The Fas Counterattack: Fas-mediated T Cell Killing by Colon Cancer Cells Expressing Fas Ligand

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Summary

Tumors escape immunological rejection by a diversity of mechanisms. In this report, we demonstrate that the colon cancer cell SW620 expresses functional Fas ligand (FasL), the triggering agent of Fas receptor (FasR)–mediated apoptosis within the immune system. FasL mRNA and cell surface FasL were detected in SW620 cells using reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemical staining, respectively. We show that SW620 kills Jurkat T cells in a Fas-mediated manner. FasR-specific antisense oligonucleotide treatment, which transiently inhibited FasR expression, completely protected Jurkat cells from killing by SW620. FasL-specific antisense oligonucleotide treatment of SW620 inhibited its Jurkat-killing activity. FasL has recently been established as a mediator of immune privilege in mouse retina and testis. Our finding that colon cancer cells express functional FasL suggests it may play an analogous role in bestowing immune privilege on human tumors. HT29 and SW620 colon cancer cells were found to express FasR mRNA and cell surface FasR using RT-PCR and immunofluorescence flow cytometry, respectively. However, neither of these cells underwent apoptosis after treatment by the anti-FasR agonistic monoclonal antibody CH11. Our results therefore suggest a Fas counterattack model for immune escape in colon cancer, whereby the cancer cells resist Fas-mediated T cell cytotoxicity but express functional FasL, an apoptotic death signal to which activated T cells are inherently sensitive.

Cancers escape immune clearance by a diversity of mechanisms. Although these include evasive strategies to avoid immune recognition, such as disruption of T cell–extracellular matrix interactions and aberration of antigen processing and presentation, tumors also engage in active modulation and suppression of immune cell function. Local immune suppression due to tumor-derived agents is a feature of many cancer types. Chemically induced mouse colon tumors have been shown to cause profound local suppression of mucosal immune function (1). The local immune suppression associated with esophageal squamous carcinoma is mediated by a tumor-derived factor that results in suppression and ultimately apoptotic cell death of activated lymphocytes (2). Well-established tumor-derived immune modulatory molecules include downregulatory cytokines, immunosuppressive aminosugars (free hexosamines) (3), and gangliosides (4, 5).

Fas ligand (FasL)[1] is a key molecule in normal immune development, homeostasis, modulation, and function (6). Ligation onto its receptor, FasR (CD95), on sensitized cells induces programmed cell death, or apoptosis. This Fas-mediated apoptotic death signal has a number of important immunological roles. Fas-mediated apoptosis is involved in such functions as thymocyte clonal deletion and tolerance acquisition (7), T cell activation–induced cell death (8), immune response termination (9), and T cell–mediated cytotoxicity (10). Dysfunction of Fas-mediated apoptosis has been associated with lymphoproliferative diseases and autoimmunity in humans (11, 12) and mice (13).

Recently, two reports established a role for FasL in the maintenance of immune privilege in mouse testis (14) and in the anterior chamber of the eye (15). FasL expressed in these tissues induces apoptosis in activated lymphocytes that infiltrate these sites.

FasL has been found to be constitutively expressed in some NK lymphomas and T cell–type large granular lymphocyte leukemias—malignancies of cells that normally express FasL upon activation (16). Because of its central role in lymphocyte modulation through delivery of an apoptotic death signal, we investigated the expression and function of FasL in gastrointestinal cancer cells as a candidate for tumor-derived immune modulation and hence tumor immune escape.

FasR expression and response are variable in human lymphoid (17, 18) and nonlymphoid malignancies (19), and

[1] *Abbreviations used in this paper:* DOTAP, *N*-[1-(2,3-Dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate; FasL, Fas ligand; FasR, Fas receptor; RT, reverse transcription.

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resistance to Fas-mediated cytotoxicity may contribute to tumor immune escape. Resistance to cellular apoptotic mechanisms in general is thought to contribute to tumorigenicity (20), and recent studies have shown that known tumor promoters, including nicotine, inhibit both FasR- and TNF-mediated apoptosis (21). Resistance to FasR has been observed in HIV- (22) and HTLV-1 (23) –infected T cells and may contribute to viral immune escape. Because of its role in receiving and transducing the FasL-mediated apoptotic signal from cytotoxic T cells, and because dysfunction in the FasR-signaling pathway results in resistance to Fas-mediated cytotoxicity, we also assessed FasR expression and function on gastrointestinal cancer cells.

Materials and Methods

Cells. OC1 and OC2 are human esophageal squamous carcinoma cell lines developed in our laboratory (24). HT29 and SW620 human colon epithelial adenocarcinoma cell lines and the Jurkat human T leukemia cell line were obtained from American Type Culture Collection (Rockville, MD). All cells were grown in DMEM supplemented with 10% FCS in a humidified 10% CO_2 atmosphere, except as otherwise indicated.

