

Metalloproteinase-mediated Release of Human Fas Ligand

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Summary

Fas ligand (FasL) is a type II integral membrane protein homologous with tumor necrosis factor (TNF). Recent studies indicate that TNF is processed to yield the soluble cytokine by metalloproteinases at the cell surface of activated macrophages and T cells. In the present study, we investigated whether FasL is also released by metalloproteinases. Treatment with hydroxamic acid inhibitors of matrix metalloproteinases specifically led to accumulation of membrane-type FasL (p40) on the surface of human FasL cDNA transfectants and activated human T cells, as estimated by surface immunofluorescence and immunoprecipitation with newly established anti-human FasL monoclonal antibodies. This surface accumulation of mFasL was associated with the decrease of soluble FasL (p27) in the supernatant as estimated by quantitative ELISA and immunoprecipitation with anti-human FasL monoclonal antibodies. These results indicate that human FasL is efficiently released from the cell surface by metalloproteinases like TNF.

Fas (APO-1, CD95) is a type I integral membrane protein initially identified by mAbs that induce apoptotic cell death upon binding to certain tumor cells (1, 2). Molecular cloning of Fas revealed that it belongs to the TNF receptor family and transduces a cell death signal (3). Fas is expressed on activated lymphocytes and in various tissues, including the liver, lung, intestine, and skin (4, 5). It has been reported that administration of an agonistic anti-Fas mAb rapidly killed mice by severely damaging the liver (6).

Recent molecular cloning of Fas ligand (FasL)¹ revealed that it is a type II integral membrane protein homologous with TNF (7). FasL is expressed on activated T and NK cells, and mediates Fas⁺ target cell lysis by these effector cells (8–10). The Fas/FasL system also been also implicated in the pathogenesis of autoimmune diseases, fulminant hepatitis, GVHD, and AIDS (11–13).

TNF- α , a prototype cytokine of the TNF family, has been well known to be released as the 17-kD secretory form from the 26-kD membrane form (14). Recent studies indicated that this processing is performed by some matrix metalloproteinases (MMPs) (15–17). It has also been recently reported that human FasL was released as a 26-kD soluble form from COS cells transfected with human FasL cDNA and activated human T cells (18). In this context, we demonstrate here that human FasL is also released by

MMPs such as TNF- α , as estimated by specific blocking by MMP inhibitors. Physiological and clinical relevance of this finding is discussed.

Materials and Methods

Cells. Mouse T lymphoma cell lines (L5178Y and WR19L) and human Fas cDNA transfectant (hFas/WR19L) (3) were kindly provided by Dr. S. Yonehara (Kyoto University, Kyoto, Japan) and cultured in RPMI-1640 medium containing 10% FCS, 100 μ g/ml each streptomycin and penicillin, and 2 mM glutamine (culture medium). A human CD4⁺ T cell clone (4HM1) of Th1 type was provided by Dr. M. Azuma (Juntendo University, Tokyo) and maintained in the culture medium supplemented with 50 U/ml of rIL-2.

Reagents. The following hydroxamic acid-based metalloproteinase inhibitors were synthesized by Kanebo Ltd. (Osaka, Japan): [4-(*N*-hydroxyamino)-2*R*-isobutyl-3*S*-(2-thienylthiomethyl)succinyl]-*L*-phenylalanine-*N*-methylamide (BB94) (19), [4-(*N*-hydroxyamino)-2*R*-isobutyl-3*S*-methylsuccinyl]-*L*-3-(5,6,7,8-tetrahydro-1-naphthyl)alanine-*N*-methylamide (KB8301), and [4-(*N*-hydroxyamino)-2*R*-phenylpropylsuccinyl]-*L*-3-cyclohexylalanine-*N*-[2-(4-sulphonamidophenyl)ethyl]amide (KB8112) (20). A non-hydroxamate derivative of KB8301, (4-hydroxy-2*R*-isobutyl-3*S*-methylsuccinyl)-*L*-3-(5,6,7,8-tetrahydro-1-naphthyl)alanine-*N*-methylamide (KB8845), was also synthesized as a negative control. These compounds are available upon request. Other protease inhibitors, including aprotinin, leupeptin, pepstatin, E64, Bestatin, captopril, and phosphoramidon, were purchased from Sigma Chemical Co. (St. Louis, MO). All these protease inhibitors were

