TRAIL/Apo2L Activates c-Jun NH$_2$-terminal Kinase (JNK) via Caspase-dependent and Caspase-independent Pathways*

(Received for publication, June 9, 1998, and in revised form, August 21, 1998)

Frank Mühlenbeck‡, Elvira Haas‡, Ralph Schwenzer‡, Gisela Schubert‡, Matthias Grell‡, Craig Smith§, Peter Scheurich‡, and Harald Wajant‡¶

From the §Institute of Cell Biology and Immunology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany and the ¶ImmuneX Research and Development Corporation, Seattle, Washington 98101

In this study we show that TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), also called Apo2L, activates the c-Jun N-terminal kinase (JNK). Interestingly, TRAIL-induced JNK activation occurs in a cell type-specific manner. In HeLa cells, TRAIL-induced JNK activation can be completely blocked with the cysteine protease inhibitor zVAD-fmk, whereas the same inhibitor has no, or even a stimulatory, effect on JNK activation in Kym-1 cells. Hence, TRAIL can engage at least two independent pathways leading to JNK activation, one that is cysteine protease-dependent and one that is cysteine protease-independent. To investigate whether the cysteine protease-dependent signaling of TRAIL leading to JNK activation is related to the apoptotic pathway engaged by this ligand, we investigated HeLa cells stably overexpressing a dominant negative mutant of FADD (Fas-associating protein with death domain) (GFP(green fluorescent protein)$\Delta$FADD). In these cells, TRAIL-induced cell death and activation of the apoptosis executioner caspase-8 (FLICE/MACH) and caspase-3 (YAMA, CPP-32, Apopain), that belong to caspase subfamily of cysteine proteases, were abrogated, whereas JNK activation remained unaffected and was still sensitive toward z-VAD-fmk. Similar data were found in HeLa cells overexpressing Apo1/Fas and GFP$\Delta$FADD upon stimulation with agonistic antibodies. These data suggest that cross-linking of the TRAIL receptors and Apo1/Fas, respectively, engages a FADD-dependent pathway leading to the activation of apoptotic caspasases and, in parallel, a FADD-independent pathway leading to the stimulation of one or more cysteine proteases capable to activate JNK but not sufficient for the induction of cell death.

Members of the tumor necrosis factor (TNF)† receptor superfamily of proteins are critically involved in inflammatory, immune regulatory, and pathophysiological reactions, including the induction of apoptosis (1, 2). The receptors of this superfamily are characterized by two to six extracellular copies of a canonical motif of cysteine-rich pseudorepeats, each comprising six conserved cysteines in a stretch of about 40 amino acids (1, 2). The ligands of these receptors belong to a complementary family of structurally related molecules, the TNF ligand family. Most of these ligands are primarily expressed as biologically active type II membrane proteins, from which soluble forms are produced by proteolytical cleavage or alternative splicing (1, 2).

A subgroup of the TNF receptor superfamily, comprising TNF-R1 (3), Apo1/Fas (4), Wsl/DR3/Apo-3/TRAMP/LARD (5–9), CAR1 (10), DR4/TRAIL-R1 (11), and DR5/TRAIL-R2/TRICK2/KILLER (12–19), can be defined by their capability to induce cell death in various cell lines. These receptors share a common intracellular 80 amino acid domain, called the death domain, that is dispensable for initiation of the intracellular signaling cascade leading to cell death (3–18). The death domain motif is also found in the cytoplasmic adaptor proteins TNF-R1-associated death domain protein (TRADD; Ref. 20), Fas-associating protein with death domain (FADD; Refs. 21 and 22), receptor-interacting protein (RIP; Ref. 23), mitogen-activated protein kinase activator with death domain (MADD; Ref. 24), myeloid differentiation marker 88 (MyD88; Ref. 25), and RIP-associated ICH1/CED-3 homologous protein with death domain (RAIDD; Ref. 26). Most of these molecules are involved in the mediation of cell death by the beforementioned death domain-containing receptors. All of these adaptor molecules are of at least bipartite structure. Aside from the death domain mediating binding to other death domain-containing proteins, these adaptor molecules have additional domains enabling the interaction with, e.g. the prodomains of apoptotic caspasases (26–28) or members of the TNF receptor-associated factor (TRAF) family (29, 30), molecules involved in activation of NF-kB and JNK (31, 32). In particular, it has been shown that the lack of FADD in FADD−/− mice (33, 34) or overexpression of a FADD molecule lacking the amino-terminal death effector domain, which mediates association with the prodomain of caspase-8, interferes with TNF-, Apo1/Fas-, and Wsl/DR3-mediated apoptosis (6, 29, 35). This suggests that activation of the apoptotic caspase cascade by these death domain-containing receptors requires recruitment of the adaptor molecule FADD into the respective receptor signaling complexes. As shown in detail for Apo1/Fas, FADD in turn recruits caspase-8 to the death-inducing receptor signaling complex leading to proteolytical activation of this proximal caspase and to initiation of the apoptotic program (27, 28, 36, 37). Interestingly, all death domain-containing receptors are in principle able to activate the transcription factor NF-kB (5, 7, 8, 13, 14, 38–40), which is frequently associated with protection from apoptosis (41–45). In the case of TNF-R1, the activation of NF-kB occurs via recruitment of RIP and TRAF2 into the