Reverse Transcription (RT) PCR Detection of FasR and FasL mRNA Expression. RNA was isolated from cells by lysis in guanidine thiocyanate (Sigma Chemical Co., St. Louis, MO) followed by phenol extraction and ethanol precipitation. cDNA was synthesized using AMV reverse transcriptase (Promega Corp., Madison, WI) and random hexanucleotide primers (Boehringer Mannheim GmbH, Mannheim, Germany).

PCR was performed on the cDNA using the following sense and antisense primers, respectively: FasR: CAGAACTTGGAAGGCCTGCATC and TCTGTTCTGCTGTGTCTTGGAC; FasL: GGATTGGGCCTGGGGATGTTTCA and TTGTGGCTCAGGGGCAGGTTGTTG; β-actin: GTGGGGCGCCCCAGGCACCA and CTCCTTAATGTCACGCACGATTTC.

PCR primers were designed using the DNASTAR Lasergene Primerselect program (DNASTAR, Inc., Madison, WI). Primer pairs were chosen to span introns in their genomic sequences, thus ensuring mRNA-specific amplification. Primers were selected that showed insignificant homology to any other genes in the EMBL DNA sequence database. The FasR primers span exons 3–6 and thus enable amplification of the three splice variants of FasR mRNA identified in normal activated lymphocytes that code for soluble forms of FasR (25).

Thermal cycling was as follows: denaturation at 96°C for 15 s; annealing at 55°C for 30 s, and extension at 72°C for 3 min. 40 cycles were performed for the FasR and FasL PCRs, 35 cycles for the β-actin PCR. Primers were used at a final concentration of 0.1 μM each, dNTPs at 50 μM, and $MgCl_2$ at 1.5 mM. 1 U of Taq DNA polymerase was used per 50-μl reaction. PCR products were analyzed by electrophoresis through 2% agarose gels and viewed under UV light after ethidium bromide staining. HaeIII-digested ΦX174 DNA size markers were used. PCR product specificities were confirmed by restriction mapping.

Immunohistochemical Detection of Cell Surface FasL. SW620 cells were cultured on glass chamber slides (Nunc, Inc., Naperville, IL). After fixation in 4% paraformaldehyde for 1 h, slides were washed twice for 5 min in a wash buffer containing 50 mM Tris-Cl, pH 7.6, 50 mM NaCl; and 0.001% saponin. Endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol for 5 min. Slides were washed as before except that the wash buffer for this and all subsequent steps included 1% normal goat serum. Slides were then blocked for 1 h in wash buffer containing 5% normal goat serum. Slides were washed and incubated overnight at 4°C with a rabbit polyclonal anti–human FasL-specific IgG (Santa Cruz Biotechnology, Santa Cruz, CA) at 0.1 $\mu g\ ml^{-1}$ in wash buffer. Antibody binding was localized using a biotinylated secondary antibody, avidin-conjugated horseradish peroxidase, and diaminobenzidine substrate, contained within the Vectastain ABC detection kit (Vector Laboratories, Inc., Burlingame, CA). Staining with isotype-matched rabbit IgG was performed as a negative control. The immunizing peptide (NH_2-terminal amino acids 2–19; Santa Cruz Biotechnology) was included at 1 $\mu g/ml^{-1}$ during the primary antibody incubation as a specific inhibitor of FasL staining in additional control staining. Slides were counterstained with hematoxylin.

Immunofluorescence Flow Cytometric Measurement of Cell Surface FasR. Mouse anti–human FasR mAb (IgG_1) was obtained from PharMingen (San Diego, CA). Adherent cells were harvested by scraping. Cells were washed in PBS and incubated with 5 $\mu g/ml^{-1}$ mAb for 30 min at 4°C and washed in PBS containing 2% FCS. FITC-conjugated secondary antibody (Dako Corp., Carpinteria, CA) was added to the cells for 30 min at 4°C. Cells were washed again in PBS containing 2% FCS. Flow cytometric analysis was performed using a flow cytometer (Epics Elite; Coulter Corp., Hialeah, FL). 10,000 cells were examined for each determination. Isotype-matched control antibody was used in negative control staining.

