Fas activates the JNK pathway in human colonic epithelial cells: lack of a direct role in apoptosis

MARIA T. ABREU-MARTIN, ANDREW A. PALLADINO, MARY FARIS, NELSON M. CARRAMANZANA, ANDRÉ E. NEL, AND STEPHAN R. TARGAN

Inflammatory Bowel Disease Center, Cedars-Sinai Medical Center, Los Angeles 90048; and Division of Clinical Immunology and Allergy, Department of Medicine, Jonsson Cancer Center, University of California Los Angeles School of Medicine, Los Angeles, California 90095

Abreu-Martin, Maria T., Andrew A. Palladino, Mary Faris, Nelson M. Carramanzana, André E. Nel, and Stephan R. Targan. Fas activates the JNK pathway in human colonic epithelial cells: lack of a direct role in apoptosis. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G599–G605, 1999.—Fas is expressed constitutively by colonic epithelial cells, and its ligand is expressed by intraepithelial and lamina propria lymphocytes. Fas ligation induces apoptosis in colonic epithelial cells and is implicated in the epithelial damage seen in ulcerative colitis. To understand the pleiotropic effects of Fas in the intestinal mucosa, we have examined signaling pathways activated by Fas in HT-29 colonic epithelial cells. HT-29 cells were stimulated with anti-Fas in the presence or absence of interferon-γ (IFN-γ). Activation of mitogen-activated protein kinase pathways was assessed by kinase assay, Western blots, and promoter-reporter assays. Electromobility shift assays were used to assess activator protein-1 (AP-1) binding activity. IFN-γ increases expression of Fas on HT-29 cells. Signaling via Fas receptor, as determined by induction of c-Jun NH₂-terminal kinase (JNK) activity and transcriptional activation of AP-1, is enhanced in IFN-γ-primed cells. Dominant-interfering mutants of the JNK pathway do not block Fas-mediated apoptosis. Signaling through Fas results in activation of JNK and AP-1 binding activity that is increased in the presence of IFN-γ. Inhibition of JNK does not block Fas-mediated apoptosis in these cells. Fas-Fas ligand interactions in the intestinal mucosa may lead to complex signal transduction cascades and gene regulation that culminate in apoptosis, cytokine secretion, or other novel functions.

C-Jun NH₂-terminal kinase; activator protein-1; extracellular signal-regulated kinase activation

FAS IS A transmembrane receptor in the tumor necrosis factor (TNF) receptor family (11, 19). Several lines of evidence suggest that Fas-Fas ligand (Fasl) interactions are important in immune-epithelial communication in the intestine. Fas is expressed constitutively by the basolateral membrane of normal colon and small intestinal epithelium (17, 20). The ligand for Fas is expressed by intraepithelial lymphocytes and lamina propria lymphocytes, and its expression is increased in the lamina propria of ulcerative colitis patients (5, 25, 26). Cross-linking of Fas on freshly isolated colonic crypts leads to accelerated apoptosis of these cells (25). There are no data, however, about the molecular events associated with ligation of Fas in colonic epithelial cells.

Previously, we described a model of Fas-mediated signals in intestinal epithelial cells using the human colon cancer line HT-29 (1). HT-29 cells display a dual response to Fas ligation: cross-linking Fas stimulates secretion of interleukin (IL)-8 and, in combination with interferon-γ (IFN-γ), rapidly undergo apoptosis. Because of the diverse effects of Fas in the intestinal mucosa and its modulation by IFN-γ, we hypothesized that Fas activates critical signal transduction pathways involved in apoptosis and proinflammatory cytokine secretion and that activation of these pathways is modified by the presence of IFN-γ.