¹Abbreviations used in this paper: cIg, control Ig; FasL, Fas ligand; mFasL, membrane FasL; sFasL, soluble FasL; MMP, matrix metalloproteinase; P+I, PMA plus ionomycin; RT, reverse transcription.

dissolved in DMSO at 10 mM as stock solutions. PMA and ionomycin were also purchased from Sigma. OKT-3 mAb was prepared from the hybridoma obtained from the American Type Culture Collection (Rockville, MD). An anti-Fas mAb (clone CH-11) was purchased from MBL (Nagoya, Japan). Preparation of the soluble Fas-Ig fusion protein has been described previously (8).

Generation of Human FasL Transfectants. Human FasL cDNA was prepared by reverse transcription (RT)-PCR from total RNA of activated 4HM1 by using an oligonucleotide corresponding to the first six codons as the 5' primer and to the last six codons as the 3' primer, according to the published sequence (21). The 5' and 3' primers were tagged with a XhoI or a NotI site, respectively. After XhoI and NotI digestion, the PCR product of 850 bp was subcloned into pBluescriptII SK+ (Stratagene, La Jolla, CA) and the nucleotide sequence was confirmed by sequencing. The 850-bp cDNA was then transferred into the XhoI and NotI sites of the CDM8 or BCMGSneo expression vector (22), kindly provided by B. Seed (Massachusetts General Hospital, Boston, MA) and H. Karasuyama (Tokyo Metropolitan Institute of Medical Science, Tokyo), respectively. Transient expression of human FasL cDNA (hFasL/CDM8) in COS cells was performed as described previously (8). Culture supernatant containing soluble FasL (sFasL) was collected after 4 d. For generating stable transfectants, hFasL/BCMGSneo was transfected into L5178Y cells by electroporation (290V; 960 μ F), using a gene pulser (Bio-Rad, Hercules, CA). After selection with 0.4 mg/ml G418 and cloning by limiting dilution, a transfectant (hFasL/L5178Y) expressing a high level of hFasL mRNA was selected by Northern hybridization.

Generation of Anti-human FasL MAbs. 4-wk old female MRL *1pr/1pr* mice (Clear Japan, Tokyo) were immunized by intraperitoneal injection of hFasL/L5178Y (10^7 cells) several times at 10-d intervals. 3 d after final immunization, the splenocytes were fused with P3U1 mouse myeloma cells, as described previously (23). After HAT selection, the antibodies that neutralize cytotoxic activity of sFasL in the hFasL/L5178Y supernatant against hFasL/WR19L were screened. Three mAbs (NOK-1, NOK-2, and NOK-3) were identified by their strong inhibitory effects and cloned by limiting dilution. NOK-1 (mouse IgG1, κ) and NOK-2 (mouse IgG2a, κ) were purified from ascites by protein G affinity chromatography. NOK-3 (mouse IgM, κ) was purified by ammonium sulfate precipitation and gel filtration.

Cytotoxic Assay. Cytotoxic activity of sFasL in the culture supernatants was tested against the human Fas transfectant by the alamar Blue method, according to the manufacturer's instructions (Alamar Biosciences, Inc., Sacramento, CA). Briefly, hFasL/WR19L or WR19L cells (10^4) were cultured with serially diluted hFasL/COS or hFasL/L5178Y supernatant in a total volume of 100 μ l. After 16 h, 10 μ l of alamar Blue was added and further incubated for 4 h. Fluorescence of the reduced alamar Blue was measured on a fluoroscan (Titertek Fluoroskan II; Labosystems Japan, Tokyo) at 590 nm by excitation at 544 nm.