---

*This work was supported by Deutsche Forschungsgemeinschaft Grant Wa 1025/3-1. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed. Tel.: 49-711-685-7446; Fax: 49-711-685-7484; E-mail: harald.wajant@po.uni-stuttgart.de.

‡This article is available online at http://www.jbc.org.

1 The abbreviations used are: JNK, c-Jun amino-terminal kinase; GFP, green fluorescent protein; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TRAIL-R, TRAIL receptor; TRADD, TNF-R1-associated death domain protein; FADD, Fas-associated protein with death domain; RIP, receptor-interacting protein; TRAP, TNF receptor-associated factor; RT-PCR, reverse transcription-polymerase chain reaction; MOPS, 4-morpholinepropanesulfonic acid; GST, glutathione S-transferase; FACS, fluorescence-activated cell sorter.
Activation of JNK by TRAIL

receptor signaling complex through association with TRADD and subsequent activation of the TRAF-associated kinase, NF-κB-inducing kinase. Subsequently, NF-κB-inducing kinase phosphorylates the IkB kinases α and β (IKKα and IKKβ), leading to activation of NF-κB (46, 47). In contrast, the molecular mechanisms involved in activation of NF-κB by other death domain-containing receptors remain to be elucidated. The most recently identified death domain-containing receptors DR4/TRAIL-R1 and DR5/TRAIL-R2/TRICK/KILLER are receptors for the cytotoxic ligand TRAIL/Apo2L (48, 49). Remarkably, TRAIL/Apo2L can also interact with two additional nonapoptotic members of the TNF receptor superfamily, the glycosphospholipid-anchored cell surface protein DcRI/TRID/TRAIL-R3 (12, 15, 18, 50) and DcR2/TRAIL-R4 (51–52), a receptor containing a truncated death domain that is still able to activate NF-κB (52). Overexpression of these nonapoptotic TRAIL receptors protects mammalian cells from TRAIL/Apo2L-induced cell death (12, 15, 18, 50–52), thereby defining a novel cell death control mechanism within the TNF receptor superfamily. In this report, we demonstrate the capability of TRAIL/Apo2L to induce JNK by a cysteine protease-dependent and by a cysteine protease-independent pathway. Moreover, using a dominant negative mutant of FADD as well as the cysteine protease inhibitor z-VAD-fmk, we are able to show that, in contrast to induction of apoptosis, the cysteine protease-dependent mode of JNK activation is FADD-independent. Thus, TRAIL/Apo2L has the capability to engage at least three different pathways: two independent pathways with distinct cysteine protease requirements leading to cell death or JNK activation and, in addition, a cysteine protease-independent pathway also linked to JNK activation.

EXPERIMENTAL PROCEDURES

Reagents and Cell Lines—The HeLa cells stably transfected with the green fluorescent protein (GFP) and a GFP-tagged dominant negative mutant of FADD, respectively, have been described elsewhere (53). For analysis of GFP a FACSStar+ (Becton and Dickinson, San Jose, Ca) has been used. Purified recombinant FLAG-tagged human TRAIL was used as complex with anti-FLAG M2 antibody (Kodak International Biotechnology, Hamburg, Germany). HeLa and Kym-1 cells were cultured in McCoy’s 5A medium supplemented with 5% (HeLa) or 10% (Kym-1) heat-inactivated fetal calf serum, 2 mM l-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin.

