Rel/NF-κB Transcription Factors Protect against Tumor Necrosis Factor (TNF)-related Apoptosis-inducing Ligand (TRAIL)-induced Apoptosis by Up-regulating the TRAIL Decoy Receptor DcR1*

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The Rel/NF-κB family of transcription factors comprises five members in vertebrates (cRel, RelA, p50, p52, and RelB) that associate in homo or heterodimers and control transcription of numerous genes by binding κB consensus sites in their promoter or enhancer. Rel/NF-κB dimers are assumed to be ubiquitously expressed but in a transcriptionally inactive complex with a protein of the IκB family. The prototypic protein of this family, IκBo, is able to both retain Rel/NF-κB dimers in the cytoplasm and inhibit their binding to DNA. The inhibition of Rel/NF-κB dimers by IκBo can be released upon stimulation by a variety of agents, including TNFα and IL-1β, which leads to the activation of high molecular weight complexes containing IκB kinase activity. The best characterized of these complexes, the IκB kinase signalosome, is composed of two IκB kinases, IκB kinase α, and IκB kinase β, and a regulatory component, NEMO. Upon IκBα or IκBβ kinase activation, IκBα is phosphorylated, ubiquitinated, and degraded, thus allowing Rel/NF-κB dimers to exert transcriptional control (1–3).

Rel/NF-κB transcription factors control the expression of a number of genes involved in immune and inflammatory responses as well as in basic cell functions such as adhesion, proliferation, and apoptosis (4, 5). Although some reports show that Rel/NF-κB factors are able to induce apoptosis (6–10), the activation of these factors seems primarily to render cells resistant against apoptosis induced by a variety of agents. To date, a few anti-apoptotic target genes of Rel/NF-κB factors have been characterized. Some of them could be relevant to a protective effect against a variety of apoptosis inducers, because they act downstream in the apoptotic pathway. That is the case for Bcl-x (22, 23) and Bcl-2 (22, 24–26), two members of the Bcl-2 family that act at the mitochondrial level, and for XIAP, cIAP-1 and cIAP-2 (27–29), the inhibitor of apoptosis proteins that inhibit the activity of several caspases (30). In contrast, two other anti-apoptotic Rel/NF-κB target genes, TRAF1 and TRAF2 (28), act upstream in the apoptotic pathway, principally at the TNF receptor level and, therefore, may account especially for the resistance against apoptosis induced by TNFα.

A few years ago, a new member of the TNF family was independently characterized by two groups and named Apo2 ligand or TRAIL, for TNF-related apoptosis-inducing ligand.

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Indeed, TRAIL activates apoptosis in many tumor cell lines by inducing the caspase cascade. TRAIL binds a family of receptors belonging to the TNF receptor superfamily. Two of these receptors, DR4 (TRAIL-R1) and DR5 (TRAIL-R2), possess a death domain in their cytoplasmic part, which enables them to engage the apoptotic machinery in a way similar to that engaged by TNF receptor 1 or Fas. Two other receptors, DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), are decoy receptors, respectively devoid of cytoplasmic domain or with a truncated death domain lacking the amino acids critical for apoptosis signaling (31–33). DcR1 and DcR2 behave as transdominant negative receptors, protecting against TRAIL-induced apoptosis either by competing for TRAIL binding on DR4 and DR5 or by forming inactive heterotrimeric receptors with DR4 or DR5 (33–36). DcR1, DcR2, DR4, and DR5 transcripts were co-detected in many normal human tissues, whereas many cancer cell lines preferentially express DR4 and DR5 but not DcR1 and DcR2 (32, 34–36). Since TRAIL induces apoptosis in a wide range of transformed cell lines but not in normal cells (37, 38), these observations suggest that decoy receptor expression may participate in the determination of whether cells are sensitive or resistant to TRAIL. In this report, we show that Rel/NF-κB transcription factors, upon overexpression or physiological activation by TNFα in HeLa cells, increase DcR1 expression, with no concomitant induction of DcR2, DR4, or DR5. This DcR1 induction confers resistance to TRAIL-induced apoptosis.