Immunofluorescence. L5178Y or hFasL/L5178Y cells (10^6 /ml) were cultured with or without various protease inhibitors (10 μ M, unless otherwise indicated) for 24–48 h. 4HM1 cells (10^6 /ml) were stimulated with ionomycin (500 ng/ml) and PMA (10 ng/ml) in the presence or absence of 10 μ M BB94 for 2–6 h. These cells (10^6) were incubated with 1 μ g of biotinylated NOK-1 for 1 h at 4°C, followed by PE-labeled avidin (PharMingen, San Diego, CA). After washing with PBS, the cells were analyzed on a FACScan (Becton Dickinson & Co., San Jose, CA), and data were processed by using the CELLQuest program.

ELISA for sFasL, TNF- α , and IFN- γ . hFasL/L5178Y cells (10^6 /ml) were cultured with or without various protease inhibitors for 24 h. sFasL in the supernatant was quantitated by sandwich ELISA using NOK-1 and NOK-3. Briefly, Immulon 2 plates (Dynatech Laboratories Inc., Chantilly, VA) were incubated overnight with NOK-3 (10 μ g/ml). After washing with 0.05% Tween/PBS, the wells were blocked by skimmed milk solution (Block Ace; Snow Brand Milk Co., Sapporo, Japan) for 2 h at 37°C, 50 μ l of the sample were added and incubated for 1 h at room temperature, biotinylated NOK-1 (5 μ g/ml) was added and incubated for 1 h at room temperature, and 50 μ l of ABC solution (Vectastain ABC kit; Vector Laboratories Inc., Burlingame, CA) was added and incubated for 30 min at room temperature. The plates were developed with 100 μ l of 1 mg/ml orthophenylenediamine in 50 mM citrate-phosphate buffer (pH 5.0) containing 0.03% H₂O₂, and stopped with 100 μ l of 2N H₂SO₄. OD at 490 nm was measured on an automated ELISA reader. Serial dilutions of purified sFasL, which was affinity purified from the hFasL/L5178Y supernatant on a NOK-1 column, was used as the standard. Detection limit of this assay was \sim 0.2 ng/ml. 4HM1 cells (10^6 /ml) were stimulated with immobilized OKT-3 (10 μ g/ml) in the presence or absence of various protease inhibitors for 24 h. sFasL in the supernatant was measured as described above. TNF- α and IFN- γ in the supernatants were evaluated with commercial ELISA (R & D Systems, Minneapolis, MN) according to the manufacturer's instruction.

Immunoprecipitation. To identify the cellular FasL, hFasL/L5178Y cells (10^6 /ml) were cultured with 100 μ Ci/ml of [³⁵S]Cys/Met (TransLabel; ICN Biomedical Inc., Costa Mesa, CA) in the presence of 10 μ M BB94 at 37°C for 16 h. After chasing in fresh culture medium with 10 μ M BB94 at 37°C for 4 h, the cells were extracted with a lysis buffer (0.5% Triton X-100, 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 μ M PMSF, and 50 μ g/ml trypsin inhibitor). To identify the sFasL, hFasL/L5178Y cells were cultured with 100 μ Ci/ml of [³⁵S]Cys/Met in the absence of BB94 at 37°C for 24 h, and the supernatant was collected. After preclearing with normal mouse control Ig (cIg)-coupled Sepharose, the lysate and supernatant were immunoprecipitated with NOK-1-coupled Sepharose for 1 h at 4°C. After washing with the lysis buffer, the bound materials were eluted and subjected to SDS-PAGE under nonreducing or reducing (5% 2-ME) conditions. For fluorography, gels were incubated with Amplify (Amersham Japan, Tokyo) for 30 min, dried, and exposed to x-ray film (XAR-5; Eastman Kodak, Rochester, NY) at -80°C for 1 d.

