclude much higher turnover rates of the antagonizing receptors, which would then require their constant production to confer resistance.

The transcription factor NFκB was recently found to prevent TNF-induced apoptosis (28–30). Therefore, the question arose whether NFκB activation can protect transformed keratinocytes from TRAIL-mediated apoptosis. To address this issue, KB and HaCaT cells were stimulated with IL-1 which is a

![IL-1 Protects from TRAIL-induced Apoptosis](image)

**FIG. 6.** IL-1 protects against TRAIL-induced apoptosis. 16 h after treatment, KB cells were stained with annexin V and subjected to flow cytometry. Cells were treated as follows: no treatment (a), IL-1β (10 ng/ml) (b), TRAIL (20 ng/ml) (c), IL-1 plus TRAIL added 15 min later (d), MG132 (50 μM) (e), MG132 plus IL-1 added 1 h later (f), MG132 plus TRAIL added 1 h later (g), and MG132 plus IL-1 plus TRAIL (h). Histograms show fluorescence intensity (x axis) versus cell number (y axis).

![IL-1 Prevents TRAIL-induced Caspase 3 Cleavage](image)

**FIG. 7.** KB cells were left untreated (a) or exposed to TRAIL (20 ng/ml) in the absence (b) or presence of 10 ng/ml IL-1β. IL-1β was added 15 min before (c) or after TRAIL exposure at the following time points: 1 h (d); 2 h (e); 4 h (f); 6 h (g); and 8 h (h). Cells were stained with annexin V and subjected to flow cytometry. Histograms show fluorescence intensity (x axis) versus cell number (y axis).

![IL-1 Prevents TRAIL-induced Caspase 3 Cleavage](image)

**FIG. 8.** IL-1 prevents TRAIL-induced caspase 3 cleavage. KB cells were left untreated (lane 1), exposed to 20 ng/ml TRAIL (lane 2), or to TRAIL after preincubation for 15 min with 10 ng/ml IL-1β (lane 3). In lane 4 IL-1 was added in combination with MG132 (50 μM). 16 h after stimulation, proteins were extracted and Western blot analysis performed using antibodies directed against CPP32/caspase 3 and α-tubulin, respectively.

![IL-1 Protects from TRAIL-induced Apoptosis](image)
potent inducer of NFκB (30). Indeed, pretreatment with IL-1β and IL-1α, respectively, rendered both KB and HaCaT cells resistant to TRAIL. The inhibitory effect of IL-1 was demonstrated by using several apoptosis read-out systems including annexin V staining (Fig. 6), cleavage of caspase 3 (Fig. 8), and apoptosis ELISA (data not shown).

IL-1-induced protection against TRAIL appears to be mediated by NFκB. First, IL-1 activates NFκB in KB cells, as demonstrated by EMSA and IκB Western blots; second, the proteasome inhibitor MG132, which inhibits the activation of NFκB (32), prevents the protective effect of IL-1. Although we cannot exclude that MG132 may also affect some pathways other than NFκB, similar dose response studies for the inhibition of IL-1-induced NFκB activation (data not shown) and the inhibition of IL-1-induced TRAIL resistance by MG132 (Fig. 10) support the assumption that IL-1 induces TRAIL resistance via NFκB. This is also confirmed by the observation that phorbol myristate acetate, another potent inducer of NFκB (32), reduces TRAIL-mediated apoptosis of KB cells (data not shown). To provide full protection, NFκB needs to be activated before initiation of the apoptosis program by TRAIL. This was shown by kinetic studies in which addition of IL-1 to KB cells, which had already been exposed to TRAIL for 2 h or longer, could not induce resistance.

Conflicting data exist as to whether triggering of the TRAIL receptors activates NFκB. Because transfection of DR4 in MCF7 cells did not lead to NFκB activation, Pan et al. (7) suggested that DR4 is not linked to NFκB. On the other hand, others have observed that transfecting cells with DR4 and DR5, respectively, induced NFκB activation (9, 37, 38). In addition, TRAIL-R4 was found to be capable of signaling NFκB activation, and it was proposed that it protects cells against the cytotoxic effect of TRAIL by this mechanism (15). Therefore we performed EMSA with KB cells that had been exposed to TRAIL. Although nuclear extracts were obtained at different time points after TRAIL stimulation ranging from 30 min to 16 h, none of the samples exhibited binding active NFκB (data not shown). These data suggest that TRAIL, in contrast to the findings with other cell types (9, 37, 38), does not activate NFκB in KB cells. Moreover, it is tempting to speculate that the failure to respond to TRAIL with NFκB activation might explain why KB cells are so sensitive to the cytotoxic effect of TRAIL. Accordingly, Jeremias et al. (39) recently reported that inhibition of NFκB enhances sensitivity for induction of apoptosis of lymphoid cells by TRAIL.

In summary, this study demonstrates that IL-1 can protect cells from TRAIL-induced apoptosis. These findings may have important practical implications for the use of TRAIL as an anti-cancer drug. Because of the unique ability of inducing apoptosis preferentially in cancer but not in normal cells, TRAIL may be highly efficient in eradicating tumor cells in vivo. In contrast to conventional cytostatic drugs, TRAIL may
exert only minimal side effects, because it has little lethal effect on normal cells. The reason for this selectivity of TRAIL remains unclear, however, differential expression of its multiple receptors by the respective cells may be of relevance but still needs to be formally proven. The present observation that transformed keratinocytes become resistant to TRAIL following IL-1 exposure is the first indication of a TRAIL receptor-independent pathway that allows cells to escape the cytotoxic effect of TRAIL. In addition, it suggests that IL-1-mediated activation of NFκB can protect cells from TRAIL-induced cytotoxicity. Tumors under these conditions could become resistant to TRAIL, because the pro-inflammatory cytokine IL-1 is secreted by a variety of tumor cells (40) and is also released by inflammatory cells participating in the tumor-host immune response (41). However, this effect may well be encountered when other cytokines or signals activate NFκB within this scenario; therefore, this pathway should be considered when applying TRAIL in vivo.

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