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Intracellular Regulation of Fas-Induced Apoptosis in Human Fibroblasts by Extracellular Factors and Cycloheximide 1

Begoña Santiago,* María Galindo,† Guillermo Palao,* and José L. Pablos2†

Fibroblasts play an important role in reparative and inflammatory processes by synthesizing extracellular matrix components and releasing growth factors and cytokines. Fibroblast apoptosis has been observed at the termination phase of reparative or fibrotic responses, but its regulation in this context is poorly known. We investigated the susceptibility of human dermal fibroblasts (DF) to Fas-induced apoptosis and its regulation by extracellular factors potentially involved in immune-mediated inflammation and repair. DF expressed all components of the Fas apoptotic pathway: surface Fas, Fas-associated protein with death domain, and caspase-8 proteins. However, Fas activation resulted in caspase-8 activation and apoptosis only in the presence of cycloheximide (CHX). DF constitutively expressed Fas-associated death domain-like IL-1-converting enzyme-like inhibitory protein (FLIP) that was drastically down-regulated by CHX. Exogenous growth factors, cytokines, and adherence to the extracellular matrix shifted the balance of FLIP-caspase-8 proteins and modified the susceptibility of DF to Fas or Fas-CHX-induced apoptosis. Short-term serum deprivation, suspension culture, and pretreatment with IFN-γ or TNF-α increased, whereas long-term serum-free culture and pretreatment with TGF-β or IL-10 decreased the apoptotic susceptibility of DF. Surface Fas expression was only modified by TNF-α and IFN-γ, whereas all studied factors modified FLIP-caspase-8 protein expression, consistently with their pro- or anti-apoptotic effects. Antisense FLIP oligonucleotides prevented resistance to Fas-induced apoptosis in DF. FLIP-caspase-8 balance seems tightly regulated in fibroblasts by extracellular factors that determine their susceptibility to Fas- or Fas-CHX-induced apoptosis. Th1 and Th regulatory cytokines display opposite effects on fibroblast apoptosis that suggest that their pro- or anti-fibrotic effects involve direct effects on fibroblast survival. The Journal of Immunology, 2004, 172: 560–566.

1 Abbreviations used in this paper: ECM, extracellular matrix; CHX, cycloheximide; DAPI, 4,6-diamidino-2-phenylindole; DF, dermal fibroblast; FADD, Fas-associated protein with death domain; FasL, Fas ligand; FLIP, Fas-associated death domain-like IL-1-converting enzyme-like inhibitory protein; FLIPL, long splice form of FLIP; polyHEMA, polyhydroxyethylmetacrylate. 

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thus suppressing apoptosis (21, 22). Although FLIP may also display caspase-8-like proapoptotic activities, in cell types constitutively expressing high levels of FLIP, it functions as an antiapoptotic factor (23). Consistently, embryo fibroblasts from FLIP knockout mice exhibit increased susceptibility to Fas-mediated apoptosis, suggesting an important role for this protein in preventing fibroblast apoptosis (24).

Human adult DF express Fas receptor, and Fas ligand is expressed by nearby resident and infiltrating cells in inflamed skin (25). However, in human DF, activation of Fas receptor triggers responses such as cell proliferation, protein kinases, and NF-kB activation instead of cell death, suggesting that Fas receptor is functional, but also the presence of intracellular inhibitors of apoptosis (26–28). FLIP is a rational candidate, because in many other cell types, down-regulation of FLIP protein correlates with enhanced susceptibility to death receptor-induced apoptosis (17, 20, 29). We have studied the expression and regulation by extracellular factors of Fas receptor, caspase-8, and FLIP proteins as key components of Fas apoptotic signaling in human DF and their potential participation in the regulation of Fas-induced apoptosis. We demonstrate that FLIP down-regulation by cycloheximide (CHX) or antisense oligonucleotides sensitizes fibroblasts to Fas-induced cell death. Exposure of fibroblasts to different factors potentially involved in inflammatory and fibrotic processes such as cytokines, growth factors, or matrix interactions could modulate the susceptibility of these cells to Fas-induced apoptosis by shifting FLIP-caspase-8 balance, suggesting a critical role for these proteins in determining the fate of activated fibroblasts in reparative or immune-mediated fibrotic responses.

