Tumor Necrosis Factor–related Apoptosis-inducing Ligand (TRAIL) Is an Inhibitor of Autoimmune Inflammation and Cell Cycle Progression

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Abstract

The tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induces apoptosis of tumor cells but not normal cells; its role in normal nontransformed tissues is unknown. We report here that chronic blockade of TRAIL in mice exacerbated autoimmune arthritis, and that intraarticular TRAIL gene transfer ameliorated the disease. In vivo, TRAIL blockade led to profound hyperproliferation of synovial cells and arthritogenic lymphocytes and heightened the production of cytokines and autoantibodies. In vitro, TRAIL inhibited DNA synthesis and prevented cell cycle progression of lymphocytes. Interestingly, TRAIL had no effect on apoptosis of inflammatory cells either in vivo or in vitro. Thus, unlike other members of the tumor necrosis factor superfamily, TRAIL is a prototype inhibitor protein that inhibits autoimmune inflammation by blocking cell cycle progression.

Key words: autoimmunity • inflammation • apoptosis • cytokine • TRAIL

Introduction

The TNF-related apoptosis-inducing ligand (TRAIL)[1] is a type II membrane protein of the TNF superfamily (1). Unlike other members of the TNF superfamily that interact with one or two specific receptors, TRAIL can potentially interact with five different receptors. These include death receptor (DR)4 (TRAIL-R1), DR5 (TRAIL-R2), decoy receptor (DcR)1 (TRAIL-R3, TRAIL receptor without an extracellular domain [TRID]), DcR2 (TRAIL-R4, TRAIL receptor with a truncated death domain [TRUNDD; references 2–8]), and a soluble receptor called osteoprotegerin (9).

Although the presence of multiple TRAIL receptors strongly suggests that TRAIL is involved in multiple processes, the precise roles of TRAIL in health and disease are unknown. In vitro studies have shown that TRAIL induces apoptosis of some, but not all, tumor cell lines (2, 6). This appears to be mediated by DR4 and DR5, which possess similar intracellular death domains as TNF receptors and CD95 (Fas/Apo-1) and are capable of activating the caspase cascade. The presence of DcR1 and DcR2, which do not contain functional death domains, blocks TRAIL-induced apoptosis (2, 6). Although both TRAIL and TRAIL receptors are constitutively expressed in various tissues (1, 2, 4, 10) and are upregulated upon cell activation (5, 11, 12), TRAIL may not induce apoptosis of most nontransformed cells (2, 6). In vivo administration of recombinant TRAIL selectively kills tumor cells but not normal cells, leaving the host organ systems unharmed (13, 14). Although this finding has generated tremendous interest in using recombinant TRAIL for cancer therapy, it also raises fundamental questions regarding the roles of TRAIL in normal nontransformed tissues. We report here that unlike many other members of the TNF family, TRAIL is a potent inhibitor of autoimmune inflammation and cell cycle progression.

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[1]*Abbreviations used in this paper:* Ad, adenovirus; AICD, activation-induced cell death; ANOVA, analysis of variance; BrdU, bromodeoxyuridine; DR, death receptor; DcR, decoy receptor; HE, hematoxylin and eosin; HSA, human serum albumin; L, ligand; NF, nuclear factor; s, soluble; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

Materials and Methods

Production of Recombinant DR5. To generate recombinant soluble (s)DR5, the cDNA that contains the full-length extracellular domain of the human DR5 (a gift from Dr. W. El-Deiry, Uni-

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versity of Pennsylvania, Philadelphia, PA; 15) was cloned into pGAPZα that contains a P_{AOX1} promotor and a six-histidine tag. Several recombinant *Pichia pastoris* clones generated via homologous recombination (16) secreted high levels of sDR5 (up to 25 mg/liter of yeast culture). The sDR5 used in this study was purified through a nickel ion column and depleted of LPS by incubation with polymyxin B–agarose (Sigma Chemical Co.). The purity of the sDR5 was confirmed by PAGE; sDR5, which had a mol wt of 26 kD, was the only protein band present. The purified sDR5 contained 1–2 ng LPS/mg of protein as determined by limulus amebocyte lysate (LAL) assay. This is comparable to the LPS level in BSA or human serum albumin (HSA) used in this study, which is 1–4 ng/mg of protein. In previous experiments, we examined whether BSA or HSA had any effect on collagen-induced arthritis using LPS-free PBS as control. No effects were observed with respect to arthritis and anticollagen immune responses (our unpublished data; 17). In vitro, purified sDR5 did not induce proliferation of lymphocytes regardless of its concentration in the culture (1–100 μg/ml; our unpublished data).

