Anti-Fas on Nonhematopoietic Tumors: Levels of Fas/APO-1 and bcl-2 Are Not Predictive of Biological Responsiveness

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ABSTRACT

Fas/APO-1 is a cell surface protein known to trigger apoptosis in a variety of cell types upon specific antibody binding. Although extensively studied on normal and malignant hematopoietic cells, little is known about Fas/APO-1 on nonhematopoietic cells. In the study presented here, we have examined Fas/APO-1 expression and function on 11 human tumor cell lines of nonhematopoietic origin. By flow cytometric analysis, Fas/APO-1 was expressed on 10 of the 11 tumors at levels comparable to those previously reported for lymphoid cells sensitive to the cytolytic effects of anti-Fas. Despite abundant cell surface expression, only 4 of the 10 Fas-positive tumors were sensitive to the cell-killing effects of anti-Fas. Moreover, anti-Fas enhanced the growth of 2 of 10 Fas-positive tumors. Additional studies using cycloheximide demonstrated that de novo protein synthesis was required for anti-Fas-triggered growth stimulation and, at least in one case, was responsible for the resistance to antibody-induced apoptosis. The biological effects initiated by anti-Fas engagement, however, were not correlated with endogenous bcl-2 expression. This report documents that: (a) Fas/APO-1 is widely expressed on cultured nonhematopoietic tumors; (b) the inherent susceptibility to anti-Fas-induced apoptosis is not correlated with expression of the Fas/APO-1 protein; (c) Fas/APO-1 engagement may result in growth enhancement; and (d) protective/growth-promoting proteins other than bcl-2 may contribute to the diverse spectrum of biological effects induced by anti-Fas engagement of the Fas/APO-1 protein.

INTRODUCTION

Apoptosis, or programmed cell death, is a physiological mechanism of cell death occurring in embryogenesis, tissue atrophy, and thymic selection. Several characteristics distinguish apoptosis from necrotic cell death, most notably a requirement for active cell participation in the generation of oligonucleosomal-length DNA fragmentation (1). Numerous stimuli have been shown to induce apoptosis in a variety of cell types, including glucocorticoid and calcium ionophore exposure (2), withdrawal of an essential growth factor (3), c-myc or wild-type p53 expression (4–7), and antibody engagement of the cell surface proteins Fas or APO-1 (8, 9).

The Fas and APO-1 proteins were independently identified by two laboratories on the basis of their ability to trigger apoptosis upon specific monoclonal antibody binding (8, 9). The complementary DNAs for Fas and APO-1 have been isolated and characterized, revealing their complete sequence identity (10–12). Analysis of the amino acid sequence revealed that Fas/APO-1 is a member of the nerve growth factor/TNF-R superfamily. The human Fas/APO-1 protein consists of an extracellular domain rich in cysteine residues, a single transmembrane spanning region, and a cytoplasmic domain containing a region of homology to the TNF-R superfamily members CD40 and TNF-R p60 (11, 12). Mutational analysis demonstrated that this homologous region is essential for transduction of the death signal, although lacking consensus sequences for all known kinases, phosphatases, and other enzymes (13). The nature of the Fas/APO-1 apoptotic signal is unknown, although the identity of the natural Fas/APO-1 ligand (presumably mimicked by antibody binding) has been recently been reported (14).

The critical role of Fas/APO-1 in the induction of cellular death has been confirmed by transfection into cells that do not express Fas/APO-1 (11), but anti-Fas susceptibility is not uniformly correlated with expression of this protein. For example, Fas/APO-1 is expressed on resting CD45RO-positive and freshly activated lymphocytes, yet programmed cell death is induced only in those lymphocytes activated for 4 days or longer (15, 16). Such findings led to the hypothesis that Fas/APO-1 may play a role in the elimination of lymphocytes undergoing excessive clonal expansion and that inactivation of this gene may lead to abnormal lymphocyte accumulation. Watanabe-Fukunaga et al. (17) have elegantly demonstrated the loss of Fas/APO-1 function in lymphocytes from ipr mice with lymphoproliferative disease. In such mice, Fas/APO-1 is functionally inactivated as a result of a single point mutation (17) or an abnormal splicing event after retrotransposon insertion (18–20). In addition to the genetic inactivation of Fas/APO-1, accumulating evidence suggests that endogenous proteins such as bcl-2 may protect against the Fas/APO-1 mediated "death signal" (21).