Materials and Methods

Induction of apoptosis in fibroblast cultures

DF were cultured by explant growth from healthy adult skin obtained during minor cosmetic surgery. Cells were cultured in 10% FCS-DMEM on plastic flasks. Fibroblasts from five individuals were used between passages 3 and 10. Fas stimulation was performed by treating cells with anti-Fas IgM mAb (clone CH11; MBL, Nagoya, Japan) at 1 μg/ml for 24 h. Controls for the anti-Fas mAb apoptotic activity were performed in PHA-activated PBMC obtained from healthy blood donors. Where indicated, 5 μg/ml CHX was simultaneously added at the same time as anti-Fas treatment. Serum deprivation was performed by extensive washing of confluent cultures that were thereafter maintained in serum-free DMEM. Suspension cultures were performed by plating cells on 10% polyhydroxyethylmetacrylate (polyHEMA)-coated dishes, and maintained under 10% FCS conditions. The effect of soluble factors on the susceptibility to Fas-induced apoptosis was studied by pretreating cells with 50 U/ml human TNF-α (Genzyme, Cambridge, MA), 10 ng/ml human TGF-β1 (R&D Systems, Abingdon, U.K.), 100 U/ml IFN-γ, or 40 ng/ml IL-10 (PreproTech EC, London, U.K.) for 24 h before washing and adding the anti-Fas mAb.

Cell death and apoptosis assays

Cell death induced by exposure to anti-Fas mAb for 24 h was quantified by direct counting of live and dead cells in Neubauer chambers, after staining with 0.2% trypan blue. All dead cells were quantified after recovery of both trypsinized adherent cells and previously detached cells floating in the medium by centrifugation. To confirm that cell death was due to apoptosis, fibroblasts were grown on coverslips under identical conditions, fixed with 4% paraformaldehyde, and labeled by TUNEL, as previously described (4). Briefly, sections were permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature, and labeled with 30 mM Trizma base, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride, and 1 mM fluorescein-dUTP, containing 0.3 U/ml TdT (Roche Diagnostics, Mannheim, Germany) for 30 min at 37°C in humid chamber. The reaction was terminated in 300 mM sodium chloride, 30 mM sodium citrate buffer for 30 min at room temperature. Control sections without TdT were included. Nuclei were simultaneously labeled with 4,6-diamidino-2-phenylindole (DAPI). Coverslips were observed under a fluorescence microscope.

Western blot analysis

Protein from 10⁶ fibroblasts was extracted in ice-cold lysis buffer (10 mM Tris-HEPES, pH 8, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 μg/ml pepstatin A, and 0.5 mM PMSF). Protein extracts (50 μg) were electrophoresed on 10% polyacrylamide gel and electrophoretically transferred to nitrocellulose filters. After blocking 2 h with 5% nonfat dried milk in TBST, the membranes were incubated overnight at 4°C with anti-FLIP (Stressgen, Victoria, British Columbia, Canada), anti-FADD (Transduction Laboratories, Lexington, KY), anti-caspase-8 (MLB), or anti-β-actin (clone AC-15; Sigma-Aldrich, Quimica, Spain) Abs in 5% nonfat dried milk-TBST. The filters were washed and incubated for 1 h with secondary Abs linked to peroxidase at 1/1500 dilution. Bands were visualized by an ECL system (Pierce, Rockford, IL) and analyzed by densitometry.

Flow cytometry analysis of surface Fas expression

Flow cytometry analysis of surface Fas receptor (CD95) expression was performed on scraped fibroblasts resuspended in PBS. Cells were incubated for 20 min at 4°C with a FITC-labeled mouse anti-human CD95/Fas mAb (clones DX-2 and DX-3; BioSource International, Camarillo, CA) or isotype-match control FITC-labeled mouse IgG. Cells were then washed and fixed in 1% paraformaldehyde. Flow cytometric analysis was performed using an EPICS Elite flow cytometer (Coulter, Miami, FL). Results were expressed as mean of the logarithm of fluorescence intensity.