RNase Protection Assay and RT-PCR—Total RNA was isolated from HeLa and Kym-1 cells (10 × 10^6) with the RNA INSTAPUR kit (Eurogentech, Seraing, Belgium) according to the manufacturer’s recommendations. The presence of transcripts of caspase-8, FasL, FADD, DR3, TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL TNF-R1, TRADD, and RIP as well as the internal controls L32 and glyceraldehyde-3-phosphate dehydrogenase were analyzed using the hApo3c Multi-Probe template set (PharMingen, Hamburg, Germany). Probe synthesis, hybridization, and RNase treatment were performed with the RibonQuant Multi-Probe RNase Protection Assay System (PharMingen, Hamburg, Germany) according to the manufacturer’s recommendations. Finally, samples were resolved by electrophoresis on denaturing polyacrylamide gels (5%) and analyzed by phosphorimaging. First-strand cDNA synthesis was performed with the First Strand Synthesis Kit (Amersham Pharmacia Biotech, Freiburg, Germany) and oligo(dT) as a primer according to the manufacturer’s protocol. Aliquots of 1 μl of cDNA were used as template in a final volume of 50 μl in a standard High Fidelity PCR (Boehringer Mannheim, Mannheim, Germany) reaction. Samples were overlaid with 50 μl of mineral oil and amplified as followed: 1) station: 94 °C, 2 min; 2) cycle: 94 °C, 15 s; 50 °C, 30 s; 72 °C, 90 s; time increase 20 s per cycle; 10–20 cycles (as indicated). For the PCR reaction 0.25 μg of each of the following primers comprising the intracellular domain of TRAIL-R1, TRAIL-R2, and TRAIL-R4 as well as the extracellular domain of TRAIL-R3, respectively, was used: TRAIL-R1: F-NotI, 5′-CAG CAC CCA TGG GTG GTG GGG ACC AGT GCA TGG AC-3′; R-NotI, 5′-CAG GAC GCA GGC CCT TAC CAG TGG AGC AAG AAT CAC ACT TAG GAC ATG GC-3′; TRAIL-R3: F, 5′-GAG ATG CAA GAG GTG AGG AGG CGC TTC-3′; R, 5′-CCA CAG TGG AGT CTT TCA AAC AAA CAC-3′; TRAIL-R4: F- BspHI, 5′-CAG CAC TCA TGC GTC GGA AGA AAT CAG TCA TTT CTT ACC TCA AA-3′; R-NotI, 5′-GTT CGT CGC GCC GCT TCC TGT CTC AGG AGA AGA TTC TTC CAT AGG CA-3′. Please obey that the primers for TRAIL-R1 and TRAIL-R2, and the TRAIL-R4 contain the restriction sites NotI, BspHI, and NotI for cloning purposes not relevant for this study. Control reactions without or with 1 μg of genomic DNA as template were performed in each experiment.

Cell Death Assays—HeLa cells (1.5 × 10^6)/well were cultivated in 96-well microtiter plates overnight. Next day TRAIL-M2 complex was titrated and 2.5 μg/ml cycloheximide were added. After 18 h culture supernatants were discarded, and the cells were washed once with phosphate-buffered saline followed by crystal violet staining (20% methanol, 0.5% crystal violet) for 15 min. The wells were washed with H_2O and air-dried. The dye was resolved with methanol for 15 min, and optical density at 550 nm was determined with a R5000 enzyme-linked immunosorbent assay plate reader (Dynatech, Guernsey, Great Britain). Kym-1 cells (1 × 10^6)/well were cultivated in 96-well microtiter plates overnight. Next day TRAIL-M2 complex was titrated and after additional 18 h of culture metabolic activity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method.