Generation of a Recombinant TRAIL Virus. A recombinant adenovirus (Ad) carrying the murine TRAIL gene (TRAIL-Ad) was generated by inserting the cloned TRAIL full-length cDNA into the plasmid pAdtet, followed by homologous recombination with human adenoviral DNA (17). In brief, the murine full-length TRAIL cDNA was generated from mouse spleen by PCR using specific primers corresponding to the 5′ and 3′ ends of the coding regions of the TRAIL gene. After adding A-overhangs by incubation with Taq polymerase, the PCR fragment was inserted into the expression vector pCRII-TOPO, which possesses T-overhangs, to create pCRII-TOPO-TRAIL. After amplification of the pCRII-TOPO-TRAIL vector DNA, the TRAIL gene was cut out with NotI and inserted into the NotI site of the vector pAdtet to create pAdtet-TRAIL. The ClaI-digested Ad5 genomic DNA was then prepared from H5.000CMVEGFP, a recombinant Ad that is depleted of E1a, E1b, and a portion of E3 region but contains the green fluorescent protein cDNA. The recombinant virus was produced by cotransfecting 293 cells with pAdtet-TRAIL and ClaI-digested Ad5 genomic DNA. White plaques of recombinant viruses were expanded and screened by PCR using TRAIL-specific primers. TRAIL-Ad was then propagated, purified through a cesium chloride gradient, and desalted on an Econo-Pac 10DG column (Bio-Rad). In vitro, TRAIL-Ad induced apoptosis of several tumor cell lines including Rat-1 cells and 293 cells; addition of sDR5 to the culture prevented TRAIL-Ad–induced apoptosis (Göke, A., and Y. Chen, unpublished results).

Induction and Examination of Arthritis. 6–8-wk-old male DBA/1 mice (The Jackson Laboratory) were immunized by multiple intradermal injections of 100 μg chicken type II collagen (Sigma Chemical Co.) in 100 μl PBS emulsified in an equal volume of CFA containing 1 mg/ml of *Mycobacterium tuberculosis* H37 RA (Difco). Mice were rechallenged with the same antigen preparation subcutaneously on the flanks 21 d later. Mice were examined daily for signs of joint inflammation and scored as follows: 0, normal; 1, erythema and mild swelling confined to the ankle joint or midfoot; 2, erythema and mild swelling extending from the ankle to the midfoot; 3, erythema and moderate swelling extending from the ankle to the metatarsal joints; 4, erythema and severe swelling extending from the ankle to the digits. The maximal disease score per foot is 4, and the maximal disease score per mouse is 16. The mean disease score per group is calculated as follows: total disease scores from all animals in the group divided by the number of animals in the group.

Histochemical Analysis. Paws were first fixed in 10% formalin, decalcified in hydrochloric acid, and embedded in paraffin. Joint sections (6 μm) were then prepared and stained with hematoxylin and eosin (HE). The degree of arthritic inflammation was scored as follows: 0, no signs of inflammation; 1, mild synovitis; 2, severe synovitis; 3, severe synovitis with mild cartilage and bone destruction; 4, severe synovitis with severe cartilage and bone destruction.

Bromodeoxyuridine Labeling of Proliferating Cells In Vivo. Mice were immunized twice with chicken type II collagen as described above. Starting from the second immunization, mice received daily intraperitoneal injections of 0.8 mg bromodeoxyuridine (BrdU) in 0.5 ml PBS. Mice were killed 2–3 wk later, and their synovial joints were collected and embedded in paraffin. Synovial sections (6 μm) were then stained with rat anti-BrdU antibody and peroxidase-labeled goat anti–rat IgG as described (18). Control antibodies and tissues were routinely used to exclude nonspecific staining.

Apoptotic Studies. For detection of apoptotic cells, ApopTag® system was used (Oncor). In brief, synovial tissues were snap-frozen and cryosectioned (6 μm). The 3′-OH ends of fragmented DNA were labeled with digoxigenin-conjugated nucleotide using terminal deoxynucleotidyl transferase. The randomly incorporated nucleotide polymers were then detected by peroxidase-labeled

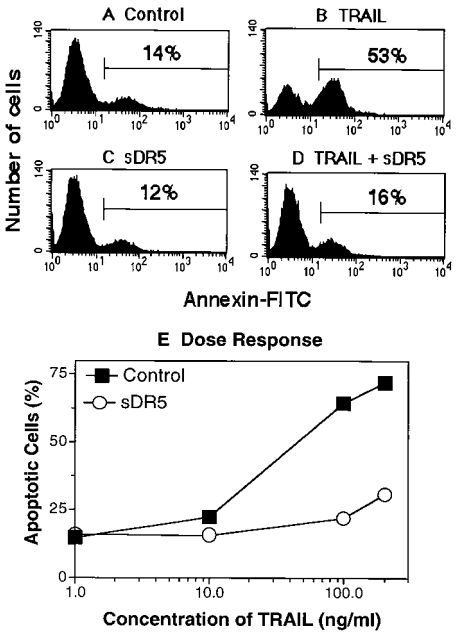


Figure 1. Recombinant sDR5 blocks TRAIL-induced apoptosis of tumor cells. Human Jurkat T cells (clone E6-1) and K562 B cells (10^6/ml) were cultured in RPMI 1640 medium containing various concentrations of TRAIL (Biomol® Research Laboratories), with or without 5 μg/ml of sDR5. 18 h later, apoptosis was determined by flow cytometry using annexin V–FITC according to the manufacturer's instructions (PharMingen). Jurkat cells were treated with either (A) 5 μg/ml of BSA, (B) 100 ng/ml of TRAIL, (C) 5 μg/ml of sDR5, or (D) 100 ng/ml of TRAIL plus 5 μg/ml of sDR5. Each histogram represents 10,000 events, with the apoptotic cells gated. (E) K562 cells were treated with various concentrations of TRAIL with (○) or without (■) 5 μg/ml of sDR5. The percentage of apoptotic cells was determined as shown above in A–D. In parallel experiments, we also tested whether sDR5 blocked Fas- and ultraviolet-induced apoptosis of Jurkat cells; no effect of sDR5 on apoptosis was detected in these systems (our unpublished data).

antidigoxigenin antibody and the chromogen diaminobenzidin. Counterstain was performed with methyl green. The apoptotic index was recorded as follows: 0, <1% of cells in the synovium are apoptotic; 1, 1–3% of cells in the synovium are apoptotic; 2, 3.1–5% of cells in the synovium are apoptotic; 3, >5% of cells in the synovium are apoptotic.