Antisense oligonucleotide transfection

Phosphorothioate-modified single-stranded oligonucleotides directed against the human FASL translation initiation codon (5′-ACTCAGCA GACATCCTAC-3′), and control nonsense phosphorothioate oligodeoxynucleotides (5′-TGGATCCGACATGTCAGA-3′) were synthesized, as previously described, by Perlmutter et al. (18). Human fibroblast cells grown to ~70% confluency were incubated with 200 nM oligonucleotides preincubated with Oligofectamine (Life Technologies, Gaithersburg, MD). Eighteen hours later, the oligonucleotide solution was replaced by normal growth medium and treated with anti-Fas for 24 h. Parallel cultures were assayed for protein expression, cell death, and TUNEL.

Statistical analysis

Comparison between mean values of cytotoxicity, densitometric data of protein levels, and mean fluorescence intensity was performed by Student’s i test or Mann-Whitney U test, where appropriate, and statistical significance was indicated where p values were <0.05. Results are expressed as mean ± SD.

Results

Fas-induced cell death in DF

Fas stimulation with 1 μg/ml anti-Fas mAb did not induce cell death in subconfluent or confluent DF lines cultured under 10% FCS conditions and examined at different time points up to 24 h. The activity of the anti-Fas mAb used was confirmed on PHA-activated PBMC, in which, at the same concentration, it induced the death of 48 ± 6% cells at 24 h, as expected. Treatment with 5 μg/ml CHX alone did not induce fibroblast cytotoxicity, but significantly increased the susceptibility of fibroblasts to anti-Fas-induced cell death at 24 h (Fig. 1a). The response to anti-Fas + CHX was highly variable between different DF lines, but a significant increase in cell death was observed in all studied lines (Fig. 1a). Serial passage of DF cultures did not significantly modify the apoptotic susceptibility of the different lines to Fas + CHX between passages 3 and 10 (data not shown).

To confirm that Fas-induced cytotoxicity was due to apoptosis, fibroblasts were grown on coverslips and labeled by TUNEL after the different stimuli. Low levels of morphologically apoptotic and TUNEL-positive nuclei were observed in untreated, anti-Fas-, or CHX-treated cells, whereas in anti-Fas + CHX-treated fibroblasts a significant increase in the proportion of apoptotic nuclei was observed (Fig. 2).

To examine the effect of growth factor deprivation on the susceptibility to Fas-induced apoptosis, we maintained confluent fibroblast cultures under serum-free conditions up to 10 days and...
stimulated with anti-Fas at 48 h or 10 days. Lines DF1, DF4, and DF5 were selected for these experiments because they displayed intermediate levels of apoptosis upon anti-Fas + CHX treatment, allowing for a better detection of the potential changes induced by serum deprivation. Serum deprivation induced a progressive increase in the number of dead fibroblasts, but most cells (>70%) remained viable, allowing us to study the effect of Fas stimulation under these conditions. At 48 h of serum deprivation, anti-Fas alone induced a significant cytotoxicity and, in the presence of CHX, it was significantly increased when compared with cultures on 10% FCS (Fig. 1b). In contrast, after 10 days of serum deprivation, fibroblasts became resistant to either anti-Fas or anti-Fas + CHX-induced cell death (Fig. 1b). TUNEL labeling of serum-deprived and anti-Fas-treated cells demonstrated concordant variations in the proportion of apoptotic cells (data not shown).

Attachment of fibroblasts to the extracellular matrix provides a survival stimulus that is lost when cells are cultured under suspension in polyHEMA-coated dishes. All fibroblast lines (DF1–5) showed a significant increase in the number of apoptotic fibroblasts under suspension culture conditions, such that at 24 h very few cells remained viable. To study the influence of suspension culture on the susceptibility to anti-Fas, we examined suspension cultures at shorter times. At 6 h, either anti-Fas alone or anti Fas + CHX induced significantly increased cytotoxicity in suspension cultures compared with plastic-attached cultures (Fig. 1c).

