Neutrophil-Derived TNF-Related Apoptosis-Inducing Ligand (TRAIL): A Novel Mechanism of Antitumor Effect by Neutrophils

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ABSTRACT

To detect the novel genes expressed uniquely in neutrophils and elucidate their function, the gene expression pattern was compared by using cDNA microarray containing 240 cytokine genes between the neutrophils and peripheral blood mononuclear cells (PBMCs) obtained from healthy human donors. Twenty-six genes, including tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), were expressed in neutrophils at a level >10 times higher than that seen in phenoxyhemagglutinin-stimulated PBMCs. The amounts of mRNA and protein of TRAIL were quantified by real-time reverse transcription-PCR and ELISA, respectively. TRAIL was expressed in resting neutrophils at the mRNA and protein levels, and its expression was enhanced after stimulation with IFN-γ. Neutrophils expressed TRAIL on the cell surface and released it into the culture media. The cytotoxicity of neutrophil-derived TRAIL against Jurkat cells was determined by flow cytometry using FITC-conjugated annexin V. When Jurkat cells were cultured with neutrophils in the presence of IFN-γ, the number of Jurkat cells undergoing apoptosis increased, and such an increase depended on the effector/target ratio. This cytotoxicity was suppressed partially by adding anti-TRAIL antibody to the media. Neutrophils may exert their own antitumor effect by TRAIL. A microarray analysis was found to be a useful tool for detecting novel genes that are suggested to play unknown roles in the neutrophil function.

INTRODUCTION

Neutrophils represent 50–60% of the total circulating leukocytes, and their cytoplasmic granules contain highly developed cytotoxic enzymes and digestive enzymes (1). The plasma membranes carry a number of receptors and other structures needed for the recognition and elimination of invading pathogens. These structures form the first line of cellular defense against various infectious agents and nonself substances that enter the body. Their targets include bacteria, fungi, protozoa, viruses, virally infected cells, and tumor cells. They also play a major role in the inflammation and tissue damage of a wide variety of noninfectious diseases, such as arthritis, inflammatory bowel disease, and ischemia-reperfusion injury. These neutrophil functions basically are regulated by several cytokines secreted from other blood cells, such as monocytes and lymphocytes (2). However, it is evident that neutrophils themselves synthesize and secrete a small amount of cytokines, including interleukin 1β (IL-1β; Ref. 3), interleukin 8 (IL-8; Ref. 4), interleukin 12 (IL-12; Ref. 2), tumor necrosis factor α (TNF-α; Ref. 5), macrophage inflammatory protein 1α (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), and transforming growth factor β1 (TGF-β1; Ref. 2). These cytokines may have an effect on macrophage stimulation and neutrophil recruitment and lymphocyte activation, proliferation, and differentiation. As a result, an analysis of expression profile of cytokine genes is thought to be useful to clarify the neutrophil function.

Although the molecular mechanism of the neutrophil function has been investigated intensively, most previous studies on the gene expression of neutrophils have focused on a few particular genes related to the known neutrophil function. Some studies have reported recently the expression of a wide variety of genes in neutrophils treated with granulocyte colony-stimulating factor (G-CSF; Ref. 6) and lipopolysaccharide (7) based on analyses using a cDNA microarray. These studies suggested that a cDNA microarray analysis could reveal unknown neutrophil functions and their molecular mechanisms by determining the changes in mRNA level of hundreds of genes after activation.

Studies on the immunologic mechanism to eliminate tumor cells in vivo have focused largely on the function of lymphocytes and macrophages as an important mediator of the host response because the in vitro tumor lysis induced by these effector cells was reported repeatedly. They included cytotoxic macrophages, natural killer cells, specific cytotoxic T lymphocytes, and antibody-dependent cell cytotoxic effectors (8). Conversely, the antitumor effect of the neutrophils, which depend on classical cytotoxic systems, also has been suggested, including antibody-dependent cell cytotoxic effectors (9, 10), Fas ligand-mediated apoptosis (11), directed cell killing by H2O2 and superoxide (12), and calprotectin (13). However, little attention thus far has been paid to the possible tumor-lytic function of neutrophils. A new antitumor molecule, TNF-related apoptosis-inducing ligand (TRAIL), has been reported recently (14, 15). TRAIL is a type II membrane protein belonging to the TNF superfamily. Several cells in the immune system, including activated T cells (16, 17), B cells (18), natural killer cells (19), dendritic cells (20), and monocytes (21), produce TRAIL. The most notable characteristics of TRAIL are to induce apoptosis only in transformed or cancer cells, whereas normal cells are resistant to TRAIL-mediated apoptosis (14, 15). Although it was reported recently that TRAIL and TRAIL receptors were expressed in neutrophils (22), it is unclear as to whether this molecule is involved in neutrophil-mediated cytotoxicity against tumor cells.