**EXPERIMENTAL PROCEDURES**

**Construction of the Bicistronic pEGFP/cRel Expression Vector**—The bicistronic pEGFP/cRel expression vector was constructed from the pEGFP-C1 vector (CLONTECH), designed to synthesize proteins fused to the C terminus of the enhanced green fluorescent protein (EGFP). To stop the translation of EGFP at the 5’ end of the multiple cloning site, we inserted a sequence containing multiple stop codons between the HindIII and XbaI sites. Next, the internal ribosomal entry site sequence of the poliovirus type 1 (39) was inserted between the Asp-718 I sites of the multiple cloning site. This construct, named pEGFP-C1 (CLONTECH), designed to synthesize proteins fused to the C terminus of the enhanced green fluorescent protein (EGFP). To stop the translation of EGFP at the 5’ end of the multiple cloning site, we inserted a sequence containing multiple stop codons between the HindIII and XbaI sites. Next, the internal ribosomal entry site sequence of the poliovirus type 1 (39) was inserted between the Asp-718 I sites of the multiple cloning site. This construct, named pEGFP, was used as a control vector. To obtain the pEGFP/cRel expression vector, the human c-rel cdna (40) was inserted in the XbaI site of the multiple cloning site.

**Cell Culture, Transfection, and Flow Cytometry Sorting**—HeLa cells from the European Collection of Cell Culture (number 93021013), were grown at 37 °C in an atmosphere of 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were transfected by FuGENE (Roche Molecular Biochemicals) according to the manufacturer’s recommendations. GFP-expressing cells were sorted 24 h after transfection with an Epics Elite cytometer (Coulter) using excitation at 488 nm and detection at 520–530 nm. On average, 40% of cells were GFP-positive. Sorting was adjusted to keep cells from the most fluorescent third of the GFP-positive population.

**Immunofluorescence**—Twenty-four to 72 h after transfection, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline and permeabilized with 0.2% Triton X-100. cRel was detected with an anti-human cRel mouse IgG1 (Sc-6955, Santa Cruz Biotechnology) and a secondary antibody, rhodamine Red-X-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). DcR1 was detected by a procedure involving tyramide amplification. Briefly, cells were successively incubated with anti-human DcR1 goat serum (Alexis Biochemicals), peroxidase-conjugated donkey anti-goat IgG (Jackson ImmunoResearch Laboratories), biotinylated tyramide (PerkinElmer Life Sciences), and peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). Peroxidase activity was revealed using 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) as substrate. Using an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech), membranes were exposed at −80 °C for 4 days. Films were scanned using a laser scanning densitometer (model GS-700, Bio-Rad Laboratories). Nuclei were stained with Hoechst 33258 (Sigma) at 1 μg/ml. After that, cells were fixed in 4% paraformaldehyde, and nuclei were stained with Hoechst 33258 (Sigma). Apoptotic and viable cells were recognized according to the condensation and fragmentation degree of their cytoplasm and nuclei. Viable cells were manually counted among 100 GFP-positive cells, in triplicate for each point. The statistical analysis was performed with analysis of variance (Statview).

**Transactivation Assays**—The reporter vector used to measure the transcriptional activity of overexpressed cRel or endogenous Rel/NF-κB factors was the pBsLuc vector containing three human immunodeficiency virus κB sites upstream of the thyminidine kinase minimal reporter and the luciferase cDNA. The luciferase activity was measured 24–36 h after transfection by the luciferase assay system (Promega) according to the manufacturer’s recommendations.