Because in preliminary experiments we observed that TGF-β or IL-10 pretreatment decreased, and TNF-α or IFN-γ pretreatment increased Fas + CHX cytotoxicity, to better quantify these effects, we selected cell lines displaying lower rates of Fas-induced cytotoxicity (DF1, DF4, and DF5) for further studies with TNF-α and IFN-γ, and those with higher rates (DF1, DF2, and DF3) for further studies with TGF-β and IL-10. Pretreatment with TNF-α or IFN-γ induced a significant increase in the susceptibility to anti-Fas + CHX (Fig. 3a), whereas TGF-β and IL-10 had the opposite effect, significantly decreasing anti-Fas + CHX cytotoxicity (Fig. 3b). To determine whether the Fas-sensitizing effect of TNF-α and IFN-γ also occurs in the absence of CHX, we tested the effect of higher concentrations of these cytokines. A smaller, but dose-dependent increase in anti-Fas-induced cytotoxicity was observed in the absence of CHX at higher concentrations of both cytokines (Fig. 3c).

Expression of Fas pathway proteins by DF

Cell surface Fas receptor was present at similar level in all DF lines by flow cytometric analysis. Because enforced expression of surface Fas receptor has been demonstrated to abrogate Fas resistance in DF (27), we studied whether pretreatment of fibroblasts with cytokines, suspension culture, or serum deprivation modified surface Fas expression. Mean surface Fas expression was increased by TNF-α or IFN-γ treatment in all fibroblast lines, whereas serum deprivation, suspension culture, TGF-β, and IL-10 did not induce significant changes (Fig. 4).

FIGURE 1. Susceptibility of DF to Fas-induced cell death. a, Cytotoxicity induced by Fas activation for 24 h in five DF lines (DF1–5) from five different individuals in the presence or absence of CHX compared with unstimulated cells (Unst.). b, Effect of serum deprivation on susceptibility to Fas-induced cell death. Subconfluent cells were cultured in DMEM or in the absence of FCS for 48 h or 10 days, and thereafter stimulated for 24 h with anti-Fas mAb. Mean ± SD are indicated. Results are representative of triplicate cultures of each of the DF lines. c, Effect of Fas activation for 6 h in fibroblasts cultured under suspension conditions on polyHEMA-coated dishes (Susp.) or plastic-attached cultures (Plastic). Results are representative of triplicate cultures of all DF lines.

FIGURE 2. Detection of apoptosis in Fas-activated DF. Fibroblasts were grown on glass coverslips and stimulated with Fas mAb for 24 h in the presence or absence of CHX. TUNEL and DAPI fluorescent labeling were directly performed on fixed coverslips. The same field was sequentially photographed under appropriate DAPI (left) or fluorescein (right) fluorescent light filters. Results are representative of three independent experiments performed with all DF lines.
intracellular mechanisms should operate to explain the modulation of the response to Fas activation by these factors.

Western blot analysis confirmed the expression of FADD and caspase-8 in protein extracts of DF (Fig. 5a). Caspase-8 activation in response to anti-Fas + CHX was also detected in apoptotic fibroblasts as a reduction in the amount of 54/55-kDa procaspase-8 and the appearance of a cleaved product of 43/41 kDa (Fig. 5b). Therefore, DF display a functional Fas-caspase-8 cell death pathway that is inhibited under normal circumstances by a CHX-sensitive factor. Constitutive expression of the long splice form (FLIPL) protein, but not the short form, was confirmed in protein extracts from fibroblasts, and after treatment with CHX, FLIP protein became undetectable, whereas expression of FADD and caspase-8 was not modified (Fig. 5a). To evaluate the potential influence of FLIP-caspase-8 balance on the observed protection or sensitization to Fas-induced apoptosis by the different stimuli, we studied the relative levels of FLIP and caspase-8 expression by Western blot in DF under such stimuli. Proapoptotic stimuli, such as IFN-γ, serum deprivation, and suspension culture, decreased FLIP levels, and, in the case of IFN-γ, they also increased caspase-8 levels (Fig. 6). TNF-α increased both FLIP and caspase-8 expression levels, with a stronger effect on caspase-8. In contrast, both cytokines with antiapoptotic effect, TGF-β and IL-10, coordinately up-regulated FLIP and down-regulated caspase-8 expression (Fig. 6).