For pulse-chase analysis, 4HM1 cells (10^6 /ml) were labeled with 100 μ Ci/ml of [³⁵S]Cys/Met in the presence of ionomycin plus PMA at 37°C for 1 h, then chased in the culture medium with or without 10 μ M BB94 for 1–6 h. Supernatants and cell lysates were prepared from the same culture and immunoprecipitated with NOK-1-Sepharose. The eluates were subjected to SDS-PAGE and fluorography as described above. Intensities of the p40 and p27 bands were measured on an image analyzer (Fujix BAS 2000; Fuji Photo Film Co. Ltd., Tokyo).

To identify the membrane FasL (mFasL), hFasL/L5178Y cells were surface-labeled with sulfo-N-hydroxysuccinimide-biotin, as described previously (23). The cells were extracted with the lysis buffer, precleared with cIg-Sepharose, and immunoprecipitated with NOK-1-Sepharose. After washing with the lysis buffer, the eluates were subjected to SDS-PAGE and electroblotted onto nitrocellulose membrane filters. The blots were subsequently incubated with Vectastain ABC solution, developed with ECL Western blotting detection reagents (Amersham Japan), and exposed to x-ray films for a few minutes.

Results and Discussion

Human FasL cDNA Transfectants Release Functional FasL. To characterize the functional properties of human FasL, we generated a stable transfectant. Human FasL cDNA was prepared by RT-PCR from total RNA of activated human T cell clone (4HM1) and introduced into a mouse T lymphoma cell line (L5178Y) that does not express Fas (8). A clone (hFasL/L5178Y) expressing a high level of FasL mRNA was selected. This cell line exhibited a high spontaneous cytotoxic activity against hFas/WR19L but not against WR19L in a 4-h ^{51}Cr release assay (data not shown), indicating the expression of functional FasL. Recently, Tanaka et al. reported the presence of a functional soluble form of human FasL (hereafter termed sFasL) in the culture supernatant of human FasL cDNA-transfected COS cells (18). We also observed that the culture supernatant of hFasL/L5178Y as well as that of human FasL cDNA-transfected COS cells

exhibited specific cytotoxicity against hFas/WR19L in a dose-dependent manner (Fig. 1 A). As measured by the sandwich ELISA described below, the hFasL/L5178Y and hFasL/COS supernatants contained sFasL at 13.5 ng/ml and 8.7 ng/ml, respectively. When dilution of the supernatant was converted to the concentration of sFasL (Fig. 1 B), sFasL derived from the L5178Y and COS transfectants exhibited almost comparable specific activity ($\text{ED}_{50} = 3.4 \text{ ng/ml}$ and 2.9 ng/ml , respectively). These were also comparable to the specific activity of natural sFasL derived from anti-CD3-stimulated 4HM1 (data not shown). In comparison, the most widely used agonistic anti-Fas mAb (CH-11) exhibited 2.5-fold lower specific activity ($\text{ED}_{50} = 8.1 \text{ ng/ml}$) than did sFasL. These results indicate that sFasL derived from various cellular backgrounds exhibits a comparable and potent specific activity, suggesting that they might undergo a similar processing.

MMP Inhibitors Induce Accumulation of FasL on Cell Surfaces. To characterize human FasL further, we generated several mAbs. To avoid possible lethality of FasL, Fas-deficient MRL *lpr/lpr* mice (6) were immunized with hFasL/L5178Y and the immune splenocytes were fused with P3U1 myeloma. After screening the ability to neutralize the FasL activity in the hFasL/L5178Y supernatant, we established three mAbs, termed NOK-1 (IgG1, κ), NOK-2 (IgG2a, κ), and NOK-3 (IgM, κ). These mAbs neutralized human FasL activity 20–50-fold more efficiently than soluble Fas-Ig fusion protein (Fig. 2). As represented in Fig. 3 A, NOK-1, as well as NOK-2 and NOK-3 (not shown), reacted with hFasL/L5178Y but not with L5178Y, as estimated by surface immunofluorescence and flow cytometry.