Fas/APO-1 has been extensively studied on normal and malignant hematopoietic cells (9, 15, 16, 22, 23), although little is known about this unique protein on nonhematopoietic cells. To expand our knowledge of Fas/APO-1 expression, as well as to examine whether bcl-2 levels are correlated with resistance to anti-Fas cell killing, we have examined 11 nonhematopoietic tumor cell lines of diverse histological type.

MATERIALS AND METHODS

Cell Lines and Culture Medium. PC-3M is a metastatic variant derived from the parent PC-3 cell line originally obtained from the American Type Culture Collection (Rockville, MD). The generation of this cell line has been described elsewhere (24). The human pancreatic tumor cell line Panc 39 was established from a well-differentiated primary adenocarcinoma, and Panc 55 was established from a liver metastasis-derived adenocarcinoma of the pancreas. The generation and characterization of these cell lines will be described elsewhere (25). The human colon carcinoma "parental" cell line KM12C was isolated from a primary colon carcinoma classified as Dukes stage B2; the KM12-SM "daughter" cell line was derived from a rare, spontaneous liver metastasis produced by parental KM12C cells growing in the cecal wall of a nude mouse; and the highly metastatic KM12-L4 "daughter" cell line was selected by four cycles of intrasplenic injections of KM12C. The generation and characterization of these cell lines will be described elsewhere (25).

The abbreviations used are: TNF-R, tumor necrosis factor receptor; PBS, phosphate-buffered saline; EBV, Epstein-Barr virus.

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4 The abbreviations used are: TNF-R, tumor necrosis factor receptor; PBS, phosphate-buffered saline; EBV, Epstein-Barr virus.


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Fas/APO-1 On Nonhematopoietic Tumors

Antibodies and Flow Cytometric Analysis. The murine monoclonal antibody anti-Fas was prepared as described previously (6). Phycoerythrin-conjugated rat anti-mouse κ-specific antibody was purchased from Becton Dickinson (Mountain View, CA) and used according to the manufacturer’s recommendations. Cells (10^6) were incubated with 1 μg anti-Fas (IgG1,κ) or isotype-matched control antibody (Caltbiochem Corp., La Jolla, CA) antibody for 30 min at 4°C, washed twice, and incubated with the secondary antibody for an additional 30 min. After washing twice, the cells were fixed in PBS containing 1% paraformaldehyde (pH 7.0) prior to analysis. A murine monoclonal antibody against the human bcl-2 protein (IgG1,κ) was purchased from DAKO Corp. (Carpinteria, CA). bcl-2 staining was carried out according to the method of Meyaard et al. (29) after a 70% cold methanol fixation using anti-bcl-2 or isotype-matched control antibody followed by phycoerythrin-conjugated rat anti-mouse κ-specific antibody (Becton Dickinson). Flow cytometric analysis was carried out using a FACScan (Becton Dickinson) with the window set to exclude dead cells and debris. Ten thousand cells were examined for each determination.

Anti-Fas-induced Inhibition of Cellular Proliferation and Viability. Cells (5 × 10^5 to 10^6 per 200 μl) were incubated in flat-bottomed 96-well plates (Corning Glass Works, Corning, NY) in triplicate or quadruplicate in the presence of either anti-Fas or control antibody (1 ng-1 μg/well) for up to 72 h. After incubation, cells were pulsed with 0.5 μCi [3H]thymidine (Amersham Chemical Co., Arlington Heights, IL) for 3 h, harvested on a Tomtec harvester (Orange, CT), and counted in a Wallac Betaplate (Gaithersburg, MD) liquid scintillation counter. Where indicated, cellular metabolic function was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction as described by Mosmann (30) in the presence of 20 μg/ml cycloheximide (Sigma Chemical Co.) during the antibody incubation period. Cell viability was measured by propidium iodide exclusion. Briefly, 5 × 10^6 cells/ml were incubated in culture media containing 1 μg anti-Fas or control antibody in 24-well plates for 72 h, washed once in PBS, trypsinized, and then stained with propidium iodide (1 μg/ml) in PBS containing 1 mg/ml glucose and 100 units/ml RNase A. Cellular viability was determined by cell counting and propidium iodide exclusion in duplicate (total number cells recovered / percentage cells excluding propidium iodide). Cells were examined on an Epics Profile, Epics Division of Coulter Co., Hialeah, FL. All functional assays were performed at least twice for each cell line.