Assessment of Anti-FasR mAb CH11-induced Apoptosis. Sensitivity of cells to Fas-mediated apoptosis was determined by treatment with the agonistic anti-FasR CH11 IgM mAb (Kamiya Biomedical Co., Thousand Oaks, CA) or isotype control IgM at 0.1 $\mu g/ml^{-1}$. After antibody treatment, DNA was isolated from cells by the following procedure: cells were lysed in 0.5 ml of a buffer consisting of 100 mM Tris-Cl, pH 8; 150 mM NaCl; 20 mM EDTA, and 0.8% sodium lauryl sarcosinate. RNA was eliminated by the addition of 10 μl of RNase A (Boehringer Mannheim GmbH; at a concentration of 10 mg/ml in a buffer containing 10 mM Tris-Cl, pH 7.6, and 15 mM NaCl that was rendered DNase free by boiling for 15 min followed by slow cooling to room temperature) and digesting for 1 h at 37°C. Proteins were degraded by digestion with 10 μl of proteinase K (Boehringer Mannheim GmbH; 20 mg/ml in water) at 50°C for 2 h. Chromosomal DNA was then purified by a single phenol extraction followed by ethanol precipitation, after which the DNA pellet was redissolved in 20 μl of TE (10 mM Tris-Cl, pH 8.0, and 1 mM EDTA). DNA integrity was assessed by electrophoresis through 2% agarose gels, DNA internucleosomal fragmentation or laddering being indicative of apoptosis.

Antisense Oligonucleotide Treatment. During antisense treatment, cells were maintained in reduced-serum medium (OptiMEM; Sigma Chemical Co.) to limit the degradation of oligonucleotides by serum-derived nucleases (26). Oligonucleotide uptake was facilitated by complexing with the cationic lipid transfection reagent *N*-[1-(2,3-Dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (DOTAP), which has been shown to enhance DNA uptake by cells (27). Complexing with DOTAP has also been shown to protect oligonucleotides from nucleolytic degradation within the cell. Cells were treated with oligonucleotides and the cationic lipid vector DOTAP (Boehringer Mannheim GmbH) at final concentrations of 10 and 13 μM, respectively for 24 h. Oligonucleotides and the transfection reagent DOTAP were complexed at 100× concentration for 5 min at

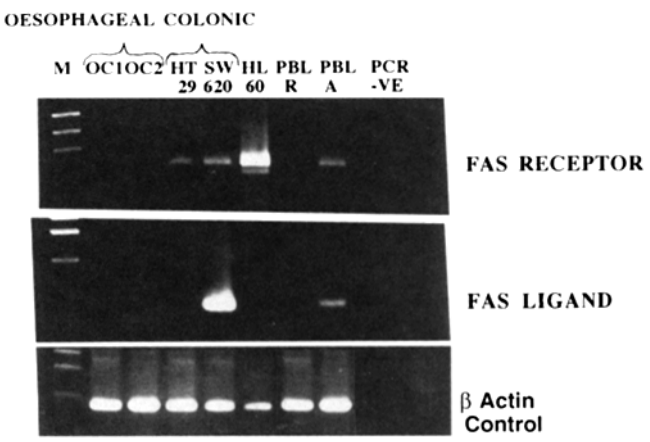

Figure 1. FasR and FasL mRNA expression in gastrointestinal cancer cells. Expression was analyzed by RT-PCR of equalized input RNA isolated from each cell line. Resting (*R*) and PHA-activated (*A*) PBL were used as negative and positive controls, respectively. β-actin control PCR was performed to monitor RT-PCR amplification efficiency. mRNA-specific amplification product bands for FasR (682 bp), FasL (344 bp), and β actin (540 bp) are indicated. The minor band (619 bp) obtained from the HL60 neutrophil control mRNA corresponds to the FasR Delta 1 mRNA splice variant, which encodes a soluble form of FasR. ΦX174-HaeIII size markers (*M*) were used.

37°C before addition to cells. After treatment, cells were washed free of the oligonucleotide/DOTAP–containing medium and resuspended in fresh medium before further manipulation. The antisense PCR primers for FasR and FasL also served as antisense oligonucleotides. The control nonsense oligonucleotide had the following nonspecific nucleotide sequence: AATTCTACTG-GTTGTTCTGCTGGT.

Coculture DNA Fragmentation Assay (The JAM Test). Target Jurkat cell death resulting from coculture with effector colon cancer cells was quantitated by measurement of target cell DNA fragmentation using the JAM test (28). The adherent colon cancer cells were seeded into the wells of a flat-bottomed 96-well microtiter plate at cell numbers appropriate to give the required E/T ratios. The cells thus seeded were incubated at 37°C for 24 h and aspirated before the addition of 2×10^4 Jurkat target cells. Target cell DNA was labeled by prior incubation with 10 μCi/ml of [^{3}H]TdR at 37°C for 3 h. Oligonucleotide-treated cells were washed in culture medium (OptiMEM) before labeling. Labeled target cells were washed and added to the seeded effector cells in a final volume of 200 μl per well. After coculture at 37°C for 8 h, the cells were removed from the wells by pipetting up and down five times and were collected by filtration onto glass fiber filters using a 96-well filtration unit. The cells were hypotonically lysed, and fragmented DNA was washed through the filter by four washes of 0.25 ml of water. The radioactivity of intact chromosomal DNA retained on each filter was measured by liquid scintillation counting. Specific cell killing was calculated using the following equation:

$$\% \text{ Specific killing} = (S - E/S) \times 100$$

where *E* (experimental) is cpm of retained DNA in the presence of effector cells, and *S* (spontaneous) is cpm of retained DNA in the absence of effector cells. Use of the value of *S* rather than total incorporated counts in the equation corrects for spontaneous target cell DNA fragmentation during the assay.