In the present study, we examine the effect of Fas on activation of the mitogen-activated protein kinase (MAPK) family of signal transduction molecules and the regulation of transcription factors downstream of MAPK activation in HT-29. We chose to study the MAPK family of signal transduction molecules because they are central regulators of cellular responses to growth factors, cytokines, and stress-induced signals (14). Recent evidence suggests that oligomerization of Fas recruits death complex that results in activation of the stress-activated branch of the MAPK family, c-Jun NH₂-terminal kinase (JNK), as well as caspase and activation of caspases (10, 31). Fas-mediated activation of JNK has been causally linked to apoptosis in T cells (8, 27), 293 cells, HeLa cells (31), and neuroblastoma cells (10). Importantly, Fas-mediated apoptosis of T cells (18) and HeLa cells (28) can occur through a caspase-dependent, JNK-independent pathway as well.

The present study demonstrates that ligation of Fas activates JNK, activator protein-1 (AP-1) binding, and AP-1 transcriptional activity in colonic epithelial cells. IFN-γ priming of these cells leads to an increase in Fas expression and enhanced signaling through these pathways. Expression of a dominant negative mutant of MAPK kinase kinase-1 (MEKK1), the upstream activator of JNK, does not block Fas-mediated apoptosis. In addition, expression of a constitutively active MEKK1 mutant leads to increased JNK activation but does not sensitize cells to Fas-mediated apoptosis. These data taken together suggest that Fas-mediated apoptosis of intestinal epithelial cells is JNK independent.

MATERIALS AND METHODS

Cell culture. HT-29 cells obtained from the American Type Culture Collection (Rockville, MD) were maintained in McCoy’s medium with L-glutamine (Meditech, Washington,
G600 FAS SIGNAL TRANSDUCTION IN COLONIC EPITHELIAL CELLS

fluence in a humidified incubator at 37°C with 5% CO₂.

DC), supplemented with 10% FCS. Cells were kept at subcon-

Reagents and antibodies. The monoclonal antibody (Ab) to

fluence in 100-mm plates. IFN-γ was added to a concentration

of 40 ng/ml for 6 h, and cells were washed three times and

kept in fresh medium overnight containing 5% bovine serum

albumin, 5 µg/ml transferrin, and 5 µg/ml insulin (Sigma, St.

Louis, MO). Anti-Fas (CH-11; 100 ng/ml) was added for the

anti-Fas Ab or irrelevant monoclonal IgG at a 1:1,000 dilution followed by PE-conju-

mediated kinase (ERK) 1 and ERK 2 Abs were purchased from

Anti-extracellular signal-

regulated kinase (ERK) 1 and ERK 2 Abs were purchased from

Zymed (San Francisco, CA).

Flow cytometry. Cells were prepared for flow cytometry by

incubating with primary anti-Fas Ab or irrelevant monoclo-

nal IgG at a 1:1,000 dilution followed by PE-conju-

ated anti-IgG in PBS with 2% FCS. Cells were fixed in PBS with

2% paraformaldehyde and were analyzed with a Becton-

Dickinson flow cytometer.

Electromobility shift assay. HT-29 cells were grown to

confluency in 100-mm plates. IFN-γ was added at a concentra-

tion of 40 ng/ml for 6 h, and cells were washed three times and

kept in fresh medium overnight containing 5% bovine serum

albumin, 5 µg/ml transferrin, and 5 µg/ml insulin (Sigma, St.

Louis, MO). Anti-Fas (CH-11; 100 ng/ml) was added for the

times indicated.

For collection of nuclear protein, cells were rinsed two

times with ice-cold PBS with 0.1% BSA. Cells were removed

from the plate with a cell scraper, transferred to a Microfuge

tube, and centrifuged at 3,000 rpm for 5 min at 4°C (12). Cell

pellets were resuspended in 900 µl of cold 1× RS buf-

fer [10 mM Tris (pH 7.4), 10 mM NaCl, 3 mM MgCl₂, 0.5 mM
dithiothreitol (DTT), 2 mM leupeptin, 1 µg/ml aprotinin, 1 µM
phenylmethylsulfonyl fluoride (PMSF), and 0.1 mM EDTA].