Flow Cytometric Determination of DNA Fragmentation. Cells containing single strand DNA breaks were analyzed by in situ nick translation according to the method of Meyard et al. (31). In brief, cells were treated with anti-Fas or control antibody for 20 h and then fixed with 1% formaldehyde followed by 70% ethanol, washed in nick translation buffer and incubated with dATP, dCTP, dGTP, and biotinylated-11dUTP (Boehringer Mannheim) in nick translation buffer containing Escherichia coli polymerase I (Boehringer Mannheim) for 1.5 h at 15°C. After incubation, the cells were stained with avidin-FITC (Becton Dickinson) to detect DNA breaks and counterstained with propidium iodide (described above) prior to analysis on a Epics Profile. DNA fragmentation is quantified as the percentage green fluorescence (dUTP incorporation) in the propidium iodide positive population (total DNA). Specific DNA fragmentation is the percentage green fluorescence in cells treated with anti-Fas after subtraction of background green fluorescence in control antibody-treated cells.

RESULTS

Fas/APO-1 Is Widely Expressed on Nonhematopoietic Tumors. This study was undertaken to expand our knowledge of Fas/APO-1 distribution and function among nonhematopoietic human tumors. To this end, we have examined the tumor cell lines shown in Table 1 for Fas/APO-1 expression by flow cytometry. As shown in Fig. 1, Fas/APO-1 was moderately to abundantly expressed 10 of 11 tumor types examined. Only the human osteosarcoma cell line, Saos-2, failed to express Fas/APO-1. The levels of expression on tumors positive for Fas/APO-1 were comparable to that of the Jurkat E-6 lymphoid cell line sensitive to the cell-killing effects of anti-Fas treatment. Our findings are consistent with those previously reported for cell lines A673 (rhabdomyosarcoma), CHU-2 (squamous carcinoma), and HT-29 (colon carcinoma) (8, 11) and establish that Fas/APO-1 expression is widely distributed among nonhematopoietic tumors of various histological types.

Fas/APO-1 Expression Is Not Predictive of Anti-Fas-mediated Cell Killing. To determine the sensitivity of the Fas/APO-1-positive tumor lines to anti-Fas-mediated apoptosis, cells were initially cultured in the presence of 0.5 μg anti-Fas or isotype-matched control antibody for 48 h before the assessment of cellular proliferation measured by [3H]thymidine uptake (Table 2). For the sake of comparison, anti-Fas-mediated cell killing is also shown for the Jurkat E-6 lymphoid line. Among the Fas/APO-1-positive nonhematopoietic tumors tested, only the colon carcinoma cell lines (KM12C, KM12-L4, and KM12-SM) and the glioblastoma cell line (U-251) demonstrated significant proliferative inhibition following anti-Fas treatment. In all cases, however, the nonhematopoietic tumors were less sensitive to anti-Fas treatment than Jurkat, despite their approximately equal cell surface expression of Fas/APO-1. As expected, Saos-2 cells lacking Fas/APO-1 expression were unaffected by anti-Fas treatment. Somewhat surprisingly, anti-Fas treatment resulted in the proliferative stimulation of several tumor cell lines when compared to isotype-matched control antibody (ME-180, A375-SM, and Panc-55). Mapara et al. (23) have noted a similar effect of anti-APO-1 antibody on human chronic B-lymphocytic leukemias.