Recently, it has been demonstrated that the release of TNF- α , which is homologous with FasL, from activated T cells and monocytes is mediated by metalloproteinase(s) (15–17, 24) and that treatment with MMP inhibitors led to surface accumulation of TNF- α on these cells (17, 24). In

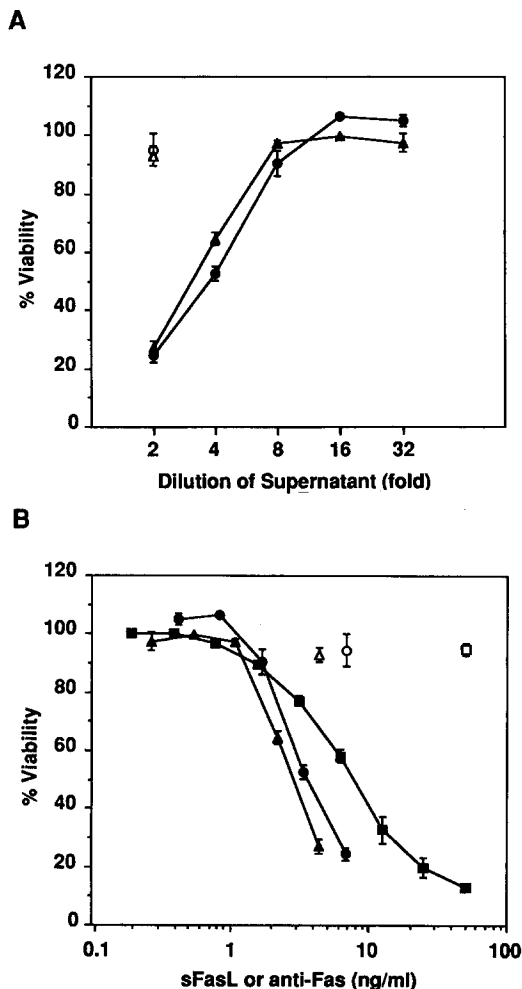


Figure 1. Release of functional sFasL from hFasL/L5178Y. Cytotoxic activity of sFasL in the supernatant of hFasL/L5178Y (●,○) or hFasL/COS (▲,△) and that of an anti-Fas mAb (■,□) was tested against hFas/WR19L (●,▲,■) or WR19L (○,△,□) cells by the Alamar blue method. Data represent mean \pm SD of triplicate samples. In B, sFasL concentration in the supernatants was determined by ELISA, and the abscissa is normalized as the concentration of sFasL and anti-Fas mAb.

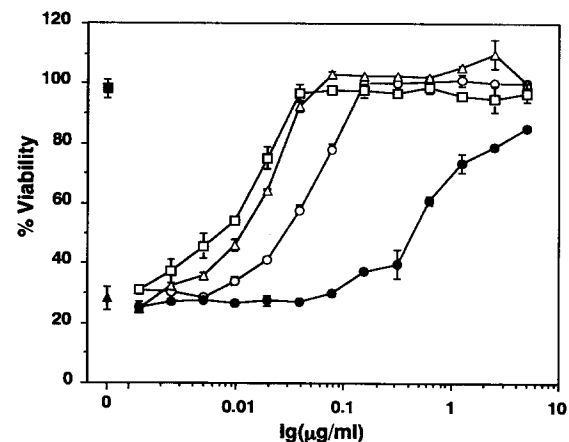


Figure 2. Neutralization of sFasL by anti-human FasL mAbs and Fas-Ig. Cytotoxic activity of sFasL in the supernatant of hFasL/L5178Y was tested against hFas/WR19L (○,△,□,●,▲) or WR19L (■) cells in the absence (▲,■) or presence of serially diluted NOK-1 (○), NOK-2 (△), NOK-3 (□), or Fas-Ig (●) by the Alamar blue method. Data represent mean \pm SD of triplicate samples.