Results and Discussion

FasR-bearing Colon Cancer Cells Are Resistant to Fas-mediated Induction of Apoptosis. RT-PCR results show that both colon adenocarcinoma cell lines HT29 and SW620, but neither esophageal squamous carcinoma cell lines OC1 or OC2, express FasR mRNA (Fig 1). The FasR RT-PCR assay was controlled by equalization of input RNA for each cell line. Comparable amplification efficiencies were achieved in all RNA samples as evidenced by the uniformity of control β-actin RT-PCR product yields. Equivalent FasR PCR product band intensities suggest that both HT29 and SW620 express levels of FasR qualitatively similar to that expressed in PHA-activated PBL. This was confirmed by detection of cell surface FasR on HT29 and SW620 cells by immunofluorescence flow cytometry after staining with a FasR-specific mAb (Fig. 2). FasR staining was absent from OC1 and OC2.

FasR expression alone does not imply sensitivity to Fas-mediated apoptosis, and other factors determine whether the FasL signal is transduced. Low FasR–expressing malignant glioma cells showed that a critical level of FasR expression is required for apoptotic signaling. Simply elevating subcritical FasR expression rendered these cells Fas sensitive (29). Mutations of p53 have been associated with lack of FasR expression in transformed cells (30). IFN-γ and TNF-α are required to potentiate FasR in some normal (31–33) and malignant (29, 34) cells. The Fas-sensitizing effect of these cytokines is partly associated with induction or upregulation of FasR expression. Indeed, IFN-γ has been shown to elevate FasR expression in HT29 (35). As our results indicate, HT29 and SW620 constitutively express levels of FasR mRNA comparable with that expressed in activated lymphocytes and cell surface FasR comparable with that expressed in Fas-sensitive Jurkat cells

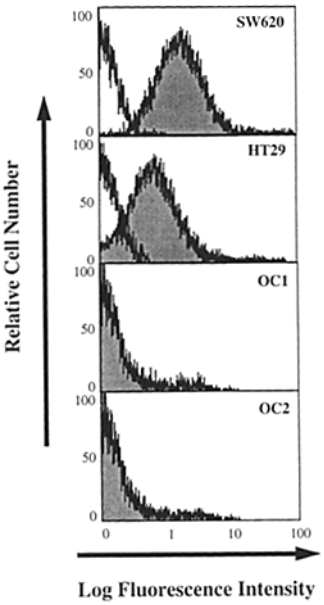

Figure 2. Cell surface FasR expression in gastrointestinal cancer cells. 10^6 cells were stained with mouse anti–human FasR monoclonal IgG followed by staining with a secondary FITC-conjugated anti–mouse IgG antibody. FasR expression was determined by flow cytometric analysis. The profiles obtained by FasR antibody staining (*shaded peaks*) relative to control antibody staining (*open peaks*) are shown.

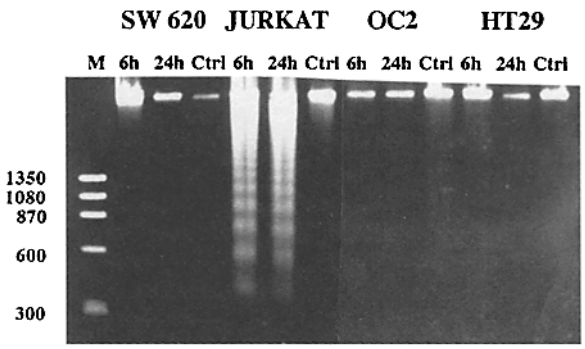

Figure 3. Resistance of gastrointestinal cancer cells to Fas-mediated apoptosis. DNA was isolated from cells after treatment with anti-FasR CH11 mAb (0.1 $\mu g/ml^{-1}$) for 6 and 24 h or with isotype control mouse IgM for 24 h (*Ctrl*). DNA integrity was assessed after electrophoresis through a 2% agarose gel. Sizes of markers (*M*) are indicated in bp. DNA fragmentation or laddering is indicative of apoptosis.

(see Fig. 5). Hence, receptor level should be adequate for Fas signaling in these cells.