**Semiquantitative RT-PCR**—Cells were transfected, sorted, and cultured as described above. They were then homogenized in Trizol (Life Technologies, Inc.), and total RNAs were isolated according to the manufacturer’s recommendations. cDNAs were synthesized using the Gene Amp RNA PCR kit (PerkinElmer Life Sciences). PCRs were performed with the Gene Amp 9600 PCR system (PerkinElmer Life Sciences) in a final volume of 50 μl of buffer containing 2.5 μl of the retrotranscription product, all four dNTPs at 150 μM, MgCl2 (3 mM for cRel, 2 mM for others), 1 unit of Taq gold polymerase (Roche Molecular Biochemicals), and each primer at 1 μM. Primers used were: cRel forward, AGAGGGAATGCGTTTATGATA; cRel reverse, CAGGGAGAAAATCGTTGAAACCA; Ixαa forward, GGCCCAAGAGCCCGGATCATGGG; Ixαa reverse, GGCGCTTCGCGCTTACCTCCT; p105 forward, GGCCCAAGACGCAGCAAGAAACA; p105 reverse, GGGCCCAATACCCCGCAGGT; DcR1 forward, GGGCCCAAGGAGCAGGTGTTCT; DcR2 forward, CCCCGGAGGAGAGGAGGT; DcR2 reverse, CTCTCCGCTGCTGGGTTTT; DcR4 forward, CCGCGGCCACACCCAGAAAT; DcR4 reverse, GTCAATGGGAGGAGCCGAAACAA; DR5 forward, GCGCCCAAAAATACCCCGCAGGT; DR5 reverse, GACGGCCGGACGAGAGGAGG; β-actin as Kasibhatla et al. (9), 3β-actin as 1.0°C (DcR2), 56.9°C (DcR5), 58.4°C (DcR1), 59.2°C (p105), 60.9°C (DR5), 61.2°C (DR4) for 1 min, and 72°C for 1 min, with an initial step of 5 min at 95°C. PCR product lengths were 415 bp (DR4), 418 bp (DcR2), 420 bp (cRel), 430 bp (p105), 437 bp (DR5), 503 bp (Ixαa), 546 bp (DcR1), and 661 bp (β-actin).

**Removing DcR1 from the Cell Surface Membrane**—HeLa cells were either transfected by pEGFP or pEGFP/cRel or treated by TNFαs (R&D Systems) at 10 ng/ml. Forty eight or 18 h later, respectively, the medium was replaced by fresh medium containing F1-PLC (Sigma) at 3 μg/ml and cycloheximide (Sigma) at 10 μg/ml. One h later, the medium was replaced by fresh medium, and the expression of DcR1 or the sensitivity to TRAIL was assayed.

**Electrophoretic Mobility Shift Assay**—Cells were incubated or not with TNFαs (R&D Systems) at 10 ng/ml for 30 min. Nuclear extracts were prepared as in Lin et al. (41). Nuclear protein concentrations were measured with the Bio-Rad protein assay. The κB consensus probe (Promega) was radioabeled according to recommendations of Promega and purified using QIAquick nucleotide removal kit (28304, Qiagen). One μg of nuclear extract was incubated with 0.05 pmol of radiolabeled κB consensus probe according to the manufacturer’s recommendations. Competitions with cold probe were performed by preincubating nuclear extracts with the κB cold probe in a 50- or 100-fold excess. For supershift experiments, nuclear extracts were preincubated with 2 μl of anti-cRel, anti-RelA, anti-p50 (antibodies used were those described by Pepin et al. (42)). DNA-protein complexes were separated from unbound probe by migration on native 4% polyacrylamide gels at 200 V for 2 h. Inhibition by Rel/NF-κB Activity—The expression vector used to inhibit Rel/NF-κB activity contains the avian Ixαa cDNA inserted in the BamHI (Invitrogen). The empty pBSLuc vector was used as a control. Rel/NF-κB factors were activated 24–36 h after transfection by treating cells overnight with 10 μg/ml TNFαs (R&D Systems).

**Apoptosis Assays**—Apoptosis was induced by treatment with various concentrations of TRAIL (R&D Systems) and 10 μg/ml cycloheximide (Sigma). After that, cells were fixed in 4% paraformaldehyde, and nuclei were stained with Hoechst 33258 (Sigma). Apoptotic and viable cells were recognized according to the condensation and fragmentation degree of their cytoplasm and nuclei. Viable cells were manually counted among 100 GFP-positive cells, in triplicate for each point. The statistical analysis was performed with analysis of variance (Statview).
RESULTS