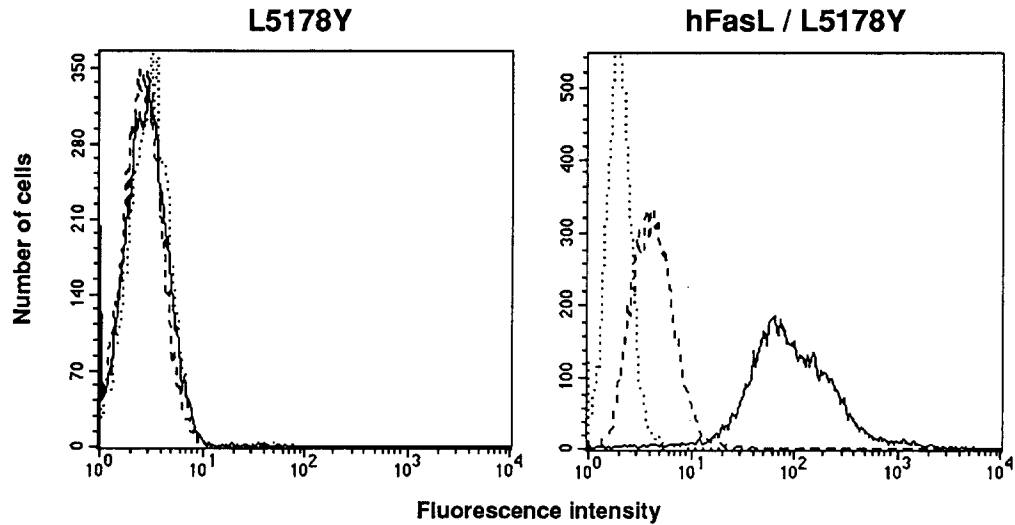
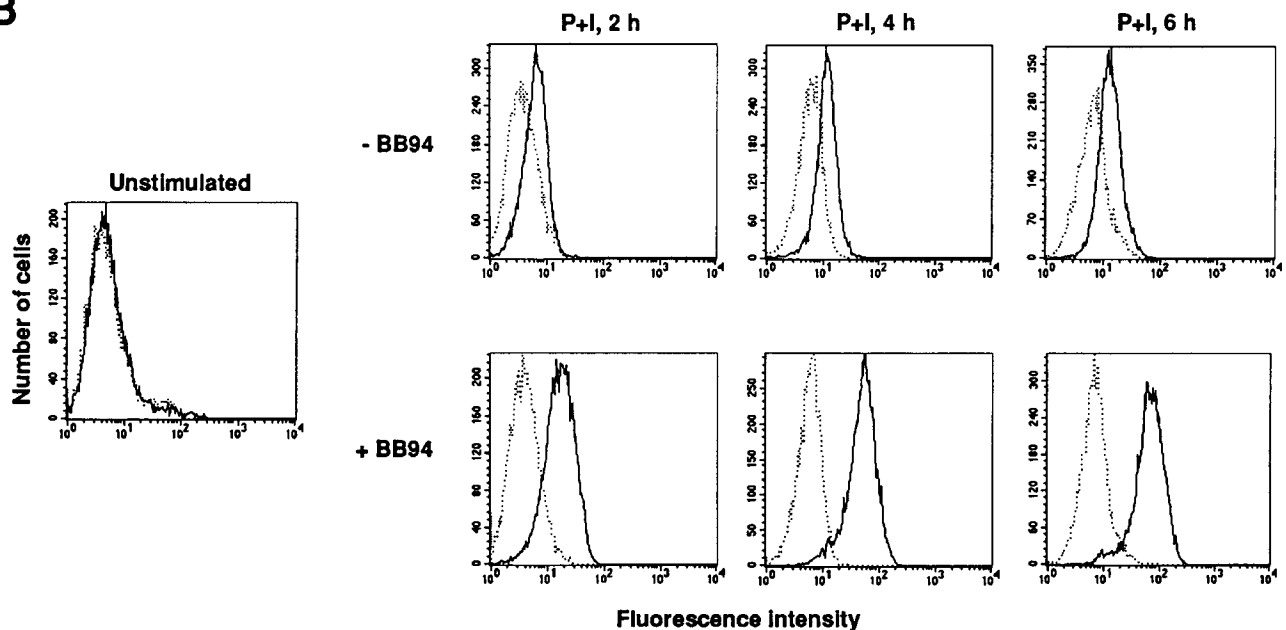
A**B**