Immune Complex JNK Assay—Following stimulation, cells (2–3 × 10^6) were lysed in 1 ml of kinase lysis buffer (200 mM Tris, pH 7.4, 5 mM MgCl_2, 1% Triton X-100, 150 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 2 mM Na_3VO_4, and 10 mM NaF) for 20 min on ice. Cell debris was removed by centrifugation at 10,000 × g for 10 min at 4 °C. JNK was immunoprecipitated with 0.5 μg of a rabbit polyclonal anti-JNK antisera (Santa Cruz Biotechnology) and 20 μl of protein A-Sepharose beads for 2 h. Beads were washed three times with kinase lysis buffer and twice in assay buffer (20 mM MOPS, pH 7.2, 10 mM EDTA, 10 mM MgCl_2, 0.1% Triton X-100, and 1 mM dithiothreitol). After the last wash, beads were left in a 1:1 suspension, and kinase reactions were carried out at room temperature for 20 min after addition of 0.5 μg GST-Jun(1–79) and ATP (100 μM ATP and 5 μCi of [γ-32P]ATP. Reactions were stopped by adding 25 μl of 6-fold concentrated Laemmli buffer and boiling for 5 min. Samples were resolved on SDS-polyacrylamide gel, transferred to nitrocellulose, and analyzed using a PhosphorImager.

RESULTS

TRAIL/Apo2L Activates JNK—It has been shown that JNK activity is induced upon cross-linking of non-death domain-containing receptors of the TNF receptor superfamily, e.g. CD40 (54), as well as by stimulation of the death domain-containing receptors TNF-R1 and Apo1/Fas (55–58). Accordingly, we were interested to know whether TRAIL/Apo2L is also able to activate this pathway. For this purpose we analyzed HeLa and Kym-1 cells, as both cell lines are susceptible toward the cytotoxic action of TRAIL and should therefore express at least one of the two death domain-containing receptors of TRAIL. To get first insights in the expression status of the various TRAIL receptors in these cells, we analyzed the transcription level by RNase protection assays and RT-PCR. Using a template set including TRAIL-R1, TRAIL-R2, and TRAIL-R3-specific probes we found significant level of TRAIL-R1 and TRAIL-R2 mRNA but no detectable amounts of TRAIL-R3 mRNA (Fig. 1A). Nevertheless, with RT-PCR transcriptions of all four TRAIL-R were detectable within a reasonable number of cycles in RNA from both cell lines (Fig. 1B). For transcription of JNK activation HeLa and Kym-1 cells were cultured for 0–5 h in the presence of an agonistic TRAIL-FLAG-M2 complex or TNF, and JNK activity was determined by an immunocomplex kinase assay using an amino-terminal (residues 1–79) c-Jun-GST fusion protein as a substrate (Fig. 2A). A low basal level of JNK activity was found in unstimulated cells, which was significantly increased by treatment with TRAIL-FLAG-M2 complex as well as TNF. However, whereas TNF induced the activation of JNK immediately and transiently for ~10–30 min, TRAIL-FLAG-M2 complex-induced activation of JNK could be discerned only after 2 h and reached a plateau after around 3–4 h of stimulation (Fig. 2A). Next, Apo1/Fas-
mediated JNK activation was investigated in HeLa cells. Because HeLa cells express only low levels of Apo1/Fas (data not shown), we analyzed a HeLa transfectant stably overexpressing this receptor. In these cells, cross-linking of Apo1/Fas using the agonistic antibody anti-Apo1 resulted in a significant activation of JNK with kinetics resembling that of TRAIL/Apo2L-induced JNK activation (Fig. 2A). Interestingly, TRAIL-FLAG-M2 complex induced apoptosis in Kym-1 but not in HeLa cells without further treatment. Nevertheless, HeLa cells became sensitive for TRAIL/Apo2L-induced cell death after addition of cycloheximide (Fig. 2B). In contrast, TRAIL-mediated JNK activation occurs in the absence of cycloheximide or other metabolic inhibitors in both cell lines. In the presence of cycloheximide TRAIL-mediated JNK activation remained unaffected, whereby the basal level of JNK activity was somewhat increased (data not shown). This suggests that, at least in HeLa cells, activation of JNK by TRAIL/Apo2L is not sufficient for induction of cell death. As expected, in both cell lines TRAIL/Apo2L-induced cell death could be inhibited by the addition of the caspase inhibitor z-VAD-fmk (Fig. 2B).