Expression of cRel in HeLa Cells via a Bicistronic GFP/cRel Expression Vector—To identify new anti-apoptotic genes under Rel/NF-κB control, we applied the DNA array technique on HeLa cells that were made apoptosis-resistant by overexpression of a Rel/NF-κB factor, cRel. The cRel expression vector constructed, pEGFP/cRel, expresses a bicistronic internal ribosomal entry-site-based mRNA encoding both cRel and the EGFP. The concordance between cRel and GFP expression in pEGFP/cRel-transfected cells was checked by immunofluorescence. Whereas cRel was undetectable in GFP-positive cells transfected by the pEGFP control vector, it was detected in all GFP-positive cells transfected by the pEGFP/cRel vector (Fig. 1A). The most fluorescent GFP-positive cells were those that express cRel at the highest level (data not shown). Hence, this vector allowed us to sort cRel-overexpressing cells by flow cytometry on the basis of their GFP fluorescence and, therefore, to perform molecular analysis on nearly pure populations.

The overexpression of cRel in pEGFP/cRel-transfected and -sorted cells was analyzed by immunoblot. As shown in Fig. 1B, a protein migrating at the expected Mr 75,000 was overexpressed in cells transfected by pEGFP/cRel. Immunofluorescence analysis revealed that the overexpressed protein was preferentially located in the nucleus (Fig. 1A), suggesting it was transcriptionally active. To further establish this point, cells were co-transfected by either pEGFP or pEGFP/cRel and a CBER luciferase vector. The luciferase activity of cRel-transfected cells was about 6.5-fold that of control cells (Fig. 1C), indicating that the overexpressed cRel was transcriptionally active. This transcriptional activity was finally confirmed by determining the expression level of two known Rel/NF-κB target genes. Semi-quantitative RT-PCR was performed for IκBα (43) and p105 (44). Expression of these two genes was induced in pEGFP/cRel-transfected and -sorted cells compared with control cells, whereas the β-actin control was expressed at similar levels in both cases (Fig. 1D). Therefore, HeLa cells transfected by the pEGFP/cRel vector overexpressed a transcriptionally active cRel protein. As already described by us and others (17–19), this overexpression renders these cells resistant against apoptosis induced by TNFα in the presence of cycloheximide (CHX). These cRel-overexpressing cells thus appear suitable for searching new target genes of Rel/NF-κB factors involved in their anti-apoptotic activity.

The Expression of DcR1, but Not DcR2, DR4, and DR5, Is Induced in cRel-overexpressing Cells—To identify new anti-apoptotic genes induced by cRel, we have made a large scale screening by using DNA arrays. Total RNAs from pEGFP- and pEGFP/cRel-transfected cells were extracted, retrotranscribed in [32P]-radiolabeled cDNAs, and successively hybridized on a filter containing ~4000 spots for human named genes (GF211, Research Genetics). Radioactivity levels were measured using a PhosphorImager (Molecular Dynamic), and the differential analysis of the results was done using the Pathways™ software (Research Genetics). Among several genes whose expression level changed above 2-fold between cRel-overexpressing cells and control cells (data not shown), only one fulfilled the criterion of being a new Rel/NF-κB anti-apoptotic target gene; this gene is DcR1, which encodes a decoy receptor of TRAIL, a cytokine of the TNF family. The expression of DcR1 was induced 2.4-fold in cell cultures, whereas that of another TRAIL receptor, DR5, remained unchanged. The other TRAIL receptors, DcR2 and DR4, were not represented on the filter.