Results

Recombinant sDR5 Blocks TRAIL-induced Apoptosis of Tumor Cells. To determine the biological roles of TRAIL in vivo and in vitro, we produced large quantities of sDR5 using the yeast *P. pastoris* system (16). The sDR5 consists only of the extracellular domain of the human TRAIL receptor DR5, and effectively blocks TRAIL-induced apoptosis of tumor cells. Fig. 1, A–D, illustrates the effect of sDR5 on TRAIL-induced apoptosis of Jurkat T leukemia cells, and Fig. 1 E demonstrates dose-dependent killing of B lymphoma cells by TRAIL, and its blockade by sDR5.

Roles of TRAIL in Autoimmune Arthritis. The effect of TRAIL blockade in vivo was first examined in a mouse model of rheumatoid arthritis (Fig. 2). DBA/1 mice were immunized to induce arthritis on days 0 and 21 with chicken type II collagen. Starting from day 21, mice were injected daily with either 100 μg sDR5 or a control protein (such as BSA or HSA). Arthritis was monitored by both clinical examination and histochemistry. As shown in Fig. 2 A, mice that received the control protein developed typical arthritis, which started ~10 d after the second immunization, and reached a maximal disease score of 7.8 by day 33. By contrast, in mice treated with sDR5, arthritis was significantly exacerbated. The mean day of onset in the sDR5-treated group was 7.5 ± 1.9 (days after second immunization), compared with 14.5 ± 1.8 in the control group ($P < 0.01$ as determined by ANOVA). The maximal disease score was increased from 7.8 in the control group to 12.4 in the sDR5-treated group. Fig. 2 B illustrates the dose-dependent effect of sDR5 on arthritis as judged by disease score per foot. It is apparent that sDR5 enhanced arthritic inflammation in most feet in a dose-dependent manner.

These results indicate that TRAIL may be an inhibitor of autoimmune arthritis. To directly test this theory, we performed intraarticular TRAIL gene transfer using a replication-defective Ad carrying the mouse TRAIL gene. The

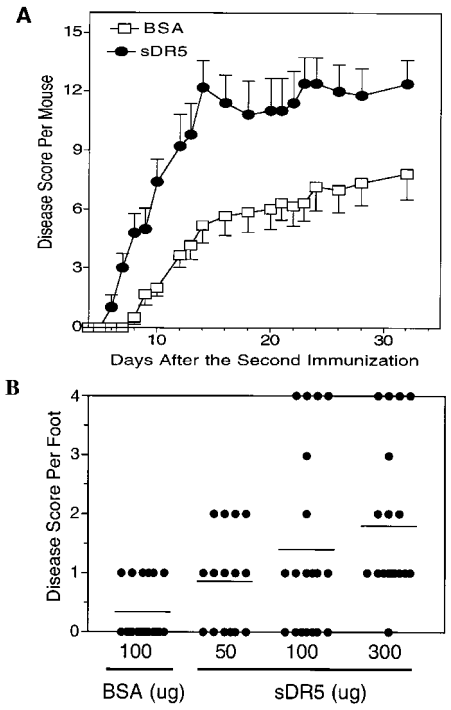


Figure 2. Exacerbation of collagen-induced arthritis by TRAIL blockade. Groups of DBA/1 mice (four to six per group) were immunized on days 0 and 21 with chicken type II collagen as described in Materials and Methods. Starting from the second immunization, mice received daily intraperitoneal injections of 50–300 μg sDR5 or BSA in 0.5 ml PBS for a total of 21 d (in parallel experiments, other control proteins such as HSA were also used; similar results as reported here were observed). (A) Disease courses in mice treated with 100 μg of BSA (□) or sDR5 (●). Each data point represents a mean ± SD from a total of five (for sDR5-treated group) or six (for BSA-treated group) mice. The experiments were repeated five times with similar results. The differences between the two groups are statistically significant ($P < 0.001$) as determined by Mann-Whitney test. (B) Disease scores of individual feet 12 d after the second immunization. A total of four groups of mice are shown: one was treated with 100 μg BSA, and the other three were treated with 50–300 μg sDR5. Each data point represents an individual foot, with 16–24 feet per group. The differences between BSA- and sDR5-treated groups are statistically significant as determined by ANOVA ($P < 0.05$ for mice treated with 50 μg sDR5, and $P < 0.01$ for mice treated with 100 or 300 μg sDR5).