In the present study, we comprehensively examined the gene expression of neutrophils by using cDNA microarray to elucidate new neutrophil functions. Our results demonstrated that neutrophils produce TRAIL and also exert a possible antitumor effect.

MATERIALS AND METHODS

Preparation of Human Neutrophils and Peripheral Blood Mononuclear Cells. Heparinized whole blood was obtained from healthy human donors after they gave their informed consent. Neutrophils were isolated by density centrifugation using Polymorphprep (Axis-Shield, Oslo, Norway) containing sodium diatrizoate, 13.8% (w/v), and dextran 500 8.0% (w/v) (density, 1.113 g/ml). Five ml of whole blood were layered on 5 ml of Polymorphprep in a 12-ml tube and centrifuged at 500 × g for 30 min at room temperature. The neutrophils in the lower band were harvested using a Pasteur pipette and washed with PBS. To obtain highly purified neutrophils, they were labeled with FITC-conjugated anti-CD16 antibody (Immunotech/Beckman Coulter, Marseille, France), and they were selected positively using magnetic cell sorting with MACS anti-FITC microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany), giving a purity of 90–100%. Neutrophils were washed with PBS and resuspended in RPMI 1640 medium (Invitrogen, Carlsbad, CA). Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation using LSM medium (Cappel, Aurora, OH) containing 6.2 g Ficoll

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and 9.4 g sodium diatrizoate per 100 ml (density, 1.077 g/ml). Heparinized whole blood was mixed with the same volume of PBS, and 4 ml of it were layered on 3 ml of LSM medium, followed by centrifugation for 20 min at 400 × g at room temperature. The PBMCs in the mononuclear layer were harvested, washed with PBS, and resuspended in RPMI media for additional experiments. Some PBMCs were purified into subpopulations, which were single positive for CD4, CD8, CD14, or CD19 using magnetic cell sorting microbeads (Miltenyi Biotec) and CD3 (pan T cell) using Pan T-cell isolation kit II (Miltenyi Biotec).

Cell Culture, Cell Activation, and Cell Lysates. Neutrophils and PBMCs were suspended in RPMI media supplemented with 10% FCS, 2 mM L-glutamine, and 40 mg/ml gentamicin sulfate and cultured in 96-well plates at 37°C in an atmosphere of 95% air/5% CO₂. For activation experiments, the cells were cultured with or without FCS at a cell density of 2 or 10 × 10⁶ cells/ml for 2–24 h in the presence of several substances, such as phytohemagglutinin (PHA; 10 μg/ml; Becton Dickinson, Sparks, MD), IFN-α (200 IU/ml; Sumitomo Pharmaceuticals, Osaka, Japan), IFN-γ (100 IU/ml; Shionogi & Co., Ltd., Osaka, Japan), lipopolysaccharide from Escherichia coli serotype 0111:B4 (5 μg/ml; Sigma Chemical Co., St. Louis, MO), human TGF-β (1 ng/ml; Habor Bio, Norwood, MA), recombinant human TNF-α (10 ng/ml; Genzyme, Cambridge, MA), G-CSF (50 ng/ml; Sankyo Co., Ltd., Tokyo, Japan), and granulocyte macrophage colony-stimulating factor (GM-CSF; 50 ng/ml; Pepro Tech, London, United Kingdom). At the end of incubation, the culture media were stored for additional experiments. To obtain cell lysates, 100 μl of cell suspension (1 × 10⁶ cells) were mixed with a lysing buffer containing 50 mM Tris HCl, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS and protease inhibitors (Protease inhibitor cocktail P8340; Sigma Chemical Co.). The cell lysate was sonicated for 45 s three times in ice-water and centrifuged at 15,000 × g for 30 min; the supernatant then was obtained for additional experiments.