TRAIL/Apo-2L induces the JNK pathway and apoptosis. A, time course of JNK activation by members of the TNF receptor superfamily. Cell lysates were prepared from HeLa, HeLa-Fas, and Kym-1 cells, stimulated for the indicated times with TNF (5 ng/ml), TRAIL-FLAG-M2 complex (200 ng/ml), and anti-Apo1 antibody (50 ng/ml), respectively. JNK activity was measured by immunocomplex kinase assay with GST-c-Jun(1–79) as substrate. B, TRAIL/Apo-2L induces apoptosis. HeLa and Kym-1 cells were plated in triplicates overnight at 37 °C in 96-well microtiter plates (HeLa, 1.5 × 10⁴ cells/well; Kym-1, 1 × 10⁴ cells/well). Next day the cells were treated for additional 16 h with various concentrations of TRAIL-FLAG-M2 complex in the presence or absence of 20 μM z-VAD-fmk. In addition, HeLa cells were treated with 2.5 μg/ml cycloheximide. Viable HeLa cells were quantified by staining with crystal violet, and viable Kym-1 cells were quantitated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay.
caspases in the activation of JNK and the p38 mitogen-activated protein kinase has been clearly demonstrated (56, 57, 59). To unravel a possible regulatory role of caspases in activation of JNK by other members of the TNF receptor superfamily, we stimulated HeLa and Kym-1 cells with TRAIL-FLAG-M2 complex, anti-Apo1, or TNF in the absence or presence of z-VAD-fmk. Interestingly, aside from its inhibitory activity on the induction of apoptosis in both cell lines (Fig. 2B), z-VAD-fmk was able to completely block the activation of JNK in HeLa cells induced by TRAIL/Apo2L and anti-Apo1, but not TNF (Fig. 3, A and C). However, in Kym-1 cells, no inhibitory effect of z-VAD-fmk on JNK activation was observed (Fig. 3, B and C). These data clearly indicate that: (i) in HeLa cells, TRAIL/Apo2L and FasL on the one side and TNF on the other side utilize different pathways for activation of JNK. (ii) TRAIL/Apo2L-induced JNK activation occurs in a caspase-dependent manner via two distinct pathways, of which one is cysteine protease-dependent and one cysteine protease-independent. The dose dependence of the inhibitory effect of z-VAD-fmk on JNK activation and induction of cell death by TRAIL is nearly identical (data not shown). As the latter is mediated by the caspase subgroup of the cysteine protease family, this argues also for an involvement of caspases in TRAIL-mediated JNK activation in HeLa cells.

**TRAIL/Apo2L- and Apo1/Fas-mediated Cell Death Is Prevented by Overexpression of GFP-FADD, but JNK Activation Still Occurs in a Caspase-dependent Manner**—From the data described so far it was unclear whether TRAIL- and Apo1/ Fas-mediated JNK activation in HeLa cells occurs via the apoptotic caspase cascade or via a distinct pathway with other cysteine protease requirements. As mentioned above, several recent reports have demonstrated that overexpression of a dominant negative mutant of FADD, which lacks the amino-terminal death effector domain and is thus unable to recruit caspase-8 to receptor signaling complexes, efficiently abrogates apoptotic signaling induced by death domain-containing receptors (13, 14, 16, 29, 35, 53). We therefore analyzed JNK activation in HeLa cells stably transfected with a GFP-tagged
version of the dominant negative mutant of FADD, GFPΔFADD (Fig. 4A). These cells are completely resistant against TRAIL/Apo2L-induced cell death but are still able to activate JNK via a caspase-dependent pathway. A, GFP and GFPΔFADD expression was analyzed in stable HeLa transfectants by FACS analysis and immunoblotting. FACS analysis was performed with living cells without further treatment. For immunoblotting GFP and GFPΔFADD were immunoprecipitated from cytosolic extracts of 1.5 × 10^6 cells using a rabbit polyclonal IgG fraction against GFP, transferred to nitrocellulose, and detected with a GFP-specific monoclonal antibody and an alkaline phosphatase-conjugated secondary antibody. B, HeLa-GFPΔFADD cells are resistant toward TRAIL/Apo2L-induced cell death. 1.5 × 10^6 cells/well were cultured in a 96-well microtiter plate overnight. The cells were then incubated for additional 16 h with the indicated concentrations of TRAIL-FLAG-M2 complex in the presence of 2.5 μg/ml cycloheximide. Viable cells were quantified after staining with crystal violet.