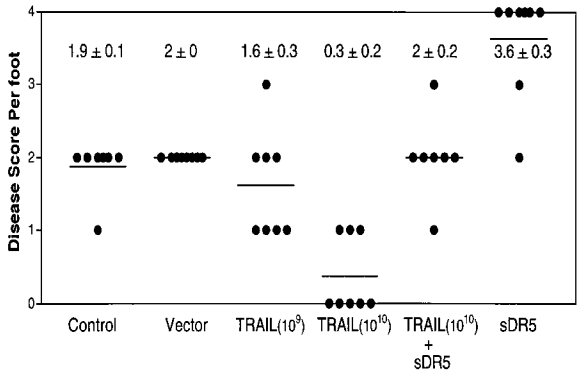


Figure 3. Inhibition of collagen-induced arthritis by TRAIL gene transfer. DBA/1 mice (four per group) were immunized with type II collagen as described in Materials and Methods. 6 d after the disease onset (10 d after the second immunization), mice were injected intraarticularly and periarticularly to the ankle and tarsal joints of the hind feet with (1) 10 μl PBS (control), (2) 10^{10} particles of Ad vector (the Ad vector contains no TRAIL gene but is otherwise identical to the TRAIL virus), (3) 10^9 particles of TRAIL virus, or (4) 10^{10} particles of TRAIL virus in 10 μl of PBS (reference 17). Starting from the day of the viral injection, two groups of mice, one nontreated and the other injected with 10^{10} particles of TRAIL virus, were subjected to daily intraperitoneal injections of 100 μg sDR5 for a total of 14 d. Data presented are disease scores of individual hind feet 6 d after viral injection. A total of eight hind feet per group are shown. Only mice that received 10^{10} particles of TRAIL virus showed significant improvement ($P < 0.001$ as determined by ANOVA). Results are representative of two experiments.

virus was injected directly into arthritic joints 6 d after disease onset as described previously (17). As shown in Fig. 3, arthritis was dramatically ameliorated after intraarticular injection of 10^{10} recombinant TRAIL viruses. Injection of 10^{9} recombinant TRAIL viruses had only a mild effect. The effect of the treatment was TRAIL specific, as it can be neutralized by sDR5 (Fig. 3). The therapeutic effect of the TRAIL virus lasted ~10 d, and arthritis returned ~2 wk after TRAIL virus injection. As expected, injection of sDR5 after the disease onset also exacerbated the inflammation (Fig. 3).

Histochemical analysis of mice treated as described above revealed dramatic differences between the groups. In mice treated with control protein, arthritis was characterized by leukocyte infiltration, mild synovitis, and pannus formation (Fig. 4, A and C); cartilage destruction and bone erosion occurred only in a small number of synovial joints. By contrast, in mice treated with sDR5, severe synovitis, hyperplasia of synovial membrane, and cartilage and/or bone destruction were observed in most synovial joints of the feet (Fig. 4, B and D) and lasted for >3 wk with few signs of remodeling or fibrosis. To directly label proliferating cells in the joint, we treated mice with nucleotide analogue BrdU and examined BrdU incorporation by immunohistochemistry. As shown in Fig. 4, E and F, $BrdU^{+}$ cells were detected in both BSA- and sDR5-treated mice, but the number of $BrdU^{+}$ cells in sDR5-treated mice was markedly increased compared with BSA-treated mice. Quantitative analysis of the histochemical data revealed significant differences between sDR5- and BSA-treated groups (pathology score 3.9 vs. 2.8, $P < 0.01$; Fig. 5 A).