cDNA Microarray Analysis. Total RNA was extracted from cell pellets by an RNA extraction kit (Iogen; Nippon Gene, Osaka, Japan) according to the manufacturer’s instructions, and approximately 20–30 μg of total RNA were labeled with cyanine 5-fluorescent dye (Cy5) or Cy3 by reverse transcription using a Labeling Core kit (TaKaRa, Ohtsu, Japan) with oligo(dT) primer. Labeled cDNA was hybridized with a cDNA microarray (Human Cytokine Chip version 2.0, TaKaRa) containing an array of 240 cytokine-associated genes at 65°C for 16–18 h. After hybridization, the microarray was washed with a buffer containing 2× standard sodium citrate (SSC) and 0.2% SDS at 55°C for 15 min three times and finally rinsed with 0.05× SSC. Fluorescence intensity of each gene was scanned by FLA-8000 (Fuji film, Tokyo, Japan), and image files were quantified using analyzing software “Array Vision” (Amersham Biosciences, Piscataway, NJ). Glyceraldehyde-3-phosphate dehydrogenase, as a constantly expressed gene, was used for normalization of the fluorescence intensity of each gene. Inappropriate spots whose fluorescence signals were less than twofold those of the background signal and were not reproducible among three independent experiments were excluded. When the analysis was carried out, the cDNA of the neutrophils from one individual was compared with the pooled cDNA of the PHA-stimulated PBMCs from five independent donors.

Quantitative Real-Time Reverse Transcription-PCR by TaqMan Method. Total RNA was extracted from cell pellets of either unstimulated or stimulated neutrophils and PBMCs, and unstimulated lymphocytes positive for one of CD3, CD4, CD8, CD14, or CD19, with the same method as a microarray analysis, followed by cDNA synthesis using a First-Strand cDNA Synthesis kit (Amersham Biosciences) with random hexamers. PCR reaction for TRAIL was performed using the following primers: forward, 5'-AGACTCTGACAGGATCAGGCTA-3' and reverse, 5'-CTCTGTGT-CAAGTAC-3' - CGAGCTGAAGCAGATGCAGGA-3', which would give a PCR product of 83 bp. This primer set was designed to amplify five exons of TRAIL. PCR reactions were performed using the following primers: forward, 5'-AGACTCTGACAGGATCAGGCTA-3' and reverse, 5'-GCCTGAGGTGTAGGCCCCATGC-3', giving a PCR product of 1582 bp.

Flow Cytometric Analysis for TRAIL. Neutrophils and PBMCs were incubated at room temperature for 20 min with phycoerythrin (PE)-conjugated mouse antihuman TRAIL monoclonal antibody (BD Pharmingen, San Diego, CA). Isotype control monoclonal antibody (Immunotech/Beckman Coulter) was used as a negative control. To determine TRAIL-induced death, apoptotic cell death was measured using FITC-conjugated annexin V (Apopotosis Detection kit; MBL, Nagoya, Japan). The fluorescence intensity of cells was measured using EPICS XL (Beckman Coulter, Miami, FL). Five thousands events were counted for each measurement.

ELISA for TRAIL. The amount of TRAIL in the culture media and cell lysates was measured by ELISA using a soluble TRAIL ELISA kit (Diaclone, Besançon, France). A monoclonal antibody specific for soluble and membrane-bound TRAIL was coated onto the wells of the microtiter plates. One hundred μl of culture media, cell lysates, or standard TRAIL samples with known concentrations were added to the wells, and a biotinylated monoclonal antibody specific for TRAIL antigen was added to the wells and simultaneously incubated for 3 h. After washing thoroughly, 100 μl of streptavidin-horseradish peroxidase were added to the wells, incubated for 30 min, and washed three times. Next, a substrate solution for peroxidase was added to induce a colored reaction product. The intensity of the colored product was determined by reading absorbance at 450 nm on a spectrophotometer.