After resuspension, 100 µl of 1× RS containing 5% Nonidet

P-40 was added to each tube and was incubated for 5 min on

ice. Lysates were centrifuged at 5,400 rpm for 5 min, and

supernatant was completely removed. Pellets were washed

once with 1× RS and centrifuged at 8,100 rpm for 5 min.

Pellets were resuspended in 60 µl of cold buffer containing 20

mM HEPES (pH 7.4), 0.42 mM NaCl, 1.5 mM MgCl₂, 0.2 mM

EDTA, 25% glycerol, 0.01% NaN₃, 0.5 mM DTT, 1 mM PMSF,

20 µM leupeptin, and 10 µg/ml aprotinin and were placed on

ice for 40 min. Samples were spun at 13,000 rpm for 10 min, supernatants were transferred to a fresh tube, and an equal

volume of buffer with protease inhibitors was added (20 mM

HEPES (pH 7.4), 50 mM KCl, 0.2 mM EDTA, 20% glycerol,

and 0.01% NaN₃).

Protein concentration was determined using the Coomassie

protein assay reagent (Pierce) as per the manufacturer’s direc-

tions. AP-1 consensus oligomer (3.5 pmol) (Promega, Madison,

WI) was labeled with [γ-³₂P]ATP using T4 polynu-

cleotide kinase to a specific activity of ~1 × 10⁶
counts·min⁻¹·μl⁻¹. Nuclear protein (4 µg) was reacted with 1

µl of labeled oligomer in 5× binding buffer containing 20%
glycerol, 5 mM MgCl₂, 2.5 mM EDTA, 25 mM DTT, 250 mM

NaCl, 50 mM Tris (pH 7.5), and 0.05 mg/ml poly(dl-

dc)-poly(dl-dc) at room temperature for 20 min and was

analyzed on a 6% acrylamide gel (80:1 ratio of acrylamide to

bis-acrylamide). Gels were dried and exposed to film (Amer-

sham, Arlington Heights, IL). For cold competition, 10-fold

excess of unlabeled oligomer was added. A Helena densitometer

was used to semiquantify nuclear protein binding activity.

Plasmin, transfections, and retroviral infections. The cDNA

MEKKdominant active (MEKK-DA) and MEKKdominant

negative (MEKKΔ K432M; MEKK-DN; cDNA gift of Dr. G.

Johnson, National Jewish Center for Immunology and Re-

search, Denver, CO) were subcloned into the retroviral vector

pSRαSVtkneo (16, 21). Amphotropic packaging plasmid

and pSRαSVtkneo containing MEKK-DA or MEKK-DN

were used to transfect 293T cells, and supernatant containing

retrovirus was collected. Retroviral vector alone or containing

MEKK mutants was used to infect HT-29 cells, and cells were

subsequently selected in G418 at 500 µg/ml. JNK assay. HT-29 cells (2 × 10⁶ cells/sample) were cul-

tured in 1% FCS overnight after IFN-γ incubation as above.

Cells were stimulated with 250 ng/ml anti-Fas for indicated
times, rinsed with ice-cold PBS, and lysed directly on the

plate [25 mM HEPES (pH 7.4), 50 mM β-glycerophosphate, 1

mM EDTA, 2.5 mM MgCl₂, 1% Triton, 10 mM p-nitrophenol

phosphate, 1 mM sodium vanadate, 10 µg/ml aprotinin, 10

µg/ml leupeptin, and 1 mM PMSF]. After centrifugation, 100

µg of sample protein were added to 15 µg of c-Jun (−79−)

GST fusion protein (kind gift of Roger Davis; see Ref. 6) bound
to glutathione-Sepharose beads (Pharmacia) and rocked for 1

h at 4°C. Beads were washed three times in wash buffer

(same as lysis buffer but with 50 mM NaCl) and resuspended

in kinase buffer [25 mM HEPES (pH 7.4), 50 mM β-glycer-

ophosphate, 1 mM EDTA, 2.5 mM MgCl₂, and 1 mM DTT] in

the presence of 10 µCi [γ-³₂P]ATP for 30 min. Samples were boiled

in 3× sample buffer containing 2-mercaptoethanol and analyzed on

12% SDS-PAGE. Equal loading of samples was determined by

Coomassie staining. Gels were dried and exposed to film.