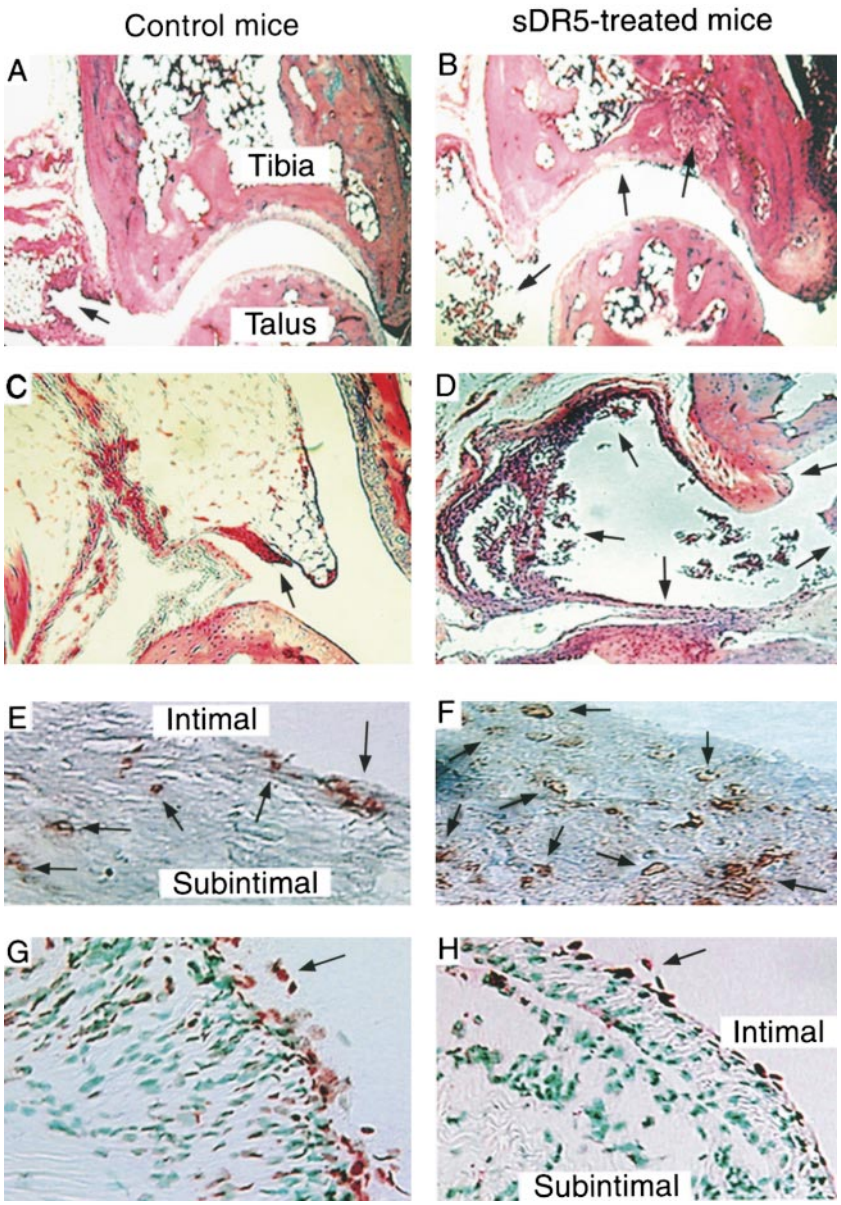


Figure 4. Histochemical profiles of arthritic joints. DBA/1 mice (eight to nine per group) were immunized for arthritis and treated with sDR5 or BSA as described in the legend to Fig. 2 A. For A–D, G, and H, mice were killed 32 d after the second immunization, and their ankle joints were analyzed for histology and apoptosis as described in Materials and Methods. For E and F, mice were injected intraperitoneally with BrdU as described in Materials and Methods, and were killed 21 d after the second immunization. BrdU staining was performed as described in Materials and Methods. (A) An ankle joint of a BSA-treated mouse with a pathology score of 2 (HE staining; original magnification: ×20). Arrow indicates signs of synovitis. (B) An ankle joint of an sDR5-treated mouse with a pathology score of 4 (HE staining; original magnification: ×20). Arrows indicate severe synovitis, hyperplasia, and cartilage and bone destruction. (C) An ankle joint of a BSA-treated mouse with a pathology score of 1 (HE staining; original magnification: ×100). Arrow indicates signs of synovitis. (D) An ankle joint of an sDR5-treated mouse with a pathology score of 4 (HE staining; original magnification: ×100). Arrows indicate severe synovitis, hyperplasia, and cartilage and bone destruction. (E) An ankle joint of a BSA-treated mouse with a disease score of 2 (BrdU staining; original magnification: ×400). Arrows indicate $BrdU^{+}$ nuclei, which are shown in brown. (F) An ankle joint of an sDR5-treated mouse with a disease score of 3 (BrdU staining; original magnification: ×400). (G) An ankle joint of a BSA-treated mouse with a pathology score of 4 (apoptotic staining; original magnification: ×200). Arrows indicate apoptotic cells, which are shown in brown. (H) An ankle joint of an sDR5-treated mouse with a pathology score of 4 (apoptotic staining; original magnification: ×200).

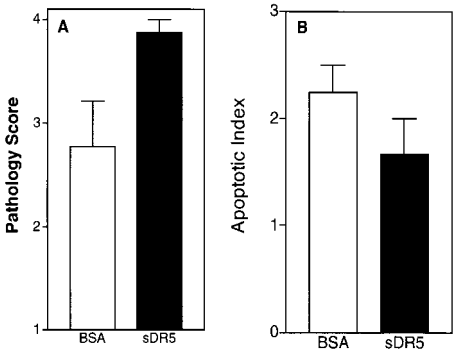
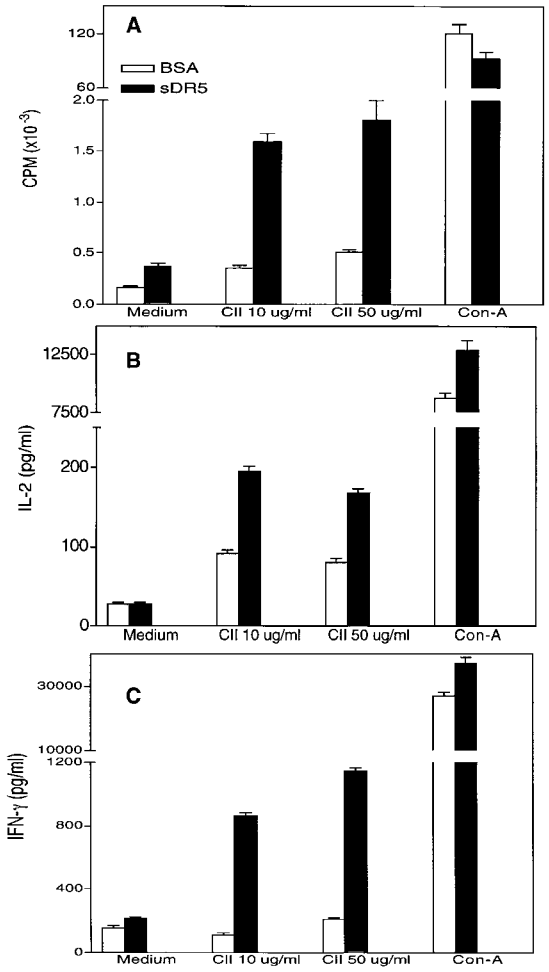