FasR function in HT29 and SW620 was assessed by treatment of these cells with the anti-FasR agonistic mAb CH11 and analysis of anti-FasR–induced internucleosomal DNA cleavage. After 6- and 24-h incubations with this mAb, neither HT29 nor SW620 showed any evidence of apoptosis using the DNA fragmentation assay (Fig. 3). After CH11 treatment, both cell types showed intact chromosomal DNA, equivalent in integrity to untreated cells incubated for 24 h. Both cell lines were as refractory to the effect of the anti-FasR agonistic mAb as the FasR-negative OC2 cell line. Under identical conditions, treatment of the Fas-sensitive Jurkat control cells with CH11 resulted in pronounced apoptotic DNA fragmentation into DNA ladders of nucleosomal oligomers of ~180 bp after 6 h of treatment relative to the intact DNA of untreated Jurkat cells incubated for 24 h. Hence, HT29 and SW620, although expressing cell surface FasR, are resistant to induction of apoptosis through agonistic engagement of FasR.

Expression of soluble, potentially antagonistic forms of FasR from splice variants of FasR mRNA lacking the transmembrane exon has been shown in some cells (36) and may contribute to Fas resistance in certain pathological conditions, including SLE (37, 38). Three splice variants of FasR mRNA have been detected in normal activated lymphocytes (25). Although a minor band was observed in the RT-PCR products from the HL60 control mRNA, which corresponds to the FasR Delta 1 mRNA splice variant, no PCR product bands derived from amplification of spliced variants Delta 1, 2, or 3 (619-, 435-, or 372-bp expected product sizes, respectively) were detectable in any of the other cell lines. Hence, expression of soluble FasR can be eliminated as a mechanism of Fas resistance in HT29 and SW620.

The level of expression of genes involved in the control of apoptosis can affect the Fas sensitivity of cells. Expression and upregulation of *bcl 2* has been implicated in a variety of cell types as protective against apoptotic cell death, including Fas-mediated apoptosis (34, 39–41). Using immunofluorescence flow cytometry, we found that although SW620 expresses bcl 2, the level of expression in HT29 was negligible (unpublished observations), so that Fas resistance in these colon adenocarcinoma cells does not corelate with bcl 2 expression.

Colon Cancer Cell SW620 Expresses FasL. RT-PCR results show that SW620, but none of the other cell lines HT29, OC1, or OC2, expresses FasL mRNA (Fig. 1). The FasL PCR was performed on the same cDNA preparations used for the FasR and β-actin control RT-PCR assays and was therefore similarly controled for equalization of input RNA and amplification efficiency. By comparing FasL RT-PCR band intensities, SW620 expresses a level of FasL mRNA qualitatively much higher than that expressed by PHA-activated PBL. Immunohistochemical staining shows that SW620 expresses cell surface FasL (Fig. 4). FasL specificity was confirmed as staining of SW620 was inhibited by inclusion of the immunizing FasL peptide as a competitive inhibitor in the primary antibody incubation. These findings show that FasL expression is not restricted to lymphoid cells and tissues and sites of immune privilege in the body.

Using Fas-sensitive Jurkat indicator cells, FasL activity was undetectable in culture fluid conditioned by SW620 cells. Jurkat cells were incubated for 24 h in SW620 cell–conditioned medium or mixtures of cell-conditioned and fresh media. This treatment failed to cause induction of apoptosis above background levels (10–15% in Jurkat cells) detectable by either the DNA fragmentation assay or flow cytometric detection of apoptotic bodies after propidium iodide staining of treated cells (unpublished observations). This suggests that the FasL expressed by SW620 is not shed by these cells.

Fas-sensitive Jurkat T Cells Are Killed by Coculture with SW620. Having demonstrated that SW620 cells express FasL, but are themselves resistant to Fas-mediated apoptosis, we wished to ascertain if the FasL was functional and therefore if SW620 could induce apoptosis in Fas-sensitive cells. In particular, we wished to know if SW620 cancer cells could kill activated, Fas-sensitive T cells in this way, which would suggest a potential mechanism of tumor immune escape. To address this question, we used Jurkat as a target cell in coculture experiments with SW620. Jurkat is a Fas-sensitive cell line of T cell origin that is constitutively activated and has been widely used experimentally as a model for activated T cells, with which it shares functional similarities. Jurkat cells are particularly appropriate target cells for investigating putative FasL activity because they are insensitive to TNF-α, another major mediator of apoptotic cell death, and to lymphotoxin α (8).