Immunobots. Nuclear protein was extracted as described

above. Nuclear protein (4 µg) was analyzed by 10% SDS-

PAGE, and proteins were transferred to nitrocellulose mem-

branes (Schleicher & Schuell, Keene, NH). Membranes

were blocked in 8% milk in PBS-Tween for 2 h and then were

incubated with primary Ab (c-Jun, JunD, JunB, Fos) at a

dilution of 1:1,500 for 1 h. Membranes were washed three
times with PBS-Tween and incubated with horseradish peroxi-
dase-linked secondary Ab (Amersham) at a 1:5,000 dilution

for 45 min. After washing, membranes were reacted with the

enhanced chemiluminescent solutions as per the manufacture-

r’s directions. The monoclonal antibody (Ab) to

Fas, CH-11, was purchased from Kamiya Biomedical (Thou-

sand Oaks, CA), and subsequently CH-11 was purchased from

Upstate Biotechnology (Lake Placid, NY). The level of

endotoxin contamination for CH-11 is 0.048 EU/µg as deter-

mined by the limulus amebocyte lysate test. IFN-γ was

purchased from R&D Systems (Minneapolis, MN). For Fas

surface staining, biotin-conjugated anti-human CD95 (Fas/

Apo-I), biotin-conjugated anti-human Fas ligand, and strep-

tavidin-phycocerythin (PE) were purchased from Pharmin-

gen. The ApoBrDU apoptosis detection kit was purchased

from Pharmingen and was used as per the manufacturer’s

directions. For Jun, Fos, and nuclear factor-xB (NF-xB)

Western blots and supershift assays, all Ab were purchased

from Santa Cruz (Santa Cruz, CA). Anti-extracellular signal-

regulated kinase (ERK) 1 and ERK 2 Abs were purchased from

Zymed (San Francisco, CA).

RESULTS

Fas is constitutively expressed by HT-29 cells, and its

expression is increased after IFN-γ incubation. We have

previously reported that cross-linking of Fas on HT-29

cells leads to IL-8 secretion and, in the presence of

IFN-γ, results in apoptosis (1). An example of the

synergism between IFN-γ and anti-Fas in causing

HT-29 apoptosis can be seen in Fig. 1A. Although

neither cross-linking Fas alone nor IFN-γ treatment

alone leads to significant apoptosis compared with

untreated cells, the combination of the two results in

apoptosis, as measured by terminal deoxy-nucleotidyl

transferase end labeling (TUNEL) assay (Fig. 1A).

In spite of the requirement for IFN-γ with respect to

Fas-mediated apoptosis of HT-29, cross-linking of Fas
alone results in IL-8 secretion, and the amount of IL-8 secreted does not change with IFN-γ preexposure, even after correction for cell loss (1). This model system, therefore, permits us to study function-specific Fas signaling.

One potential role for IFN-γ in this system is to upregulate the expression of Fas. Upregulation of Fas may lead to a critical mass of Fas receptors required for apoptosis. To determine the level of Fas expression on the surface of HT-29, flow cytometry was performed.
with a Fas-specific monoclonal Ab. Fas receptors are present on resting HT-29 cells (Fig. 1B, left). After a 6-h incubation with IFN-\(\gamma\), the level of Fas expression is increased and approaches that of Jurkat cells, which are sensitive to Fas-mediated killing. Caco-2 cells, which are not sensitive to Fas-mediated apoptosis, do not express Fas receptors at baseline or after incubation with IFN-\(\gamma\). These data suggest a correlation between the level of Fas expression and the ability of cells to undergo Fas-mediated apoptosis in colonic epithelial cells.