Figure 5. Pathological and apoptotic studies of arthritic joints. Mice were treated and killed as described in the legend to Fig. 4, and their paws were examined for the degrees of inflammation and apoptosis as described in Materials and Methods. A minimum of three comparable synovial sections per mouse were analyzed. A total of eight (for sDR5-treated group) and nine (for BSA-treated group) mice were used. For pathology scores (A), the differences between the two groups are statistically significant as determined by ANOVA ($P < 0.01$). For apoptotic index (B), the differences between the two groups are not statistically significant as determined by ANOVA ($P = 0.21$).

Roles of TRAIL in Autoimmune T and B Cell Responses In Vivo. Collagen-induced arthritis is initiated by collagen-specific lymphocytes. To determine whether exacerbation of arthritis by sDR5 is associated with functional alterations of collagen-specific lymphocytes, we examined both cellular and humoral anticollagen immune responses. Mice were treated as described above, and their blood and lymphoid tissues were collected 2–6 wk after immunization. As shown in Fig. 6, A–C, lymph node cells from control mice proliferated moderately in response to collagen and produced both IL-2 and IFN-γ. Remarkably, both lymphocyte proliferation and cytokine production were enhanced in mice treated with sDR5. Con A–treated cultures were included as positive controls to illustrate the levels of polyclonal T cell responses. Anticollagen antibody responses were determined by collagen-specific ELISA as in Fig. 6, D and E. Anticollagen IgG2a was dramatically increased in mice treated with sDR5, whereas anticollagen IgG1 was only moderately increased on day 14. These results indicate that chronic TRAIL blockade in mice enhanced both cellular and humoral immune responses, which might in turn exacerbate autoimmune arthritis. It is to be noted that short-term TRAIL blockade in DBA/1



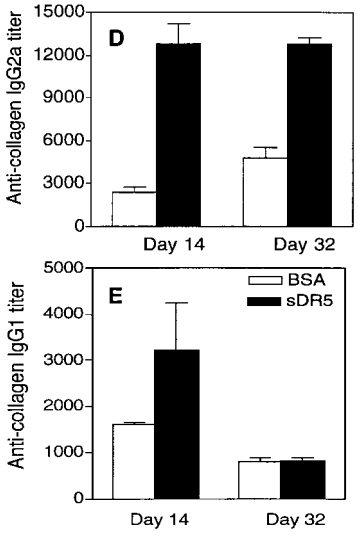


Figure 6. Effect of TRAIL blockade on anticollagen immune responses. DBA/1 mice (five to six per group) were treated as described in the legend to Fig. 2 A. To test cellular immune responses (A–C), mice were killed 32 d after the second immunization, and their inguinal lymph nodes were collected. For cytokine assays, lymph node cells (1.5×10^6 per well) were cultured in 0.2 ml of serum-free medium (X-vivo 20; BioWhittaker) with or without 10–50 μg/ml of chicken type II collagen (CII) or 1 μg/ml of Con A. Culture supernatants were collected 40 h later, and IL-2/IFN-γ concentrations were determined by sandwich ELISA as described (reference 46). To test lymphocyte proliferation (A), lymph node cells (0.5×10^6 cells per well) were first cultured for 72 h, then pulsed with [^{3}H]thymidine for an additional 16 h. Radioactivity (presented as cpm) was determined using a Wallac β-plate counter. To test humoral immune responses (D and E), mice were bled retroorbitally 14 and 32 d after the second immunization, and anticollagen IgG1 and IgG2a antibodies were determined by ELISA using chicken type II collagen as antigen. Each data point represents a mean ± SD from five (for sDR5-treated group) or six (for BSA-treated group) mice. The experiments were repeated three times with similar results. When a similar ELISA was performed using sDR5 as detecting antigen, no anti-sDR5 antibodies were detected (data not shown). In parallel experiments, mice were killed 14 and 21 d after the second immunization, and were tested as described in A–C. Similar differences between control and sDR5-treated mice were observed (data not shown). Additionally, sDR5 had no effect on LPS-induced proliferation of splenocytes in vitro (our unpublished data). (A) Proliferative responses as determined by [^{3}H]thymidine incorporation. (B) IL-2 production. (C) IFN-γ production. (D) Anticollagen IgG2a titers. (E) Anticollagen IgG1 titers. White bars, mice treated with BSA; black bars, mice treated with sDR5.

mice did not alter the structure or cellular composition of lymphoid organs as judged by histochemistry and flow cytometry (our unpublished data).