Figure 3. Accumulation of Fas ligand on cell surface by BB94 treatment. (A) L5178Y and hFasL/L5178Y cells that had been cultured in the presence (solid lines) or absence (broken lines) of 10 μ M BB94 for 2 d were stained with biotinylated NOK-1 plus PE-avidin. Dotted lines indicate background staining with PE-avidin alone. (B) Human CD4⁺ T cell clone 4HM1 that had been unstimulated or stimulated with P+I for 2–6 h in the presence or absence of 10 μ M BB94 was stained with biotinylated NOK-1 plus PE-avidin (solid lines). Dotted lines indicate background staining with PE-avidin alone.

this context, we tested various protease inhibitors for their ability to accumulate FasL on the surface of hFasL/L5178Y. As represented with BB94 in Fig. 3 A, the treatments with hydroxamic acid-based MMP inhibitors (BB94, KB8301, and KB8112), which are similar to those used for inhibiting the TNF- α processing (15–17), greatly enhanced the staining with NOK-1. In contrast, a nonhydroxamic acid control compound (KB8845) or various inhibitors of other proteases, including angiotensin-converting enzyme (captopril, 100 μ M), enkephalinase (phosphoramidon, 100 μ M), serine proteases (aprotinin, 10 μ M), serine/cysteine proteases

(leupeptin, 100 μ M), cysteine proteases (E64, 100 μ M), aspartyl proteases (pepstatin, 100 μ M), and aminopeptidases (Bestatin, 100 μ M), were not effective (data not shown). The 2-d BB94 treatment did not affect the steady-state level of FasL mRNA in hFasL/L5178Y as estimated by Northern hybridization (not shown), ruling out the possibility of enhanced transcription.

It has been demonstrated that the expression of FasL mRNA and protein is induced in human T cells by stimulation with PMA plus ionomycin (P+I). We also observed that a human CD4⁺ T cell clone, 4HM1, expressed FasL in

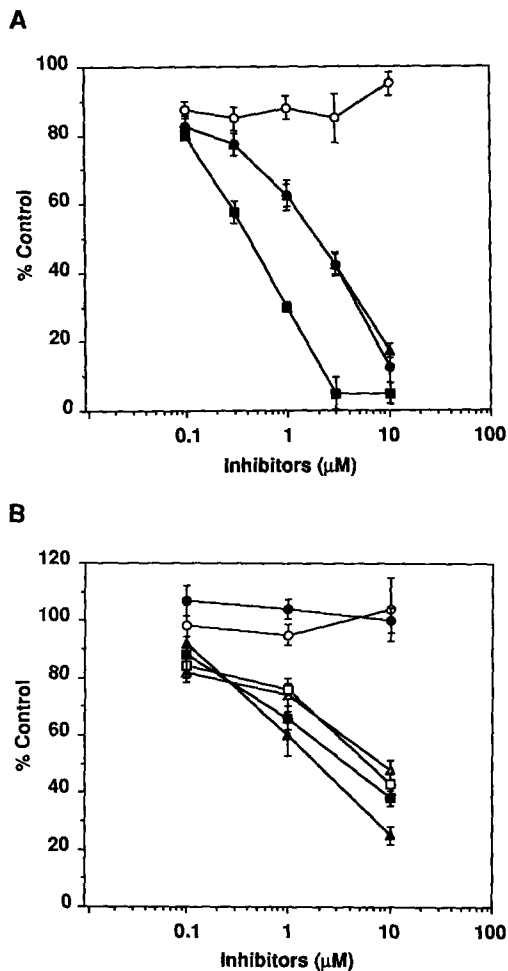


Figure 4. Blocking of FasL release by MMP inhibitors. (A) hFasL/L5178Y cells were cultured in the presence of the indicated doses of KB8845 (○), KB8301 (●), BB94 (▲), or KB8112 (■) for 24 h. sFasL in the supernatants was measured by ELISA. Data are indicated as a percentage of control of sFasL in the absence of inhibitors. (B) Human CD4⁺ T cell clone 4HM1 was stimulated by immobilized OKT-3 in the presence or absence of the indicated doses of BB94 (○, Δ, □), or KB8112 (●, ▲, ■). After 24 h, IFN-γ (○, ●), sFasL (Δ, ▲), and TNF-α (□, ■) in the supernatants were measured by ELISA. Data are represented as a percentage of control of each cytokine in the absence of inhibitors.