Ligation of Fas in HT-29 results in JNK activation, which is enhanced by IFN-\(\gamma\) priming. The MAPK signal transduction family is involved in cellular responses to diverse extracellular stimuli. Ligation of Fas has been shown to cause activation of the JNK branch of the MAPK family and has been causally linked to apoptosis in different cell types (10, 31). Based on these observations, we hypothesized that Fas activates the JNK branch of the MAPK pathway in HT-29 cells. Because our HT-29 model requires IFN-\(\gamma\) for Fas-mediated apoptosis but not for IL-8 secretion, we further hypothesized that the magnitude of JNK activation in response to Fas would be altered by the presence of IFN-\(\gamma\). Ligation of Fas leads to a fourfold activation of JNK at 60 min after treatment with IFN-\(\gamma\) followed by cross-linking of Fas to lead to a rapid induction of JNK activity at 15 min that is ~10-fold higher than control cells and is sustained at 60 min.

The specificity of JNK activation by Fas ligation was demonstrated by parallel study of ERK activation. There was no significant activation of ERK based on Western blot analysis of phosphorylated ERK in response to ligation of Fas in the presence or absence of IFN-\(\gamma\) (Fig. 2B). The demonstration of increased JNK

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Fig. 2. A: c-jun NH2-terminal kinase (JNK) activity of anti-Fas-stimulated HT-29 cells. Cells were incubated in the presence or absence of IFN-\(\gamma\) on day 1 followed by anti-Fas (1,000 ng/ml) stimulation on day 2 for indicated times. Each lane represents 200 µg of total cellular protein reacted with glutathione-S-transferase (GST)-c-jun-(1—79) fusion protein in the presence of [\(\gamma\]-\(^32\)P]ATP. These data are 1 representative experiment of 3. B: extracellular signal-regulated kinase (ERK) activity in HT-29 cells in response to ligation of Fas. Cells incubated in the presence or absence of IFN-\(\gamma\) on day 1 followed by anti-Fas (100 ng/ml) stimulation on day 2 for indicated times. Each lane represents 20 µg of total cellular protein reacted with Ab against ERK1 and ERK2. ERK1 (mol wt 44) was not detected. ERK2 (mol wt 42) is detected and undergoes a slight upward shift upon phosphorylation (bracket). These data are 1 representative experiment of 2.

MAPK family and has been causally linked to apoptosis by 100-fold excess of unlabeled AP-1 oligomer. These data are 1 representative experiment of 4.

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Fig. 3. Effect of cross-linking Fas on activator protein-1 (AP-1) nuclear binding. Nuclear protein (4 µg) from HT-29 cells was exposed to conditions as indicated and reacted with \(^{32}\)P-labeled AP-1 oligomer, with IFN-\(\gamma\) (40 ng/ml) given day before collection and anti-Fas used at 100 ng/ml for indicated times. Last lane (c.c.) shows competition with 100-fold excess of unlabeled AP-1 oligomer. These data are 1 representative experiment of 4.