Roles of TRAIL in Apoptosis and Cell Cycle Progression. Some members of the TNF family are capable of inducing apoptosis of normal and/or tumor cells. TRAIL has been shown to induce apoptosis of some, but not all, tumor cell lines (2–8). The effect of sDR5 on arthritis can be explained by its blockade of TRAIL-induced apoptosis of inflammatory cells. To test this theory, we first examined the effect of TRAIL blockade on apoptosis of synovial cells in vivo. As shown in Fig. 4, G and H, apoptotic cells were readily detectable in arthritic synovia of both control and sDR5-treated mice 32 d after the second immunization. However, no statistically significant differences between the two groups were observed when the degrees of apoptosis were compared (Fig. 5 B). In parallel experiments, mice were also killed 14 and 21 d after the second immunization and examined for apoptosis as in Fig. 4, G and H. Again, no significant differences in the degree of apoptosis were observed between control and sDR5-treated groups (data not shown). Both TNF and CD95 ligand (CD95L) have been shown to mediate activation-induced cell death (AICD) of lymphocytes (19–21). Therefore, we also examined the role of TRAIL in the death and survival of lymphocytes (Fig. 7). Splenocytes were isolated from BALB/c mice and activated in vitro with Con A for 3 d. Live cells were then purified through a Ficoll gradient and restimulated in vitro with anti-CD3 mAb. Apoptosis and cell cycle progression were analyzed by flow cytometry using the DNA dye propidium iodide (22). As shown in Fig. 7 A, anti-CD3 mAb induced apoptosis of ~16% of the cells; this was completely prevented by anti-CD95L mAb, confirming an essential role for CD95L in AICD (19–21). By contrast, sDR5 moderately increased AICD induced by anti-CD3 mAb (Fig. 7 A). Unexpectedly, the total number of cells, especially cells in the S-G2/M phases of the cell cycle, were dramatically increased in cultures containing sDR5 (Fig. 7 B). This increase in S-G2/M cells was TRAIL specific, as it was partially blocked by recombinant TRAIL (Fig. 7 B). These results strongly suggest that although TRAIL may not induce apoptosis of lymphocytes, it does play important roles in regulating cell cycle progression. To test this theory directly, we examined the effect of recombinant TRAIL and sDR5 on cell cycle progression of lymphocytes. T lymphocytes were stimulated with anti-CD3 mAb as in Fig. 7 A, and G1 to S phase progression was determined by [^{3}H]thymidine incorporation (cells entering the S phase of the cell cycle synthesize DNA and take up thymidine). As shown in Fig. 7, C and D, anti-CD3 mAb induced a marked increase in thymidine uptake. This was significantly inhibited by TRAIL (Fig. 7 C) but enhanced by sDR5 (Fig. 7 D). Again, neither TRAIL nor sDR5 affected apoptosis of cells in the culture (our unpublished data). Studies are underway to elucidate the intracellular signaling pathways that are responsible for TRAIL-mediated cell cycle inhibition. As AICD is regulated by cell cycle progression, inhibition of cell cycle by TRAIL may modulate the sensitivity of cells to AICD as shown in Fig. 7 A.