Using the DNA gel fragmentation assay, we observed that DNA from cocultured Jurkat and SW620 cells showed pronounced nucleosomal DNA laddering relative to either cell line incubated alone or to Jurkat cells cocultured with FasL-negative HT29 colon cancer cells. These preliminary results were suggestive of apoptotic killing of the Jurkat cells by the Fas-resistant, FasL-expressing SW620 cells. A more sensitive and controled coculture cell killing assay,

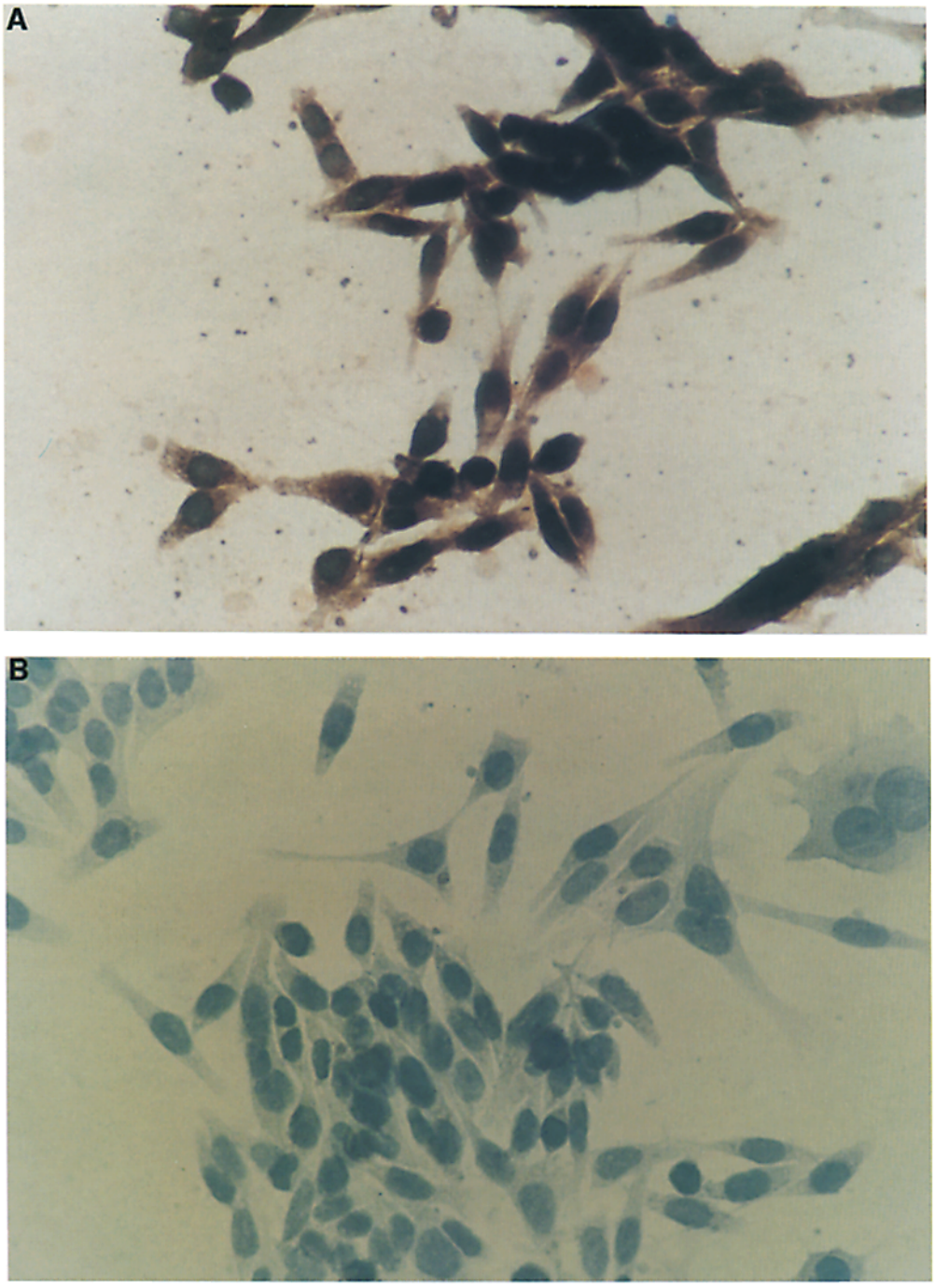

Figure 4. Colon cancer cell SW620 expresses cell surface FasL. SW620 cells were stained with a rabbit polyclonal anti–human FasL-specific IgG and counterstained with hematoxylin (*purple*). Positive staining (*brown*) was obtained (*A*), whereas isotype control rabbit IgG failed to stain the cells (*B*). Positive staining was reduced by coincubation of the primary antibody with the FasL immunizing peptide (not shown).

the JAM test (28), was adopted to resolve the source of the apoptotic DNA fragments observed after coculture of Jurkat with SW620 cells. By prelabeling the target Jurkat cell DNA with [^{3}H]TdR, specific DNA fragmentation of the target cells in response to the cocultured effector SW620 cells was quantified. As seen in Fig. 6 *A*, SW620 effected 30% specific killing of Jurkat cells at an E/T ratio of 25:1 in an 8-h incubation. Cell killing increased with increasing E/T ratio, and pronounced killing (>20%) occurred even at a low E/T ratio equivalent to 5:1.