To clarify whether the inhibitory/stimulatory effects of anti-Fas treatment were the result of alterations in cell cycle progression or cellular growth, survival experiments were performed. For these studies, tumors positive for Fas/APO-1 were treated with anti-Fas or isotype-matched control for 72 h prior to the determination of viable cells.
Fluorescence Intensity

Fig. 1. Fas/APO-1 expression on nonhematopoietic tumors measured by flow cytometry. Cultured tumor cell lines were stained with anti-Fas as described in "Materials and Methods." The data are presented as the log peak fluorescence intensity of the various cell lines stained with isotype-matched control (open peak) or anti-Fas antibody (filled peak).

Table 2 Effects of anti-Fas on nonhematopoietic tumors

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Anti-Fas</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM12C</td>
<td>25,898 ± 1,378 (^b)</td>
<td>40,918 ± 3,522</td>
</tr>
<tr>
<td>KM12-SM</td>
<td>42,598 ± 3,716 (^b)</td>
<td>56,180 ± 6,715</td>
</tr>
<tr>
<td>KM12-L4</td>
<td>22,731 ± 3,670 (^c)</td>
<td>34,065 ± 2,372</td>
</tr>
<tr>
<td>ME-180</td>
<td>20,223 ± 720 (^c)</td>
<td>12,552 ± 1,735</td>
</tr>
<tr>
<td>U-251</td>
<td>29,564 ± 1,800 (^c)</td>
<td>53,221 ± 2,131</td>
</tr>
<tr>
<td>A375</td>
<td>46,553 ± 4,758</td>
<td>49,922 ± 5,132</td>
</tr>
<tr>
<td>A375-SM</td>
<td>79,558 ± 2,813 (^c)</td>
<td>57,253 ± 1,284</td>
</tr>
<tr>
<td>Saos-2</td>
<td>32,286 ± 2,886</td>
<td>32,842 ± 753</td>
</tr>
<tr>
<td>Panc-39</td>
<td>3,638 ± 1,404</td>
<td>5,201 ± 624</td>
</tr>
<tr>
<td>Panc-55</td>
<td>28,337 ± 1,578 (^c)</td>
<td>19,454 ± 1,787</td>
</tr>
<tr>
<td>PC-3M</td>
<td>91,128 ± 1,416</td>
<td>95,540 ± 6,986</td>
</tr>
<tr>
<td>Jurkat</td>
<td>8,477 ± 1,553 (^b)</td>
<td>28,644 ± 1,588</td>
</tr>
</tbody>
</table>

\(^a\) Cells were treated as described in "Materials and Methods" with 0.5 \(\mu\)g anti-Fas or control antibody for 48 h prior to assessment of \(^{3}H\)thymidine incorporation.

\(^b\) Significantly different from control using a two-tailed \(t\) test for paired samples \((b, P ≤ 0.01; c, P ≤ 0.05)\).

Cellular recovery (number of cells/ml able to exclude propidium iodide). Due to the difficulty of prolonged ex vivo passage, the human pancreatic adenocarcinomas were not examined in this system. Results from a representative experiment are shown in Fig. 2. In tumors where anti-Fas treatment resulted in a significantly decreased \(^{3}H\)thymidine uptake (KM12C, KM12-SM, KM12-L4, and U-251), a significantly reduced viable cellular recovery was also observed. A significant increase in viable cellular recovery was observed in anti-Fas-treated ME-180 and A375-SM compared to antibody controls, corroborating the previous observation of enhanced proliferation by thymidine incorporation. PC-3M growth was, again, unaltered by anti-Fas treatment (Table 2). In contrast to previous findings, viable cell recovery of the A375 cell line was significantly reduced in 3/3 experiments following a 72-h treatment with anti-Fas. A similar treatment regimen failed to significantly decrease nucleic acid incorporation at 48 h (Table 2), reflecting a delayed biological response of this tumor to cell death initiated by anti-Fas binding. These results provide evidence that anti-Fas affects cell survival rather than cell cycle progression and that the levels of Fas/APO-1 expression are not predictive of a growth-inhibitory response to specific antibody treatment.