**Discussion**

Systemic activation of Fas receptor induces apoptosis in vivo in a limited range of cell types that includes hepatocytes, lymphoid, endothelial, and glomerular cells (30–32). Apoptosis in response...
to local Fas activation in vivo has also been demonstrated in epithelial cells, but not in connective tissue fibroblasts (33). However, fibroblasts display surface Fas receptors, and a role for Fas/Fas ligand (FasL) interactions in fibroblast apoptosis under inflammatory conditions has been supported by different studies. Fas-FasL interactions seem to participate in the resolution of liver myofibroblast expansion after acute injury (34). Furthermore, local transfer of FasL to inflamed rheumatoid synovium is followed by extensive apoptosis of synovial fibroblasts (35). In vitro, human fibroblasts display surface Fas and activate nonapoptotic pathways in response to Fas activation, but are highly resistant to Fas-mediated cell death (26–28). Enforced expression of Fas receptor abrogates this resistance, but it is not clear whether this mechanism is physiologically relevant (27). Therefore, the participation of Fas in the elimination of fibroblasts observed in pathological inflammatory or reparative responses could only be explained if the susceptibility to Fas-mediated apoptosis of fibroblasts is modulated by extracellular factors associated with these processes.

The role of intracellular regulation of death receptor-mediated apoptosis by recruitment and homo- or hetero-oligomerization of proteins at the death-inducing signaling complex has been extensively studied, but its precise role in pathology is still a matter of debate. The roles of FADD and caspase-8 in activating a proapoptotic cascade are clearly defined; however, the interference of FLIP isoforms on this pathway remains controversial. FLIP was described as a dominant inhibitor of Fas-induced apoptotic, but not nonapoptotic responses, the latter being enhanced by enforced FLIP expression (36, 37). Recent data propose a dual function of FLIP as a pro- or antiapoptotic protein, depending on the relative expression levels in different cell types (23, 38). In different normal or tumoral cell types, down-regulation of FLIPL abrogates Fas resistance (18, 39–41). In fibroblasts, high constitutive levels of FLIP, suggest that it operates as an apoptosis inhibitor, and consistently, embryonic fibroblasts from FLIP-deficient mice display higher apoptotic responses to Fas (24). In this study, we demonstrate that constitutive expression of FLIP by human fibroblasts under basal conditions is a major factor of resistance to Fas-induced apoptosis, and its down-regulation by CHX or antisense oligonucleotides abrogates this resistance. Furthermore, exposure to different factors with a defined participation in tissue injury and immune mediated fibrotic responses shifted the normal FLIP-caspase-8 balance and modulated the susceptibility of these cells to

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**FIGURE 5.** Expression of Fas-apoptotic pathway proteins by DF. a, Protein extracts from fibroblasts cultured with or without 5 μg/ml CHX for 6 h were analyzed by Western blot with anti-caspase-8 (CASP-8)-, anti-FADD-, anti-FLIP-, or anti-β-actin-specific Abs. b, Protein extracts from fibroblasts stimulated with anti-Fas or anti-Fas + CHX for 24 h were analyzed by Western blot with anti-caspase-8-specific Ab. Results are representative of three independent experiments, including all DF lines.

**FIGURE 6.** Expression of FLIP and caspase-8 protein DF pretreated with cytokines, serum deprivation, or cultured on suspension. Protein extracts were analyzed by Western blot. a, Effect of pretreatment with TNF-α, IFN-γ, TGF-β, or IL-10 for 24 h. b, Densitometric analysis of FLIP and caspase-8 expression levels normalized to β-actin. The ratio between the level of each protein in treated and untreated cells is shown. c, Effect of serum deprivation for 48 h or 10 days, and suspension culture on FLIP and caspase-8 protein expression. Results are representative of three independent experiments, including all DF lines.

**FIGURE 7.** Susceptibility to cell death of DF treated with FLIP antisense oligonucleotides. a, Western blot analysis of FLIP protein from FLIP antisense (AS)- and control nonsense (NS)-transfected DF. b, Cytotoxicity of Fas in DF treated with FLIP antisense or nonsense oligonucleotides, or CHX. c, Percentage of Fas-induced apoptosis, as quantified by TUNEL in DF cultures treated with FLIP antisense or nonsense oligonucleotides, or CHX. Results are representative of three independent experiments in three different DF lines.
FIGURE 8. Schematic diagram of the mechanisms that regulate Fas-induced apoptosis in DF and their modulation by CHX or extracellular factors. Caspase-8 activation and cleavage upon Fas activation are counterbalanced by FLIP protein, which is constitutively expressed in DF and restraints apoptosis. CHX, TNF-α, and IFN-γ up-regulate apoptosis by modulating FLIP, caspase-8, and surface Fas expression. Adherence to the ECM, growth factors (GF), TGF-β, and IL-10 down-regulate apoptosis by up-regulating FLIP expression. TNF-α induces effects of opposite sign by up-regulating FLIP, caspase-8, and surface Fas expression, but its net effect is proapoptotic in DF.