C, HeLa-GFPΔFADD cells were treated with the indicated concentrations of TRAIL-FLAG-M2 complex alone or in combination with z-VAD-fmk (20 μM) for 4 h. JNK activity was measured in an immunocomplex kinase assay using GST-c-Jun(1–79) as a substrate. D, GFPΔFADD prevents activation of caspase-3 and caspase-8. 2 × 10^6 HeLa, HeLa-GFP, and HeLa-GFPΔFADD cells were treated with TRAIL-FLAG-M2 complex (200 ng/ml) for 5 h in the presence or absence of cycloheximide (1 μg/ml). Protein lysates and immunoblotting of proteins were performed as described under “Experimental Procedures.” Blots were probed with a polyclonal IgG fraction directed against caspase-3 or a monoclonal caspase-8-specific antibody.

**DISCUSSION**

Stimulation of the TNF receptor superfamily members TNF-R1 (55), CD40 (54), HVEM/ATAR (60), Apo1/Fas (56–58), the TRANCE receptor RANK (61), as well as triggering of the interleukin-1R leads to activation of the stress activated kinase JNK. Transient expression experiments with members of the TRAF family, and dominant negative mutants derived thereof,
as well as studies with transgenic mice (62, 63), have suggested that TRAF molecules are indispensable for JNK activation by these receptors. Interestingly, the TNF receptor superfamily member Apo1/Fas, representing the prototype of a death-inducing receptor, does not associate with TRAFs, but is nevertheless able to induce JNK activation (56–58, 64–66). Both receptors, TNF-R1, which uses TRAF2 for signaling, and Apo1/Fas, which signals TRAF independently, belong to a death-inducing subgroup of the TNF receptor superfamily that is defined by a common intracellular domain, called the death domain. It was therefore of interest to define whether TRAIL/Apo2L, which binds to two additional members of this receptor subgroup, is also able to activate JNK and, if yes, whether TRAIL/Apo2L-induced JNK activation resembles the TNF-R1 or the Apo1/Fas pathway. HeLa and Kym-1 cells are highly susceptible toward the cytotoxic action of a TRAIL-FLAG-M2 complex (Fig. 2B) and express all four TRAIL receptors, in particular the two recently cloned death domain-containing receptors for TRAIL/Apo2L (Fig. 1). In addition, cross-linking of the TRAIL receptor(s) in these cell lines resulted in a stimulation of JNK (Fig. 2A). TRAIL-induced JNK activation could be detected 1–2 h after stimulation, reaching a plateau after 3–4 h. Hence, the time course of JNK activation was clearly different from the rapid and transient JNK stimulation by TNF (Fig. 2A), but resembled the sustained JNK activation found after stimulation of Apo1/Fas (Fig. 2A). It has been reported that both the overexpression of the serpin family protease inhibitor CrmA and treatment with the cysteine protease inhibitor z-VAD-fmk can interfere with Apo1/Fas-mediated p38 and JNK activation (56, 57, 59, 66). We therefore analyzed the effect of z-VAD-fmk on TRAIL/Apo2L-induced activation of JNK. TRAIL/Apo2L- and anti-Apo1-, but not TNF-induced, JNK activation was blocked by treatment with z-VAD-fmk (20 μM) for 4 h. JNK activity was measured in an immunocomplex kinase assay using GST-c-Jun(1–79) as a substrate.