Fig. 4. Effect of dominant-interfering and dominant-active mutants of MEKK1 on Fas-mediated apoptosis. A: JNK assay and MEKK1 Western blot of HT-29 sublines. Top: JNK activity for sublines as indicated in which 100 µg of total cellular protein were reacted with GST-c-jun-(1—79) fusion protein in the presence of [\(\gamma\]-\(^32\)P]ATP. Bottom: Western blot in which 20 µg of total cellular protein were extracted from parental HT-29 and HT-29 sublines stably infected with retrovirus containing the neomycin resistance gene alone, MEKK-dominant negative (DN), or MEKK-dominant active (DA). Protein was separated on 10% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with anti-MEKK1 Ab. Only cells infected with pSRaMSVtkNeo-MEKK-DN or MEKK-DA retrovirus express the truncated MEKK1 protein. B: JNK activity in HT-29 cells expressing MEKK-DN after cross-linking Fas. Cells were incubated in the presence or absence of IFN-\(\gamma\) on day 1 followed by anti-Fas (250 ng/ml) stimulation on day 2 for indicated times. Neo, cells infected with control retrovirus. Each lane represents 20 µg of total cellular protein reacted with GST-c-jun-(1—79) fusion protein in the presence of [\(\gamma\]-\(^32\)P]ATP. These data are 1 representative experiment of 3. C: apoptosis in response to cross-linking Fas in HT-29 sublines. HT-29 sublines expressing Neo control, MEKK-DN, or MEKK-DA were incubated in the presence or absence of IFN-\(\gamma\) on day 1 followed by anti-Fas (250 ng/ml) stimulation on day 2 for 4 h as indicated. Apoptosis was determined using the ApoBrdU apoptosis detection kit. Percent of cells staining positive for apoptosis is indicated. This is 1 representative experiment of 3.
activity within 15 min after cross-linking of Fas is consistent with a temporal relationship, albeit not a causal relationship, between JNK activation and apoptosis.

Activation of the JNK pathway in HT-29 cells results in increased AP-1 nuclear protein binding. Cross-linking Fas leads to activation of JNK, resulting in the phosphorylation of the AP-1 transcription factor c-jun (6). To further understand the molecular pathways involved in Fas signal transduction in colonic epithelial cells, we studied the ability of Fas to induce nuclear protein binding to an AP-1 consensus sequence. We hypothesized that IFN-γ and cross-linking Fas would result in increased AP-1 binding with kinetics similar to JNK activation. To test this hypothesis, nuclear extracts from cells treated with anti-Fas alone or after IFN-γ preincubation were tested for their ability to bind an AP-1 consensus sequence (Fig. 3). Compared with untreated cells, nuclear protein from cells stimulated with anti-Fas had gradually increasing AP-1 activity that peaked at 60 min (Fig. 3). In contrast, nuclear extracts from cells treated with anti-Fas in the presence of IFN-γ had much greater AP-1 binding compared with untreated cells. In the presence of IFN-γ, peak AP-1 binding occurred 15 min after ligation of Fas. These data are consistent with the JNK data with regard to the temporal relationship of AP-1 binding and JNK activation. An excess of unlabeled AP-1 oligomer eliminates the DNA-protein complexes, but unlabeled irrelevant oligomers such as NF-kB and Sp1 do not compete with the DNA-protein complexes, verifying specificity of nuclear protein binding to AP-1 (data not shown). Western blots of nuclear protein showed that the absolute amounts of c-jun, JunD, or Fos nuclear protein do not change with IFN-γ preincubation or in response to cross-linking Fas (data not shown). Thus ligation of Fas in the presence of IFN-γ causes an early increase in AP-1 binding activity followed by downregulation coincident with the onset of apoptosis.

Expression of MEKK1-DN does not inhibit apoptosis. Because the apoptotic combination of anti-Fas and IFN-γ induces JNK activation, we asked whether JNK activation was required for HT-29 apoptosis. To address this question, HT-29 cells were stably infected with retrovirus containing a dominant negative mutant of MEKK1, MEKK-DN. This construct has been shown to inhibit downstream JNK activation in response to several stimuli (3, 9). Expression of the truncated MEKK-DN protein was demonstrated by Western blot analysis (Fig. 4A, bottom). HT-29 expressing MEKK-DN have a 50% reduction in JNK activity after IFN-γ and cross-linking of Fas compared with cells infected with vector control (Fig. 4B). HT-29 cells expressing MEKK-DN or the vector control do not show any differences in Fas-mediated apoptosis (Fig. 4C). These results suggest that a non-JNK-dependent pathway is present in HT-29 cells that signals apoptosis in cells exposed to anti-Fas and IFN-γ or that partial inhibition of JNK is insufficient to block Fas-mediated apoptosis.