FasR-specific Antisense Oligonucleotide Treatment Protects Jurkat Cells from Killing by SW620. To determine whether the killing of Jurkat cells by coculture with SW620 was Fas mediated, we used Fas-specific antisense oligonucleotide treatment to render Jurkat cells temporarily FasR negative for use as control targets. The efficacy of FasR antisense oligonucleotide treatment was verified by immunofluorescence flow cytometry of treated Jurkat cells after staining with a Fas-specific mAb. In the FasR antisense oligonucleotide–treated Jurkat cells there was a reduction of cell sur-

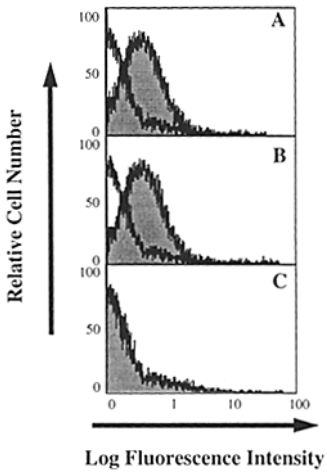

Figure 5. Inhibition of FasR expression in Jurkat T cells by FasR-specific antisense oligonucleotide treatment. DOTAP-mediated Fas-specific or nonspecific control oligonucleotide treatment of Jurkat cells was performed for 24 h in optimized reduced-serum medium (OptiMEM). After oligonucleotide treatment, 10^6 cells were stained with mouse anti-human FasR monoclonal IgG followed by staining with a secondary FITC-conjugated anti-mouse IgG antibody. FasR expression was determined by flow cytometric analysis. The profiles obtained by FasR antibody staining (*shaded peaks*) relative to control antibody staining (*open peaks*) are shown for untreated (*A*), nonsense oligonucleotide–treated (*B*), and FasR-specific antisense oligonucleotide–treated Jurkat T cells (*C*).

face FasR staining to background levels. In the control nonsense oligonucleotide–treated cells, staining for FasR was equivalent to that of untreated Jurkat cells (Fig. 5). This indicates that antisense oligonucleotide treatment effectively and specifically abolishes FasR expression in Jurkat cells.

In the JAM cell-killing test, it was found that FasR antisense oligonucleotide treatment completely protected Jurkat cells from killing by SW620 at all the tested E/T ratios (Fig. 6 *A*). Control nonspecific oligonucleotide treatment had no effect on killing of Jurkat cells by SW620. As FasR antisense–treated Jurkat cells were demonstrated to be FasR negative as opposed to the FasR-positive nonsense–oligonucleotide treated cells (Fig. 5), these results strongly suggest that SW620 kills Jurkat cells in a Fas-dependent manner.

FasL-specific Antisense Treatment Inhibits SW620 Killing of Jurkat T Cells. To confirm that SW620-induced killing of Jurkat T cells was mediated by FasL, the SW620 cells were tested for cytotoxic activity after pretreatment with antisense oligonucleotides specific for the FasL gene. The JAM test was performed as described at an E/T ratio of 10:1, previously shown to result in significant cell killing (Fig. 6 *A*), with FasL-specific antisense and control nonsense oligonucleotide–treated SW620 effector cells. Although our results indicated that SW620 did not shed FasL at detectable levels, the SW620 cells were washed rigorously to eliminate the possibility of residual soluble FasL remaining in the culture medium of the oligonucleotide-treated cells. The results show that FasL antisense treatment resulted in significant inhibition, by ~60%, of the killing effect of