Changes in the expression of all four TRAIL receptors were subsequently investigated by semi-quantitative RT-PCR. As shown in Fig. 2A, the levels of DcR1 mRNAs increased in cRel-expressing cells compared with control cells, confirming the DNA array results. In contrast, no change in the expression of the other TRAIL receptors was detected: the levels of DcR2, DR4, and DR5 mRNAs were similar in cRel-expressing cells and in control cells (Fig. 2A). Since it has been shown that in
some cell types DcR1 is expressed but not localized at the membrane (45), we examined the localization of DcR1 in cRel-expressing cells by immunofluorescence. The comparative observation of GFP-positive cells transfected by pEGFP or pEGFP/cRel revealed that DcR1 was specifically accumulated in cRel-expressing cells (Fig. 2 B). DcR1 being anchored at the surface membrane by a phosphatidylinositol tail (35), it can be specifically removed from the cell surface by treating cells with an extracellularly added phosphatidylinositol-specific phospholipase C (PI-PLC) (35, 46). After a 1-h treatment with PI-PLC in the presence of CHX (to avoid any DcR1 neosynthesis), DcR1 became undetectable by immunofluorescence in pEGFP/cRel-transfected cells (Fig. 2 B), indicating that it was indeed localized at the cell surface. Taken together, these results indicate that cRel induces the expression of the TRAIL decoy receptor DcR1 on the surface membrane by a phosphatidylinositol tail (35), it can be specifically removed from the cell surface by treating cells with an extracellularly added phosphatidylinositol-specific phospholipase C (PI-PLC) (35, 46). After a 1-h treatment with PI-PLC in the presence of CHX (to avoid any DcR1 neosynthesis), DcR1 became undetectable by immunofluorescence in pEGFP/cRel-transfected cells (Fig. 2 B), indicating that it was indeed localized at the cell surface. Taken together, these results indicate that cRel induces the expression of the TRAIL decoy receptor DcR1 at the membrane, with no concomitant induction of DcR2, DR4, and DR5.

**DcR1 Expression Is Directly Controlled by Physiological Levels of Rel/NF-κB Factors**—To establish whether physiological levels of Rel/NF-κB transcription factors directly participate in the control of DcR1 expression, endogenous Rel/NF-κB activity was induced in parental HeLa cells by TNFα or inhibited by IκBα overexpression, and DcR1 expression was assessed in both cases. Rel/NF-κB activation by TNFα was checked by gel shift assays. Thirty minutes of TNFα treatment increased Rel/NF-κB binding on a κB consensus probe (Fig. 3 A). Supershift experiments indicate that the affected Rel/NF-κB dimers were composed of at least cRel, RelA, and p50 (Fig. 3 A). The transcriptional activity of these complexes was assayed by transfecting the pκB-Luc reporter vector. Fig. 3B shows a 3.5-fold increase in transcriptional activity upon TNFα treatment. Both the basal and TNFα-induced transcriptional activities were inhibited by overexpressing IκBα (Fig. 3B). Changes in TRAIL receptor expression upon TNFα treatment and/or IκBα overexpression were then investigated by semiquantitative RT-PCR. The results show that TNFα treatment induced the accumulation of DcR1 transcripts but not DcR2, DR4, and DR5. This
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**Fig. 4.** cRel protects HeLa cells against TRAIL-induced apoptosis by up-regulating DcR1. The resistance of pEGFP- or pEGFP/cRel-transfected cells was assayed 48 h after transfection. **A**, a microscopic examination of cells after 6 h of treatment with 10 ng/ml TRAIL + 10 μg/ml CHX indicates that nearly all pEGFP-transfected cells were apoptotic, i.e. with condensed cytoplasm and nucleus (arrowheads in the **bottom panel**), whereas pEGFP/cRel-transfected cells were still alive, well spread on the dish, with normal nuclei (arrows in **bottom panel**). **B**, quantification of viable cells among GFP-positive cells after different times of treatment with 10 ng/ml TRAIL + 10 μg/ml CHX. **C**, analysis of the involvement of DcR1 in the protective effect of cRel against TRAIL-induced apoptosis. Two days after transfection by pEGFP or pEGFP/cRel, cells were treated or not with 3 μg/ml PI-PLC and 10 μg/ml CHX for 1 h, and then apoptosis was induced by 10 ng/ml TRAIL and 10 μg/ml CHX for 6 h. After fixation and nuclear staining by Hoechst, viable cells were counted among GFP-positive cells. * *, **, and *** respectively, mean p < 0.05, 0.005, and 0.0005.