Differential Anti-Fas Response among Paired Tumor Cell Lines with Equivalent Fas/APO-1 Cell Surface Expression. Initial experiments revealed a differential response to anti-Fas-mediated cell killing among the paired colon carcinoma cell variants despite their
approximately equal Fas/APO-1 expression (Figs. 1 and 2). Interestingly, the growth-inhibitory effects of anti-Fas were most pronounced against the parental, nonmetastatic tumor line KM12C, while the metastatic variants KM12-SM and KM12-L4 remained relatively refractory to these effects. To test whether this differential biological response was antibody dose-dependent, titration experiments were performed. As shown in Fig. 3, the sensitivity of KM12C to anti-Fas-mediated growth inhibition was highly significant \((P \leq 0.010)\) at both 100 ng and 1000 ng/ml using a two-tailed \(t\) test for paired samples. The metastatic variants KM12-SM and KM12-L4 were significantly less inhibited by anti-Fas treatment than the KM12C cell line over a range of antibody concentrations when examined by multiple regression analyses. To determine whether the differences in biological response also reflected a decrease in DNA damage after anti-Fas treatment, flow cytometric quantification of DNA fragmentation was carried out using \textit{in situ} nick translation (31). One of three consistent experiments is shown in Fig. 4. Concordant with the growth inhibition experiments, parental KM12C cells demonstrated 28% specific DNA fragmentation, while the KM12-SM and KM12-L4 lines demonstrated approximately 19 and 1% DNA fragmentation, respectively, after a 20-h treatment with anti-Fas. Likewise, when the parental and metastatic amelanotic melanomas A375 and A375-SM were examined, the disparate biological effects (growth inhibition \textit{versus} growth stimulation, respectively) were maintained in both antibody dose-titration and DNA fragmentation experiments. For example, parental A375 cells demonstrated 36 ± 4% specific DNA fragmentation in two separate experiments, while no specific DNA fragmentation was observed in the A375-SM variant (data not shown). Because anti-Fas engagement initiated dissimilar biological effects in parent-daughter cell lines expressing equivalent cell surface Fas/APO-1, the role of accessory proteins in the mediation of Fas/APO-1 signaling was explored.

\textbf{Inhibition of Protein Synthesis Alters Anti-Fas Biological Effects: bcl-2 Levels Are Not Associated with Cell Death or Growth Stimulation.} To address whether selective protein synthesis can account for anti-Fas mediated growth stimulation or the resistance to anti-Fas-mediated apoptosis, nonhematopoietic tumors were treated with anti-Fas for 20 h in the presence of the protein synthesis inhibitor cycloheximide and cellular metabolic function measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction. As shown in Table 3, the stimulatory effects of anti-Fas on ME-180 and A375-SM cells were completely abolished in the presence of cyclo-

![Fig. 3](image3.png)

\textbf{Fig. 3.} Dose titration of anti-Fas on the cellular proliferation of paired colon carcinoma tumor cell lines. Cultured tumor cell lines were treated with anti-Fas or isotype-matched control antibody at the concentrations indicated for 48 h and then pulsed with [\(^{3}H\)]thymidine as described in "Materials and Methods." \textit{Points}, mean cpm [\(^{3}H\)]thymidine incorporation. \textit{Bars}, SEM.

\textbf{Fig. 4.} DNA fragmentation after anti-Fas treatment measured by flow cytometry. Cultured tumor cell lines treated with anti-Fas or control antibody for 72 h were fixed, and \textit{in situ} nick translation was performed using biotin 11-dUTP as described in "Materials and Methods." Fragmented DNA was detected using fluorescein isothiocyanate-avidin staining and total DNA by propidium iodide staining.