Fas-mediated apoptosis. We observed that growth factors, cytokines, and matrix interactions, which activate widely heterogeneous signaling pathways, all modulate fibroblast Fas-mediated apoptosis by regulating this common pathway, underlining the biological relevance of FLIP-caspase-8 balance in fibroblasts. A diagram summarizing the proposed regulatory mechanisms is shown in Fig. 8.

Interestingly, an inverse regulation of FLIP-caspase-8 expression was observed for IFN-γ, TGF-β, and IL-10. Previous data have suggested a role for STAT and SMAD signaling in response to IFN-γ or bone morphogenetic proteins, respectively, in the regulation of caspase-8 expression and activation (42–45), but this is the first demonstration of the coordinate and opposite effects of these cytokines on FLIP-caspase-8 expression. The observed effects of TNF-α appear more complex. Although TNF-α facilitates Fas-mediated apoptosis in fibroblasts, which can be explained by its effects on surface Fas and caspase-8 up-regulation, it also induced FLIP expression. This effect has been observed and shown to be dependent on NF-κB activation in other cell types, but the net effects of TNF-α on death receptor susceptibility are variable in different cell types (20, 46). In DF, the net effect of TNF-α was proapoptotic, which is consistent with other observed antiﬁbrotic effects of this cytokine in skin fibrosis (13, 14).

Our data suggest that immune mediators present in T cell-mediated responses with known ﬁbrogenic or antiﬁbrotic potential regulate ﬁbroblast survival in response to death receptor activation. In animal models, Th1 responses are characterized by higher tissue damage and little ﬁbrosis, whereas in responses dominated by Th2 and regulatory cytokines, ﬁbrosis is a prominent feature (9, 12, 47–50). In the clinical setting, IFN-γ has demonstrated speciﬁc antiﬁbrotic therapeutic properties, and TGF-β antagonists are also being developed as antiﬁbrotic agents (11, 50). Most previous studies have attributed the contrasting ﬁbrogenic potential of Th1, Th2, or regulatory cytokines to their effects in the transcription of matrix genes (13–15). Interestingly, the role of IL-10 in ﬁbrosis has remained controversial because it potently down-regulates inﬂammation and matrix synthesis, but contrary, in some models, it displays proﬁbrotic effects (9, 10, 51). We demonstrate a potent effect of IL-10 in increasing ﬁbroblast survival after Fas challenge. Therefore, discordant effects of IL-10 may reﬂect the variable participation of ﬁbroblast survival or ECM synthesis in different models of ﬁbrogenesis.

Our data show that ﬁbroblast survival in response to Fas activation and transcriptional activation of ECM genes is coordinately regulated by proﬁbrotic and antiﬁbrotic cytokines, which suggests that in addition to ECM protein synthesis, ﬁbroblast survival can be differentially regulated during inﬂammatory responses in which ﬁbrosis is a variable component. Both effects are not mutually exclusive, and selective expansion of ﬁbroblast populations with high constitutive expression of matrix proteins has been long proposed as a mechanism of ﬁbrosis (6, 52). The precise tuning of FLIP-caspase-8 balance in ﬁbroblasts by different extracellular factors points to their biological relevance as a determinant of ﬁbroblast survival. Because our study was performed in normal ﬁbroblasts, the potential involvement of this pathway in determining susceptibility to Fas-mediated apoptosis under pathological conditions characterized by abnormal ﬁbroblast expansion as well as its potential as therapeutic target warrants further studies.

References
10. Louis, H., A. Le Moine, E. Quertrimont, M. O. Peny, A. Geerts, M. Goldman, O. Le Moine, and J. Deviere. 2000. Repeated concanavalin A challenge in mice...