Western Blot Analysis. Culture media and cell lysates were applied to 10% polyacrylamide gels, and SDS-PAGE was performed under reduction conditions. The proteins then were electroblotted onto nitrocellulose membranes and probed with mouse anti-TRAIL monoclonal antibody (1 μg/ml; A molecule selected for additional study in this article.  

| Table 1 Cytokine genes highly expressed in human peripheral neutrophils |
|------------------------|------|
| Gene                   | Ratio |
| Granulocyte colony-stimulating factor receptor (G-CSF receptor) | 1373.51 |
| IL-1 receptor type 2    | 378.81 |
| Pre-B-cell colony-enhancing factor (PREF)                     | 139.67 |
| Chemokine receptor 4 (CXCR4)                                  | 127.55 |
| Thioredoxin-like (TXNL)                                        | 113.10 |
| Tissue inhibitor of metalloproteinase 2 (TIMP2)               | 86.65 |
| Calreticulin                                                      | 66.55 |
| IFN-γ receptor 1                                                | 66.42 |
| Selectin L                                                       | 66.15 |
| Granulocyte macrophage colony-stimulating factor receptor (GM-CSF receptor) | 58.47 |

b A molecule selected for additional study in this article.
TRAIL mRNA levels of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in resting neutrophils and peripheral blood mononuclear cells (PBMCs). The amounts of TRAIL mRNA in resting neutrophils (n = 12), PBMCs (n = 12), and lymphocytes single positive for one of CD3, CD4, CD8, CD14, and CD19 (n = 3 each) from peripheral blood of healthy human donors were quantified by real-time reverse transcription-PCR. The expression levels were described as the ratio of that in the phytohemagglutinin-stimulated normal PBMCs (1.0). The bars in each scatter diagram show the median values. Each dot was determined in duplicate.

Cytotoxic Assay of Neutrophils against Human Leukemic Cells. Jurkat cells, a human T-cell leukemia cell line, were cultured in RPMI media and Fig. 3) or 24 h (data not shown) with several cytokines, which have been shown to induce activation or high expression of the genes shown in neutrophils, gene expression was compared using a cDNA microarray. To find unique genes expressed differentially in neutrophils, gene expression was compared using a cDNA microarray between the neutrophils and PHA-stimulated PBMCs, which were expected to express many cytokine genes at increased levels. Table 1 shows the representative results of three independent experiments, indicating that some genes were expressed at a level >10 times higher in neutrophils than that seen in PBMCs. The expression profile reflected the known neutrophil function, including G-CSF receptor, thioredoxin-like (TXNL), catalase, selectin L, superoxide dismutase 2, GM-CSF receptor, integrin α-L, and CSF1 receptor. Cytokine genes, such as pre-B-cell colony-enhancing factor, IL-16, IL-8, and TRAIL, also were expressed at a high level in neutrophils. Of the genes shown in Table 1, we selected the TRAIL gene as the most attractive gene to elucidate novel neutrophil function in cancer immunology.

Quantification of TRAIL mRNA in Neutrophils and PBMCs. The amount of TRAIL mRNA in neutrophils and PBMCs was determined by real-time reverse transcription-PCR, which confirmed a high expression of the TRAIL gene in neutrophils (Fig. 1). The results showed that TRAIL gene expression in neutrophils was significantly higher than that of each of PBMCs, CD3 (pan T cell), CD4-, CD8-, CD14-, or CD19-positive cells (neutrophils versus PBMCs; p = 0.0027, CD3; p = 0.0094, CD4; p = 0.014, CD8; p = 0.0094, CD14; p = 0.0094, CD19; and p = 0.043). Neutrophils and PBMCs were selected with several cytokines, which have been shown to induce activation or differentiation in neutrophils and PBMCs, including IFN-α, IFN-γ, lipopolysaccharide, GM-CSF, G-CSF, TNF-α, and TGF-β. In neutrophils, TRAIL expression increased at 2 h after stimulation with IFN-α (Fig. 2A) and IFN-γ (Fig. 2B and Fig. 3). PBMCs showed increased TRAIL expression at 4 h (Fig. 2C and Fig. 3) or 24 h (data not shown) after IFN-γ stimulation. Neutrophils did not survive at 24 h after stimulation; therefore, gene expression was not determined at the time...
point. IFN-α and lipopolysaccharide also enhanced TRAIL expression in PBMCs, whereas other cytokines, including TNF-α, G-CSF, GM-CSF, and TGF-β, did not induce such expression in either neutrophils or PBMCs (data not shown).