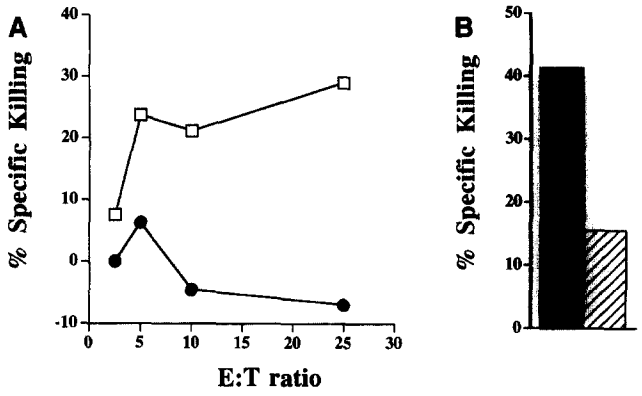

Figure 6. FasL-expressing colon cancer cell SW620 kills Jurkat T cells in a Fas-dependent manner. [^{3}H]TdR-labeled Jurkat target cells were cocultured with SW620 effector cells at the indicated E/T ratios. Target cell death was determined 8 h later by measuring DNA fragmentation of the ^{3}H-labeled target cell DNA (JAM test). Specific cell death was calculated relative to spontaneous cell death occurring in target cells alone. Each percent of specific killing value represents the mean of quadruplicate coculture cell-killing assays. (*A*) FasR-specific antisense oligonucleotide treatment of Jurkat T cells results in complete protection from killing by SW620 colon cancer cells. Nonspecific "nonsense" control oligonucleotide–treated Jurkat cells (FasR positive) are killed by coculture with SW620 cells (*open squares*). FasR-specific antisense oligonucleotide–treated Jurkat cells (FasR negative) are completely immune to killing by SW620 (*solid circles*). (*B*) FasL-specific antisense oligonucleotide treatment inhibits killing of Jurkat T cells by SW620. FasL-specific antisense oligonucleotide treatment results in 60% inhibition of killing of Jurkat T cells by SW620 (*hatched bar*) relative to nonspecific "nonsense" oligonucleotide–treated SW620 cells (*solid bar*). An E/T ratio of 10:1 was used.

SW620 on Jurkat T cells relative to the nonsense oligonucleotide–treated control SW620 cells (Fig. 6 *B*). These data suggest that killing of Jurkat cells was mediated by FasL expressed by SW620 cells.

The Fas Counterattack. We demonstrate that colon cancer cell SW620 expresses functional FasL and kills the activated T cell, Jurkat, in a Fas-dependent manner. We also show that colon cancer cells SW620 and HT29 express

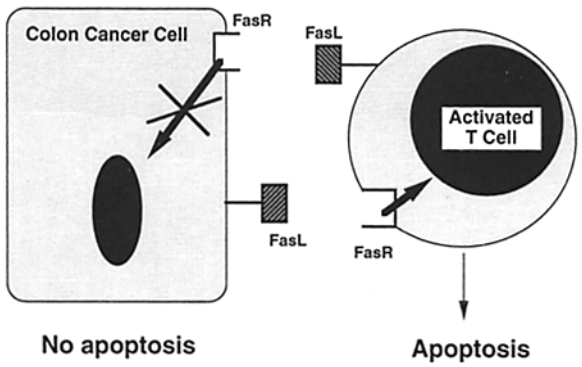

Figure 7. Proposed Fas counterattack model of tumor immune escape in colon cancer. The colon cancer cell expresses FasR but is resistant to Fas-mediated T cell cytotoxicity. However, the colon cancer cell expresses functional FasL, which rapidly induces apoptosis in Fas-sensitive activated T cells, which infiltrate the tumor.

FasR but are resistant to Fas-mediated apoptosis. Although the cause(s) of Fas resistance in these cells remains to be elucidated, our results eliminate underexpression of cell surface FasR or expression of soluble FasR as reasons for resistance.

Our results suggest a Fas counterattack model as a mechanism of immune escape in colon tumors (Fig. 7). It shows how a tumor may exploit an intrinsic cell death program of the activated T cells that infiltrate it. Essentially the cancer cell counterattacks the activated cytotoxic T cell that challenges it with one of the T cell's own principal cytotoxic armaments: FasL. The counterattack is rendered more effective as the cancer cell itself is resistant to the cytotoxic effect of FasL, whereas the attacking T cell is inherently sensitive to its apoptotic death signal.

This mechanism is analogous to the recently established role of FasL in mediating immune privilege in mice. FasL expressed in tissues and at sites of immune privilege, such as the testis (14) and the anterior chamber of the eye (15), induces apoptosis in activated lymphocytes that infiltrate these sites. Expression of functional FasL by human tumors could conceivably confer immune-privileged status on such malignancies.

Other selective advantages could theoretically accrue from expression by a tumor of such an important biological death factor to which the tumor cells themselves are resistant. FasL expression could conceivably facilitate the establishment of tumors or tumor metastases at sites where the indigenous cells express FasR and can therefore be rendered subject to the FasL-mediated cytotoxicity of the tumor. In this respect it is of interest that the FasL-expressing SW620 cell line was derived from a lymph node metastasis of a primary colon carcinoma.

Subsequent to the investigation of FasL expression and function in SW620, using RT-PCR, two other colon adenocarcinoma cell lines, T84 and CaCo2, were found to express FasL mRNA. This suggests that FasL expression may be a prevalent feature of colon carcinoma.

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