![Fig. 4](image4.png)
Table 3 Effects of anti-Fas on nonhematopoietic tumors in the absence of protein synthesis

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Medium</th>
<th>Anti-Fas</th>
<th>Control</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM12C</td>
<td>0.646 ± 0.048</td>
<td>0.477 ± 0.021</td>
<td>0.671 ± 0.069</td>
<td>29</td>
</tr>
<tr>
<td>KM12-SM</td>
<td>0.627 ± 0.027</td>
<td>0.626 ± 0.015</td>
<td>0.723 ± 0.016</td>
<td>13</td>
</tr>
<tr>
<td>KM12-L4</td>
<td>0.729 ± 0.010</td>
<td>0.903 ± 0.021</td>
<td>0.962 ± 0.021</td>
<td>6</td>
</tr>
<tr>
<td>ME-180</td>
<td>0.310 ± 0.021</td>
<td>0.305 ± 0.008</td>
<td>0.315 ± 0.028</td>
<td>3</td>
</tr>
<tr>
<td>PC3M</td>
<td>0.524 ± 0.023</td>
<td>0.348 ± 0.005</td>
<td>0.486 ± 0.026</td>
<td>28</td>
</tr>
<tr>
<td>A375</td>
<td>0.156 ± 0.007</td>
<td>0.103 ± 0.007</td>
<td>0.122 ± 0.003</td>
<td>16</td>
</tr>
<tr>
<td>A375-SM</td>
<td>0.320 ± 0.009</td>
<td>0.294 ± 0.006</td>
<td>0.314 ± 0.006</td>
<td>6</td>
</tr>
</tbody>
</table>

*Cells were incubated with 0.5 μg/ml anti-Fas or control antibody for 20 h prior to assessing cellular metabolic function using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay as described in "Materials and Methods." Cycloheximide (20 μg/ml) was present in all treatment groups for the duration of the assay. Percentage inhibition was derived by comparing anti-Fas treatment to controls.

heximide, suggesting an essential role for protein synthesis in anti-Fas-triggered growth enhancement. In the case of PC-3M, cycloheximide induced modest sensitivity to anti-Fas treatment (30 ± 4% inhibition in three experiments), whereas A375 cells were only slightly inhibited (14 ± 2% inhibition in three experiments). The synthesis of a protective protein, therefore, may be involved in the resistance of some tumors to anti-Fas-mediated killing. It was of particular interest to determine whether the differential anti-Fas response would be retained among the KM12 parent-daughter lines in the absence of protein synthesis. In fact, the response to anti-Fas proved to be similar regardless of cycloheximide addition (Fig. 2, Table 3), suggesting that in the KM12 cell line the enhanced resistance of the metastatic variants did not result from a differentially expressed protective protein. Recent experimental evidence has indicated that overexpression of the intracellular protein bcl-2 can protect against the cell killing effects of anti-Fas (21) and that endogenous bcl-2 levels correlate with anti-Fas-mediated apoptosis or growth stimulation in hematopoietic tumors (23). To determine if endogenous bcl-2 levels are correlated with anti-Fas-mediated biological responses in any of the nonhematopoietic tumors examined, bcl-2 was measured in situ using flow cytometry (29). As shown in Fig. 5, although the hematopoietic cell line Jurkat expressed moderate levels of bcl-2, expression was virtually nondetectable in all nonhematopoietic cell lines except U-251 and ME-180. Thus, in the nonhematopoietic tumors examined, bcl-2 levels do not correlate with anti-Fas biological effects.

DISCUSSION

Previously, we and others (9, 13, 15, 16) have investigated Fas/APO-1 expression and function in normal and malignant hematopoietic cells. Recently Leithauser et al. (32) have examined Fas/APO-1 expression in a variety of nonhematological tumors by immunohistochemistry, however, our study is the first to examine both Fas/APO-1
distribution and susceptibility to anti-Fas-mediated apoptosis among such tumors. Our studies confirm that Fas/APO-1 expression is widely distributed among solid tumors of various histological types and, in accordance with previous studies using hematopoietic cells, the inherent susceptibility to anti-Fas-induced apoptosis is not correlated with expression of this protein. Furthermore, the biological effects of anti-Fas engagement widely differ among parent-daughter tumor cell lines expressing equivalent cell surface Fas/APO-1. Although the presence of Fas/APO-1 is clearly a prerequisite for biological responses triggered by specific antibody binding, signaling may not uniformly result in programmed cell death. Several solid tumor cell lines respond to anti-Fas treatment with growth stimulation rather than cell death. Anti-Fas/APO-1-mediated growth stimulation has also been reported in several cases of chronic lymphocytic leukemia (23) and is not completely unexpected in light of the vastly pleiotropic effects initiated by antibody/ligand binding in other members of the nerve growth factor/TNF receptor superfamily.