Flow Cytometric Analysis of the TRAIL Expression on Neutrophils and PBMCs. The expression of TRAIL on the cell surface of neutrophils and PBMCs was examined by flow cytometry. As shown in Fig. 4, TRAIL was detected on the surface of neutrophils stimulated with IFN-γ for 2 h, although the expression levels varied considerably among individuals. In a few individuals, unstimulated neutrophils also showed a TRAIL expression (data not shown). In contrast to neutrophils, PBMCs did not show a considerable increase of TRAIL at 2 h after stimulation, but a significant increase was observed at 24 h (Fig. 4).

Quantification of the TRAIL Protein Produced by Neutrophils and PBMCs with ELISA. The amounts of TRAIL protein in the cells and in culture media were determined by ELISA after stimulation with IFN-γ. TRAIL was detected in the media of the neutrophil culture at 2 h after stimulation and increased by 4 h (Fig. 5A). PBMCs did not release any detectable amount of soluble TRAIL at 2 h, but they did show considerable increase at 24 h after stimulation (Fig. 5B). Fig. 5, C and D show the changes in the amount of TRAIL protein in cell lysates. The amount of TRAIL in neutrophils only tended to increase by 4 h after IFN-γ stimulation (Fig. 5C). In PBMCs, a significant increase was observed at 24 h (Fig. 5D).

Detection of TRAIL Protein Produced by Neutrophils with Western Blot Analysis. TRAIL protein in a cell lysate of neutrophils was examined by Western blot analysis. The M₆ 45,000 form of TRAIL was detected in unstimulated fresh neutrophils, and its amount increased after IFN-γ stimulation for 2 h (Fig. 6). The M₆ 24,000 cleaved form of TRAIL also was detected in the lysates of unstimulated and IFN-γ-stimulated neutrophils.

Cytotoxicity of Neutrophil-Derived TRAIL against Leukemic Cells. To evaluate the functional activity of neutrophil-derived TRAIL against cancer cells, IFN-γ-stimulated neutrophils were mixed with Jurkat cells, and the number of Jurkat cells undergoing apoptosis was determined. When Jurkat cells were mixed with neutrophils and cultured for 8 h in the presence of IFN-γ, the percentage of Jurkat cells undergoing apoptosis increased depending on the neutrophil number (Fig. 7A, white bars). This cytotoxicity was inhibited partially when anti-TRAIL antibody was added to the culture media (Fig. 7A, black bars). A significant increase of neutrophil-induced cytotoxicity also was observed when leukemic cells were mixed with neutrophils that had been stimulated with IFN-γ followed by extensive washing to remove IFN-γ (Fig. 7B, open circles; p = 0.014). By adding the...
of IFN-γ after, the neutrophils (three individuals) and PBMCs (4) were incubated in the presence of IFN-γ/H9253 cells (PBMCs) after IFN-γ stimulation. The neutrophils and PBMCs from five individuals were incubated in the presence of IFN-γ (100 IU/ml) at a cell density of 1 × 10^7 cells/ml for 0–24 h. Culture media of neutrophils (A) and PBMCs (B) were obtained at the indicated times. The amounts of soluble TRAIL were determined using ELISA. Thereafter, the neutrophils (three individuals) and PBMCs (4) were incubated in the presence of IFN-γ (100 IU/ml) at a cell density of 1 × 10^7 cells/ml for 0–24 h. The cells were harvested at the indicated times, and cell lysates were obtained. The amounts of cellular TRAIL in neutrophils (C) and PBMCs (D) were determined by ELISA.

To elucidate novel neutrophil function and its molecular mechanism, the expression profile of genes, especially cytokine genes, was analyzed using a cDNA microarray, and any unique genes that expressed differentially in neutrophils were identified. Resting neutrophils expressed 26 genes at a level that was 26 times higher than that of PHA-stimulated PBMCs. They included hematopoietic growth factor receptors (G-CSF receptor, GM-CSF receptor, and CSF-1 receptor), superoxide-related molecules (thioredoxin-like, catalase, and superoxide dismutase 2), and adhesion molecules (selectin L and integrin α-L), all of which are related closely to the known neutrophil functions. Of the 26 genes highly expressed in neutrophils, the TRAIL gene was selected as the most interesting gene for the additional study. Itoh et al. (25) reported that most genes for secretary proteins such as cytokines are not particularly active in circulating granulocytes, and TRAIL functions. Of the 26 genes highly expressed in neutrophils, the Itoh gene was selected as the most interesting gene for the additional study.