**Fig. 5.** Rel/NF-κB factors protect HeLa cells from TRAIL-induced apoptosis by up-regulating DcR1. To inhibit their endogenous Rel/NF-κB factors, parental HeLa cells were co-transfected with pCR3/IκBα (or with pCR3 as control) and with pEGFP as a transfection marker. Thirty-six h later, cells were treated or not first with 10 ng/ml TNFα overnight to activate the endogenous Rel/NF-κB factors and second with 3 μg/ml PI-PLC and 10 μg/ml CHX for 1 h to degrade the DcR1 molecules present at the cell surface; third, apoptosis was induced by a 5-h treatment with 10 ng/ml TRAIL and 10 μg/ml CHX. The number of viable cells among GFP-positive cells was counted in each case. ** means p < 0.005.

**A** Protective Effect of NF-κB against TRAIL-induced apoptosis—Since DcR1 ectopic expression has been shown to protect HeLa, 293, and MCF7 cells from TRAIL-induced apoptosis (34, 35), we hypothesized that the induction of DcR1 by cRel overexpression or by Rel/NF-κB activation by TNFα should make cells resistant to TRAIL-induced apoptosis. To test this hypothesis, cells were transfected by pEGFP or pEGFP/cRel and 48 h later treated with TRAIL in the presence of CHX. Viable and apoptotic cells were identified according to their morphology after fixation and nuclear staining by Hoechst; viable cells were spread on the dish and displayed normal nuclei, whereas apoptotic cells were markedly rounded with condensed or fragmented nuclei (Fig. 4A). TRAIL was applied during 4, 5, or 6 h at a concentration of 10 ng/ml (+CHX), and viable cells were counted. The results show that after 6 h of TRAIL+CHX treatment, only 20% of control cells were viable versus 60% of cRel-expressing cells (Fig. 4B).

To demonstrate the involvement of DcR1 in the protective effect of cRel against TRAIL-induced apoptosis, we assayed the protective effect of cRel after removal of DcR1 from the cell surface membrane by a PI-PLC treatment. Fig. 4C shows that the treatment of cRel-expressing cells with PI-PLC completely reverted the protection against TRAIL-induced apoptosis acquired on cRel overexpression. Hence, in HeLa cells, cRel exerts its anti-TRAIL protective activity by up-regulating DcR1.

To evaluate whether physiological levels of Rel/NF-κB transcription factors were also able to make HeLa cells resistant against TRAIL-induced apoptosis, we examined the sensitivity to TRAIL of parental HeLa cells in which Rel/NF-κB activity was induced by TNFα. Cells were pretreated or not by TNFα overnight, and then apoptosis was induced by a 6-h TRAIL+CHX treatment. In the absence of TNFα pretreatment, only 10% of the cells were alive after TRAIL+CHX treatment. In contrast, when cells were pretreated by TNFα, 45% were still alive after TRAIL+CHX treatment (Fig. 5). To investigate the involvement of Rel/NF-κB factors in the protective effect conferred by the TNFα pretreatment, the same experiment was performed on cells transfected by the IκBα ex-
pressure version. In cells overexpressing IκBα, the percentage of viable cells after TRAIL + CHX treatment was reset to 10%, i.e. to the level reached in the absence of TNFα pretreatment (Fig. 5). Therefore, physiological levels of Rel/NF-κB were as effective as cRel overexpression in protecting HeLa cells from TRAIL-induced apoptosis. To evaluate the role of DcR1 in this Rel/NF-κB protective effect, cells were treated by PI-PLC before inducing apoptosis by TRAIL. This treatment totally abrogated the protective effect against TRAIL-induced apoptosis acquired on Rel/NF-κB activation by TNFα (Fig. 5B). Taken together, these results indicate that cRel overexpression or Rel/NF-κB activation by TNFα makes cells resistant against TRAIL-induced apoptosis by up-regulating DcR1.