Our experimental findings suggest that resistance to cell killing by anti-Fas in nonhematopoietic tumors can result from one or more of at least three factors: lack of cell-surface Fas/APO-1 expression (Saos-2), synthesis of a protective protein (cell line PC-3 m), or alterations in Fas/APO-1 intracellular signaling pathways (cell lines A375, A375-SM, ME-180). Lack of Fas/APO-1 expression has been observed on other tumors, including EBV-negative Burkitt lymphoma lines, EBV-positive Burkitt cell lines with a group I phenotype, and the cervical carcinoma cell line HeLa (11, 22). In the case of the Fas/APO-1-negative Burkitt lymphoma cell lines noted above, Falk et al. (22) have demonstrated that Fas/APO-1 expression can be induced upon activation of latent EBV viral gene expression, strongly suggesting the potential for transcriptional activation/depression in the molecular regulation of this gene. Induction of Fas/APO-1 expression has not been shown for HeLa cells, but γ-interferon has been shown to markedly upregulate mRNA and protein expression in the HT-29 colon carcinoma line (11). In preliminary studies, we have been unable to demonstrate Fas/APO-1 upregulation in Saos-2 after γ-interferon treatment. Further studies are warranted to extend our knowledge of Fas/APO-1 regulation by other soluble immune regulators.

Protection against apoptosis via synthesis of an intracellular protein is a well-established paradigm. bcl-2 is a novel M,
25,000 integral membrane protein that functions to inhibit programmed cell death (33). Abundant evidence suggests that dereglulation of bcl-2 is a genetic lesion that appears consistently in the development of follicular lymphoma (34, 35). Interestingly, expression of bcl-2 has been shown to inhibit DNA fragmentation induced by a variety of stimuli, including anti-Fas, in transfected cell lines (20, 36–38). Levels of bcl-2 appear to be correlated with the biological effects of Fas/APO-1 in human malignant B-cells (23). Our studies, however, do not suggest a correlation between bcl-2 levels and anti-Fas-mediated apoptosis or growth stimulation in the nonhematopoietic tumors examined. On the other hand, protein synthesis inhibitor experiments indicate a potential role for constitutively produced proteins in the regulation of Fas/APO-1-mediated cellular growth (ME-180 and A375-SM) and death (PC-3M). Such proteins may include manganese superoxide dismutase (39), Bax (40), bcl-λs (41), or bcl-δs (41).

The potentially critical role of programmed cell death in the pathogenesis of malignant disease has been underscored by recent experimental observations that wild-type p53 (6, 7) and bcl-2 (33–35) can induce or inhibit, respectively, apoptosis. Based on our knowledge of these systems, we propose that Fas/APO-1 may normally function as a suppressor of tumor formation by the induction of apoptosis (analogous to the overexpression of p53) and that the inactivation of Fas/APO-1 signaling can lead to abnormal cellular survival as a consequence of the failure to undergo apoptosis (analogous to bcl-2 overexpression). As Fas/APO-1 expression and function have been shown to be required for the Ca2+-independent mediation of T-cell cytotoxicity (42), disruption of Fas/APO-1 signaling may also provide a selective survival advantage in the escape of immune surveillance. To date, there are no published reports concerning Fas/APO-1 mutations in malignant cells; however, genetic defects resulting in the functional inactivation of Fas/APO-1 have been described in lpr mice with autoimmune disease (17). Alternately, or perhaps additionally, changes in the intracellular signaling pathways initiated by anti-Fas engagement may account for the disparate and unexpected effects of Fas/APO-1 in cell lines such as A375-SM and ME-180. Whatever the molecular mechanisms involved, further analysis of the Fas/APO-1 protein and studies using its natural cognate ligand will provide additional insight into the role of this unique protein in autoimmune disease, oncogenesis, and tumor progression.

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{
* 6 L. Owen-Schaub, unpublished observation.}


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