DISCUSSION

To elucidate novel neutrophil function and its molecular mechanism, the expression profile of genes, especially cytokine genes, was analyzed using a cDNA microarray, and any unique genes that expressed differentially in neutrophils were identified. Resting neutrophils expressed 26 genes at a level that was >10 times higher than that of PHA-stimulated PBMCs. They included hematopoietic growth factor receptors (G-CSF receptor, GM-CSF receptor, and CSF-1 receptor), superoxide-related molecules (thioredoxin-like, catalase, and superoxide dismutase 2), and adhesion molecules (selectin L and integrin α-L), all of which are related closely to the known neutrophil functions. Of the 26 genes highly expressed in neutrophils, the TRAIL gene was selected as the most interesting gene for the additional study. Itoh et al. (25) reported that most genes for secretary proteins such as cytokines are not particularly active in circulating granulocytes, and only 2 genes, pre-B-cell colony-enhancing factor and IL-8, were detected of the 748 genes analyzed. These two genes also demonstrated a high expression level in our study (Table 1), whereas Itoh et al. did not examine TRAIL expression in their article.

TRAIL is a recently identified type II integral membrane protein belonging to the TNF family. The TRAIL gene is located on chromosome 3 at position 3q26 (14, 15). Several cells in the immune system, including activated T cells (16, 17), B cells (18), natural killer cells (19, 26), dendritic cells (20), and monocytes (21), express TRAIL. The full-length TRAIL on cell membrane and soluble TRAIL rapidly induce apoptosis in cancer cells (14, 15) and virus-infected cells (27), whereas normal cells are resistant to them. These findings suggest that TRAIL may play an important role in cancer immunity and that TRAIL-bearing cells kill cancer cells selectively. At least five receptors for TRAIL have been identified in humans. Two of them, DR4 (TRAIL-R1) and DR5, are capable of transducing an apoptotic signal (28). The other three receptors (TRAIL-R3, TRAIL-R4, and a soluble receptor called osteoprotegerin or TRAIL-R5) lack death domains and may serve as a decoy receptor to regulate TRAIL-mediated cell death. The increased expression of these decoy receptors on normal cells is speculated to be one reason for the selective cytotoxicity of TRAIL against cancer cells (29). Although TRAIL reportedly is produced from several types of cells, its expression and functional roles in human peripheral blood neutrophils remain unclear. Some reports have shown recently the expression of TRAIL and TRAIL receptors in human neutrophils (22, 30). Renshaw et al. (22) reported that neutrophils were susceptible to TRAIL-mediated apoptosis, which may provide a mechanism for clearance of neutrophils from sites of inflammation and be involved in the regulation of inflammation. They also detected the expression of TRAIL in neutrophils but did not mention the antitumor effect through TRAIL.

The present results showed that TRAIL was expressed in resting neutrophils, and its expression was enhanced at mRNA and protein levels after stimulation with IFN-α and -γ. TRAIL was detected on anti-TRAIL antibody to the media, this IFN-γ-induced cytotoxicity also was inhibited (p = 0.00026). These results demonstrated that neutrophils exerted cytotoxicity against leukemic cells and that neutrophil-derived TRAIL was, at least in part, responsible for this antileukemia effect. The neutrophil-mediated cytotoxicity against Jurkat cells in this experiment was almost the same level as the recombinant soluble TRAIL that was added at a concentration of 4–40 ng/ml (data not shown).