DISCUSSION

In this report, we show that Rel/NF-κB transcription factors up-regulate DcR1, a truncated TRAIL receptor unable to induce apoptosis, without changing the expression of the other TRAIL receptors, the death-inducing receptors DR4 and DR5, and the other decoy receptor DcR2. This was demonstrated either by constitutively overexpressing cRel or by physiologically inducing a Rel/NF-κB activity with TNFα. Furthermore, we show that cells overexpressing cRel or treated with TNFα become resistant to TRAIL-induced apoptosis. This resistance is due to the up-regulation of DcR1 by Rel/NF-κB factors, because resistance is abolished when DcR1 is removed from the cell surface by a PI-PLC treatment. Therefore, Rel/NF-κB factors may contribute to adjusting the sensitivity of cells to TRAIL-induced apoptosis by controlling the ratio of TRAIL-decoy to death receptors.

Whether Rel/NF-κB factors are able to protect against TRAIL-induced apoptosis is controversial in the literature. In support of a protective effect and in agreement with our results, it was shown that IL-1β protects keratinocytes from TRAIL-induced apoptosis via the activation of NF-κB (46, 47). Moreover, T cells and epithelial colon cancer cells were shown to be sensitized to TRAIL-induced apoptosis when NF-κB was inhibited by sulfasalazine (48). In contrast, Hu et al. (49) concluded from two sets of experiments that Rel/NF-κB factors cannot protect against TRAIL-induced apoptosis (49). The first set of experiments showed that the induction of Rel/NF-κB by overexpression of NF-κB-inducing kinase (NIK) or IκB kinase β did not protect against apoptosis induced by DR4 overexpression. However, even if DcR1 was induced in that situation, it could not have evoked its protective effect, because apoptosis was triggered without exposing cells to TRAIL. The second set of experiments indicates that overexpression of an IκBα superrepressor did not sensitize cells to TRAIL, whereas it did sensitize them to TNFα. However, these experiments were done on a subpopulation of HeLa or MCF7 cells, selected for their resistance against TRAIL-induced apoptosis. If these cells had become TRAIL-resistant because they highly expressed a molecule specifically interfering with the TRAIL pathway, such as DcR1, further blocking NF-κB could not sensitize them to TRAIL but could indeed sensitize them to TNF. Therefore, this study cannot exclude a protective role of Rel/NF-κB factors against TRAIL-induced apoptosis via DcR1 but suggests that some of the other anti-apoptotic Rel/NF-κB target genes involved in the TRAIL resistance, such as BFL-1/A1, Bcl-x, IAP proteins, or TRAF1 and -2, do not participate in the TRAIL resistance. However, the literature is also controversial regarding the involvement of these factors in the resistance against TRAIL. Bcl-x would indeed not participate in this resistance, since it was shown in diverse B and T tumor cells that its overexpression at levels that protect against etoposide does not protect against TRAIL (50). cIAP-1 and -2 would in contrast mediate the protective effect of Rel/NF-κB against TRAIL-induced apoptosis in keratinocytes (47). Therefore, depending on the cell type or the context, the strategy evoked by Rel/NF-κB factors to protect against TRAIL-induced apoptosis would differ; it would engage either molecules specific to the TRAIL pathway, such as DcR1, or more pleiotropic molecules also involved in the protection from apoptosis induced by other cytokines of the TNF family. In addition, the resistance against TRAIL can be controlled independent of Rel/NF-κB factors. For instance, the expression level of cFLIP, a caspase 8 inhibitory protein (52) that can potentially inhibit apoptosis induced by several death receptors (53–56), was shown to be responsible of the resistance of melanoma cells and keratinocytes against TRAIL (37, 51).

Little is known on the regulation of TRAIL-receptor expression. The tumor suppressor protein p53 was shown to be involved in the up-regulation of DcR1, DcR2, and DR5 (57–59). Our results suggest that Rel/NF-κB factors would, in contrast, specifically up-regulate DcR1 but not DcR2, DR4, and DR5. Post-transcriptional mechanisms could also participate in the control of TRAIL receptor localization at the membrane. For example, MRC-5 fibroblasts express negligible amounts of DcR1 at their surface, but some molecules are present in the nucleus (45). Therefore, the sensitivity of a cell to the killing effects of TRAIL may be regulated by complex mechanisms involving transcriptional and post-transcriptional controls of the balance of decoy and death receptor expression at the membrane as well as expression of some anti-apoptotic proteins such as cFLIP or IAPs.

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