Although JNK activation does not seem to be necessary for Fas-mediated apoptosis, we asked whether JNK activation was sufficient to induce apoptosis in HT-29 cells or bypass the requirement for IFN-γ pre-treatment. HT-29 cells were derived that expressed a constitutively active form of MEKK1, MEKK-DA. This mutant form of MEKK1 induces apoptosis in fibroblasts and T cells (8, 13). HT-29 cells expressed MEKK-DA protein as demonstrated by Western blot analysis and had fourfold JNK activation at baseline compared with vector control cells (Fig. 4A). Activation of JNK was not sufficient to induce apoptosis and did not sensitize cells to Fas-mediated apoptosis as demonstrated by TUNEL staining (Fig. 4C). These data suggest that JNK activity is neither necessary nor sufficient to induce apoptosis in HT-29 cells.

DISCUSSION

The important role of FasL interactions in the intestinal mucosa is beginning to emerge. Known functions mediated by Fas in colonic epithelial cells include proinflammatory cytokine secretion and apoptosis, but the full range of Fas effects in the intestinal mucosa is not known (25). This study examines the subcellular signaling pathways triggered by cross-linking Fas on colonic epithelial cells. We chose HT-29 as a model system because cross-linking Fas stimulates secretion of IL-8 but, in the presence of IFN-γ, cells rapidly undergo apoptosis (1). This model system, therefore, permits us to study function-specific signaling in response to ligation of Fas.

In this paper, we have demonstrated that cross-linking Fas in colonic epithelial cells stimulates the stress-activated branch of the MAPK pathway, resulting in JNK activation, phosphorylation of c-jun, and increased AP-1 binding activity. The parallel ERK branch of the MAPK pathway is not activated by ligation of Fas. In other systems, activation of JNK and downregulation of ERK are sufficient to cause apoptosis (30). Importantly, however, inhibition of the MEKK1–JNK pathway in HT-29 does not inhibit apoptosis. These data are consistent with the existence of multiple pathways resulting in Fas-mediated apoptosis of colonic epithelial cells. Recent work by Yang et al. (31) demonstrates that Fas can activate parallel pathways resulting in JNK activation or caspase activation and that both can result in apoptosis (31). We have also expressed a constitutively active form of MEKK1 that induces apoptosis in diverse cell types (8, 13). HT-29 cells expressing dominant-active MEKK1 did not have a higher spontaneous rate of apoptosis and were not sensitized to Fas-mediated apoptosis. These data suggest that JNK is not causally involved in apoptosis in HT-29 cells.

Our results suggest a model in which Fas-expressing intestinal epithelial cells are not competent to undergo apoptosis without costimulation by IFN-γ. At least one effect of IFN-γ in this system is to increase the level of surface expression of Fas. Although the level of Fas expression does not correlate directly with the ability of cells to undergo apoptosis (24, 29), in HT-29 cells and Caco-2 cells Fas expression parallels sensitivity to apoptosis. Our findings of increased second signaling in IFN-γ-treated cells can be explained by an increase in Fas expression. In addition, IFN-γ may be exerting its effect on any of the components of the Fas death machinery. Binding of FasL leads to aggregation of Fas.
and recruitment of a death complex that results in JNK activation and sequential degradation of caspases (2, 4, 7, 22). Cells deficient in the IFN-γ-regulated STAT-1 transcription factor are defective in TNF-α-mediated apoptosis as a result of a deficiency in caspases (15). It is plausible, therefore, that the individual components of the Fas death machinery such as FADD or caspases exist in rate-limiting quantities and are increased by the presence of IFN-γ. An understanding of the different pathways utilized by intestinal epithelial cells to signal cytokine secretion and apoptosis may eventually permit selective targeting of these pathways as a treatment for chronic inflammation or cancer (23).

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Address for reprint requests and other correspondence: M. T. Abreu-Martin, Inflammatory Bowel Disease Center, 8700 Beverly Blvd., D4063, Los Angeles, CA 90048 (E-mail: abreu@csmc.edu).

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