transient transfection assays in 293 cells. In the case of TNF, the induction of cell death via the caspase cascade and the TRAF2-dependent activation of JNK by TNF-R1 bifurcate at the level of the receptor-associated protein TRADD (43). Hence, it is apparent that JNK activation is dispensable for TNF-induced cell death. However, several reports point to a critical role of JNK activation for induction of apoptosis by other stimuli (e.g. Refs. 67–69). The question therefore arose whether JNK activation has an essential function for TRAIL/Apo2L-induced cell death in the cellular system studied here. Two recent studies have described the generation of FADD--/ mice (33–34). In one of these studies it has been shown in embryonic fibroblasts that the lack of FADD interferes with TNF-R1, Apo1/Fas, and DR3, but not with TRAIL-R1-induced apoptosis (33). This argues for a FADD-independent mechanism of TRAIL-R1-induced apoptosis; however, it is still unclear whether FADD is involved in TRAIL-R2-induced apoptosis as suggested by some studies based on transient transfection assays. We have shown recently that HeLa cells expressing a dominant negative GFPΔFADD fusion protein are completely resistant toward induction of apoptosis by TNF, agonistic Apo1/Fas-specific antibodies, and TRAIL (53). This indicates that FADD itself or a FADD-related factor is involved in TRAIL-induced apoptosis in these cells. More important for this study, TRAIL-mediated processing of the apoptotic caspase-8 and caspase-3 (Fig. 4D) is blocked by overexpression of GFPΔFADD. As JNK activation by TRAIL/Apo2L is not impaired in the GFPΔFADD-expressing cells, but is still z-VAD-fmk-sensitive (Fig. 4C), the caspase protease(s) involved in this process must be distinct from caspase-3 and caspase-8. Moreover, in HeLa cells, TRAIL-induced processing of caspase-3 and caspase-8, and therefore induction of cell death, only occur in the presence of cycloheximide, whereas JNK activation takes place without further treatment. Together, these data suggest (i) that a FADD-dependent as well as a FADD-independent pathway of caspase protease activation exist and (ii) that both pathways activate different subsets of caspase protease, one not sufficient to induce apoptosis but essential for JNK activation and one dispensable for JNK activation but involved in apoptosis (Fig. 6). Transcripts of all known TRAIL receptors containing an intracellular domain (TRAIL-R1/DR4, TRAIL-R2/DR5, TRAIL-R4) have been easily detected in HeLa and Kym-1 cells using RNase protection assays and/or RT-PCR (Fig. 1). It is therefore rather unlikely that the caspase protease-dependent and cysteine protease-independent modes of JNK activation in HeLa and Kym-1 cells reflect the utilization of distinct TRAIL receptors. Moreover, the similarities to the Apo1/Apo1 system rather point to distinct cellular pathways involved in JNK activation, because triggering of Apo1/Fas, the only known receptor for Apo1L, also results in cell type-specific, caspase-dependent and caspase-independent activation of JNK. At least in HeLa cells, TRAIL-R and Apo1/Fas-mediated JNK activation is independent of FADD. How this pathway is molecularly linked to the respective receptors remains to be elucidated. Two possible candidates are the recently cloned molecules RAIDD and DAXX, proteins that are involved in TNF-R1 and Apo1/Fas signaling. However, overexpression of dominant negative forms of these molecules in HeLa cells exerted no effect on apoptosis or JNK activation initiated by TRAIL/Apo2L (data not shown).