Fig. 5. Changes in the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in culture media and in the cell lysates of neutrophils and peripheral blood mononuclear cells (PBMCs) after IFN-γ stimulation. The neutrophils and PBMCs from five individuals were incubated in the presence of IFN-γ (100 IU/ml) at a cell density of 1 × 10^7 cells/ml for 0–24 h. Culture media of neutrophils (A) and PBMCs (B) were obtained at the indicated times. The amounts of soluble TRAIL were determined using ELISA. Thereafter, the neutrophils (three individuals) and PBMCs (4) were incubated in the presence of IFN-γ (100 IU/ml) at a cell density of 1 × 10^7 cells/ml for 0–24 h. The cells were harvested at the indicated times, and cell lysates were obtained. The amounts of cellular TRAIL in neutrophils (C) and PBMCs (D) were determined by ELISA.
the cell surface and also in culture media. A similar increase of TRAIL expression after IFN stimulation was reported in peripheral blood monocytes (21, 26). Mariani and Kramer (18) suggested that membrane-bound TRAIL is cleaved by cysteine proteases such as E64 and leupeptin, not by metalloproteinase, and released from the cell surface (soluble TRAIL). It is possible that TRAIL is produced in neutrophils, binds to the cell surface, and then is immediately cleaved by proteases to release soluble forms, as described for other death receptor ligands (31, 32). However, the difference in the cytotoxic function between membrane-bound and soluble TRAIL is unclear (33). In the case of Fas, only membrane-bound Fas ligand is known to exert a full biological activity, whereas soluble Fas ligand has only a weak activity. This fact may explain the absence of tissue damage despite high levels of circulating soluble Fas ligand in patients with several kinds of neoplasia (33).

When Jurkat cells were cultured with neutrophils in the presence of IFN-γ, the number of Jurkat cells undergoing apoptosis increased, and such increase depended on the effector:target ratio. Jurkat cells are a well-known leukemic cell line that is susceptible to TRAIL-mediated cell killing (34). This neutrophil-mediated cytotoxicity was suppressed partially by adding anti-TRAIL antibody to the media. These results indicate the possibility that neutrophils can exert antitumor cytotoxicity by TRAIL. Whereas investigations of cell-mediated killing against tumor cells have focused largely on lymphocytes and macrophages, only slight attention has been paid to the antitumor effect of neutrophils. The reported mechanisms of neutrophil-mediated antitumor effect includes antibody-dependent cell cytotoxic effectors (9, 10), Fas ligand-mediated apoptosis (11), direct cell killing by H₂O₂ and superoxide (12), and calprotectin (13). Neutrophils stimulated with IFN-γ and G-CSF expressed Fcγ receptor I (CD64), which binds to monomeric IgG with a high affinity. Fcγ receptor I has been reported to be the main cytotoxic trigger molecule involved in antibody-dependent cell cytotoxic effectors with G-CSF-primed neutrophils (9, 10). Activated neutrophils increased the transcription and production of TNF-α and Fas ligand (11). The immune responses, including the modification of TNF-α and soluble Fas ligand produced by neutrophils, were shown to play an important role in regulating the apoptotic process of Helicobacter pylori-infected human gastric epithelial cells (11). Soluble Fas ligand also increased the neutrophil infiltration in a tumor area, thus leading finally to the acute elimination of tumor cells by the immune system. Direct cell killing is an important defense mechanism in the control and elimination of tumor cells. The cytologic observation that a substantial number of neutrophils surrounded tumor cells in ascites from cancer patients suggests that neutrophils play an active role in tumor cell clearance (12). The production of H₂O₂ and superoxide by inflammatory neutrophils also plays a role in tumor lysis (12). Calprotectin, an abundant calcium-binding protein complex in neutrophils, can induce growth inhibition and apoptosis against a variety of tumor cell lines and normal cells such as fibroblasts (13). In our study, the inhibition of neutrophil-mediated cell killing by anti-TRAIL antibody was only partial, thus suggesting that other mechanisms, including those mentioned previously, might be involved in cytotoxicity induced by IFN-γ-stimulated neutrophils.

The present study demonstrated that neutrophils stimulated with IFN-γ produced TRAIL and that activated neutrophils exerted their cytotoxic effect against leukemic cells, at least in part, via TRAIL. The TRAIL-mediated antitumor effect is a novel neutrophil function. Additional studies to examine closely the TRAIL-mediated killing by the neutrophils of cancer cells in an in vivo system may reveal a suppressive function of neutrophils in tumor formation, thus leading to the development of a new strategy in cancer treatment.

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ANTITUMOR EFFECT OF NEUTROPHIL-DERIVED TRAIL


Neutrophil-Derived TNF-Related Apoptosis-Inducing Ligand (TRAIL): A Novel Mechanism of Antitumor Effect by Neutrophils

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