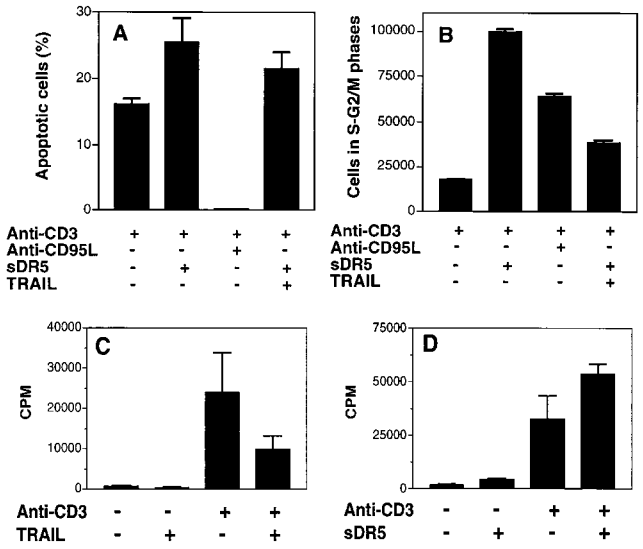


Figure 7. Inhibition of DNA synthesis and cell cycle progression by TRAIL. Splenocytes were prepared from 6–8-wk-old BALB/c mice (The Jackson Laboratory) and cultured in DMEM for 3 d in the presence of 10% FBS and 2.5 μg/ml of Con A. Live cells were then purified through a Ficoll gradient and cultured in 96-well plates at 3×10^5 cells per well in 200 μl of DMEM containing 10% FBS, with or without the following reagents: 100 ng/ml of TRAIL, 5 μg/ml of sDR5, 5 μg/ml of anti-CD95L mAb (MFL-3), and anti–mouse CD3 mAb (which was coated on the plate by preincubating the plate with 10 μg/ml of the antibody at 4°C for 16 h). For apoptosis and cell cycle analyses (A and B), cells were cultured for a total of 24 h, fixed in 70% ethanol, and stained with 50 μg/ml of propidium iodide. For thymidine incorporation assays (C and D), cells were cultured for 24 h, then pulsed with 10 μCi/ml of [^{3}H]thymidine for an additional 16 h. Cells were then harvested, and radioactivity was determined using a Wallac β-plate counter. (A) Percentage of apoptotic cells as determined by flow cytometry. The spontaneous apoptotic rate in cultures containing no anti-CD3 mAb was 12%, which was subtracted from the data presented here. For cultures that contained TRAIL and anti-CD3 mAb, the percentage of anti-CD3–induced apoptosis was 16 ± 1%, which was comparable to that of the cultures treated with anti-CD3 mAb alone. The differences between anti-CD95L mAb–treated culture and all other cultures are statistically significant as determined by ANOVA ($P < 0.0001$). (B) Number of S-G2/M cells per well as determined by flow cytometry. For cultures that contained TRAIL and anti-CD3 mAb, the number of cells in the S-G2/M phases was 70–90% of that of the anti-CD3 mAb–treated culture. The total numbers of live cells per well recovered from each group were as follows: cultures with anti-CD3 mAb alone, 0.3×10^5; cultures with anti-CD3 mAb plus sDR5, 1.6×10^5; cultures with anti-CD3 mAb plus anti-CD95L mAb, 1.1×10^5; cultures with anti-CD3 mAb plus sDR5 plus TRAIL, 0.6×10^5. The differences between all four groups are statistically significant as determined by ANOVA ($P < 0.01$). (C and D) DNA synthesis as determined by [^{3}H]thymidine incorporation. Data presented are means cpm ± SD of triplicate cultures. For cultures containing anti-CD3 mAb, the differences between the two groups are statistically significant as determined by ANOVA ($P < 0.01$). The experiments were repeated twice with similar results. The concentrations of TRAIL and sDR5 used in these experiments were selected based on the dose-dependency studies performed in our laboratory. When 0.2–1 μg/ml of sDR5 was used, less significant effects on cell cycle progression were observed. Similarly, when 50–150 ng/ml of TRAIL was tested, much less significant effects on cell cycle were detected (data not shown).

Discussion

Although it has been established that TRAIL induces apoptosis of tumor cells but not normal cells, the roles of TRAIL in health and disease are virtually unknown. Results presented here strongly suggest that TRAIL is a potent inhibitor of autoimmune arthritis. The inhibitory effect of TRAIL on arthritis may be a result of inhibition of cell cycle progression and/or cytokine production. Blocking endogenous TRAIL with sDR5 eliminates this inhibition and enhances proliferation of autoreactive lymphocytes (as shown in Fig. 6 A) or synovial cells (as shown in Fig. 4). This may in turn contribute to the exacerbation of arthritic inflammation and joint tissue destruction.

Thus, one of the functions of TRAIL in vivo is to maintain immune homeostasis and to downregulate immune responses, including autoimmune responses. This is in startling contrast to TNF, which initiates and exacerbates autoimmune diseases. In fact, anti-TNF therapy is effective in preventing arthritic inflammation both in humans and animals (23–26). Results reported here indicate that the role of TRAIL in autoimmunity may be more closely related to that of CD95L or CD30L. Mutations in CD95/CD95L genes lead to the development of systemic autoimmune diseases in both humans (27, 28) and mice (29–32), although they paradoxically prevent several organ-specific autoimmune diseases (33–39). We and others have shown that upregulating CD95 or CD95L function in synovial joints ameliorates autoimmune arthritis (17, 40). Similarly, CD30L may also play an antiinflammatory role in autoimmune diseases (41). Autoreactive $CD8^+$ T cells deficient in CD30L elicit more severe autoimmune insulitis in mice (41). Thus, unlike TNF but similar to CD95L and CD30L, TRAIL may be a member of an inhibitor protein subfamily that prevents autoimmune diseases by downregulating immune responses.

However, our results also indicate that the mechanism of TRAIL action in vivo is different from that of CD95L. Whereas CD95L induces apoptosis of activated T cells, TRAIL appears to inhibit their proliferation without eliminating them through apoptosis. This finding is consistent with recent reports that, unlike CD95L, TRAIL induces apoptosis of tumor cells but not normal cells (2, 6). Systemic administration of recombinant TRAIL, but not CD95L, selectively kills tumor cells while sparing normal host cells (13, 14). Our observation that TRAIL can inhibit DNA synthesis provides direct evidence that TRAIL can prevent G1 to S phase progression of lymphocytes. Therefore, we propose that unlike TNF or CD95L, TRAIL inhibits activation and expansion of lymphocytes in vivo, but does not delete them from the system. The molecular basis of TRAIL-induced inhibition of cell cycle progression needs to be further examined. It has been reported that some of the TRAIL receptors can activate both caspase (through Fas-associated death domain protein [FADD]/TNF receptor 1–associated death domain protein [TRADD]) and nuclear factor (NF)-κB pathways in tumor cells (42–44). Therefore, it is necessary to determine whether this also occurs in normal cells, and if so, whether this is responsible for the cell cycle–arresting effect reported here. It is to be noted that NF-κB activation occurs in collagen-induced arthritis, and that inhibition of NF-κB activation in T cells prevents the disease (45). On the other hand, various signal transduction pathways of TRAIL receptors are yet to be defined. Novel unidentified pathways may be responsible for the inhibitory effect of TRAIL in inflammation and cell cycle progression.

In summary, we have established that TRAIL, unlike its apoptotic effect on tumor cells, inhibits cell cycle progression of nontransformed lymphocytes, and that unlike TNF, which promotes inflammation, TRAIL inhibits autoimmune inflammation and prevents self-tissue destruction.

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