Acknowledgments—We thank Ingolf Berberich, University of Würzburg, Germany, for GST-Jun and Marcus Peter, Heidelberg, Deutsches Krebsforschungszentrum, Germany, for anti-Apo1 antibody.

REFERENCES

1. Armitage, R. J. (1994) Curr. Opin. Immunol. 6, 407–413
2. Smith, C. A., Farrah, T., and Goodwin, R. G. (1994) Cell 76, 959–962
3. Tartaglia, L. A., Ayres, T. M., Wong, G. H., and Goeddel, D. V. (1993) Cell 74, 845–853
4. Itoh, N., and Nagata, S. (1993) J. Biol. Chem. 268, 10932–10937
5. Korneluk, R. G., Jiang, Y., Goeddel, D. V., Giles, M. K., Pan, K. T., Grinham, C. J., Brown, R., and Farrow, S. N. (1996) Nature 384, 372–375
6. Chinnaianay, A. M., O’Rourke, K., Yu, G. L., Lyons, R. H., Garg, M., Duan, D. R., Xiong, L., Gentz, R., Ni, J., and Dixit, V. M. (1996) Science 274, 950–952
7. Marsters, S. A., Sheridan, J. P., Donahue, C. J., Pitti, R. M., Gray, C. L., Goddard, A. D., Bauer, K. D., and Ashkenazi, A. (1996) Curr. Biol. 6, 1669–1676
8. Bodmer, J. L., Burns, K., Schneider, P., Hofmann, K., Steiner, V., Thomé, M., Bornand, T., Hahne, M., Schroter, M., Becker, K., Wilson, A., French, L. E., Browning, J. L., MacDonald, H. R., and Tschopp, J. (1997) Immunity 6, 79–86
9. Screeton, G. R., Xu, X. N., Olsen, A. L., Cowper, A. E., Tan, R., McMichael, A. J., and Bell, J. I. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4615–4619
10. Brojatsch, J., Naughton, J., Rolls, M. M., Zingler, K., and Young, J. A. (1996) Cell 87, 845–855
11. Pan, G., O’Rourke, K., Chinnaianay, A. M., Gentz, R., Ehner, R., Ni, J., and Dixit, V. M. (1997) Science 276, 111–113
12. Pan, G., Ni, J., Wei, Y.-F., Yu, G.-L., Gentz, R., and Dixit, V. M. (1997) Science 277, 815–818
13. Chaudhary, P. M., Pabst, M., Masim, A., Bokoch, G., and Hood, L. (1997) Immunity 7, 821–839
14. Schneider, P., Thomé, M., Burns, K., Bodmer, J.-L., Hofmann, K., Kataoka, T., Holler, N., and Tschopp, J. (1997) Immunity 7, 831–836
15. Muzio, M., Ahmad, M., Srinivasula, S. M., Ferrando-Alvarez, T., Cohen, G. M., and Alnemri, E. S. (1997) J. Biol. Chem. 272, 25417–25420
16. Wakabayashi, H., Degli-Esposti, M. A., Johnson, R. S., Smolak, P. J., Waugh, J. Y., Siboni, N., TIMOUR, M., Garhart, M. J., Schooley, K. A., Smith, C. A., Goodwin, R. G., and Rauch, C. T. (1997) EMBO J. 16, 5386–5397
17. Screeton, G. R., Mongkolpapaya, J., Xu, X.-N., Cowper, A. E., McMichael, A. J., and Bell, J. I. (1997) Curr. Biol. 7, 693–696
18. Sherer, J. P., Marsters, S. A., Pitti, R. M., Gurney, A., Skubatch, M., Baldwin, D., Ramakrishnan, L., Gray, C. L., Baker, K., Wood, W. L., Goddard, A. D., Godowski, P., and Ashkenazi, A. (1997) Science 277, 108–121
19. Wu, G. S., Burns, T. D., McDonald, E. R., III, Jiang, W., Meng, R., Krantz, I. D., Kao, G., Han, D. D., Zhou, J. Y., Muschel, R., Hamilton, S. R., Spinner, N. B., Markowitz, S. W., Wu, G., and el-Deiry, W. S. (1995) Nat. Genet. 16, 85–89
20. Hsu, H., Xiong, J., and Goeddel, D. V. (1995) Cell 81, 495–504
21. Chinnaianay, A. M., O’Rourke, K., Tewari, M., and Dixit, V. M. (1995) Cell 81, 818–821
22. Boldin, M. P., Varfolomeev, E. E., Pancer, Z., Mert, I., Camonis, J. H., and Wallach, D. (1995) J. Biol. Chem. 270, 7795–7798
23. Stanger, B. Z., Leder, P., Lee, T. H., Kim, E., and Seed, B. (1995) Cell 81, 513–523
24. Schievella, A. R., Chen, J. H., Graham, J. R., and Lin, L. L. (1997) J. Biol. Chem. 272, 12069–12075
25. Muzio, M., Ni, J., Feng, P., and Dixit, V. M. (1997) Science 278, 1612–1615
26. Duan, D. R., and Dixit, V. M. (1997) Nature 385, 86–89
27. Muzio, M., Chinnaianay, A. M., Kischkel, F. C., O’Rourke, K., Shcherwenko, A., Ni, J., Scaffidi, C., Brez, J. D., Zhang, M., Gentz, R., Mann, M., Kramer, P. H., Eberhard, M., and Dixit, V. M. (1996) Cell 85, 817–827
28. Boldin, M. P., Goncharov, T. M., Goltsvev, Y. V., and Wallach, D. (1996) Cell 83, 803–815
29. Hsu, H., Shu, H. B., Han, G., and Goeddel, D. V. (1996) Cell 84, 299–308
30. Hsu, H., Huang, J. N., Shu, H. B., Baichwal, V., and Goeddel, D. V. (1996) Immunity 4, 387–396
31. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995) Science 269,
TRAIL/Apo2L Activates c-Jun NH$_2$-terminal Kinase (JNK) via Caspase-dependent and Caspase-independent Pathways
Frank Mühlenbeck, Elvira Haas, Ralph Schwenzer, Gisela Schubert, Matthias Grell, Craig Smith, Peter Scheurich and Harald Wajant

J. Biol. Chem. 1998, 273:33091-33098.
doi: 10.1074/jbc.273.49.33091

Access the most updated version of this article at http://www.jbc.org/content/273/49/33091

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 69 references, 34 of which can be accessed free at http://www.jbc.org/content/273/49/33091.full.html#ref-list-1