response to these stimulations, as estimated by Northern hybridization and specific cytotoxicity against hFas/WR19L (data not shown). Consistently, NOK-1 did not react with unstimulated 4HM1 but did react with P+I-stimulated 4HM1 (Fig. 3 B). In the presence of BB94, the level of surface FasL was progressively increased. KB8301 and KB8112 but neither KB8845 nor other protease inhibitors gave a similar result (data not shown). Induction of FasL mRNA was not affected by BB94, as estimated by Northern hybridization (not shown). These results suggested that the MMP inhibitors induced the surface accumulation of FasL on the transfectants and activated T cells by inhibiting the processing by some MMP.

MMP Inhibitors Block sFasL Release. By utilizing NOK-1 and NOK-3, we established a sandwich ELISA system for sFasL (see Materials and Methods), which enabled us to es-

timate sFasL release quantitatively. We then examined the effect of MMP inhibitors on sFasL release. The hydroxamic acid-based MMP inhibitors (BB94, KB8301, and KB8112) but not the non-hydroxamic acid control (KB8845) inhibited the sFasL release from hFasL/L5178Y in a dose-dependent manner (Fig. 4 A). The other protease inhibitors tested were not effective (not shown). In Fig. 4 B, we also tested the effect of BB94 and KB8112 on the release of sFasL, TNF-α, and IFN-γ from OKT-3-stimulated 4HM1. These MMP inhibitors inhibited the release of sFasL and TNF-α comparably but not the release of IFN-γ. This result not only rules out an inhibitory effect of these MMP inhibitors on T cell activation but also suggests a close correlation between the TNF processing enzyme and the FasL processing enzyme.

Biochemical Characterization of FasL Processing. The processing of FasL was biochemically characterized by immunoprecipitation and SDS-PAGE analysis. To identify the mFasL, hFasL/L5178Y cells were metabolically labeled with [³⁵S]Cys/Met in the presence of BB94, which leads to accumulation of mFasL. As represented in Fig. 5 A, NOK-1 specifically precipitated the 40-kD band (p40) under reducing and nonreducing conditions from the BB94-treated hFasL/L5178Y but not from L5178Y (not shown). This result appears to represent the mFasL as described in COS cell transfectant (18). In some cases, an additional 80-kD band (Fig. 5 A) and a faint 120-kD band were also detected under nonreducing conditions, which might represent a dimer and trimer, respectively, as described for sFasL (18). On the other hand, sFasL was immunoprecipitated by NOK-1 from the culture supernatant of ³⁵S-Cys/Met-labeled hFasL/L5178Y as a 27-kD band (p27) under non-reducing and reducing conditions (Fig. 5 A). Although oligomers of sFasL were barely detected in our SDS-PAGE analysis, FasL activity in the hFasL/L5178Y supernatant was eluted around *M_r* of ~80 kD in gel filtration (data not shown), suggesting that sFasL is present as a trimer, as demonstrated in the supernatant of COS cell transfectant (18).

NOK-1 immunoprecipitates from the surface biotinylated hFasL/L5178Y lysate typically exhibited two bands of 40 and 27 kD, which apparently represent mFasL and sFasL, respectively (Fig. 4 B). The 27-kD band (p27) was also detected occasionally in the lysate of [³⁵S]Cys/Met-labeled hFasL/L5178Y cells (not shown). This suggests that some sFasL is anchored on the cell surface as a part of partially processed mFasL trimer like lymphotoxin-α anchored by lymphotoxin-β (25). As represented in Fig. 5 B, an additional 60-kD band, whose identity is unknown, was also detected occasionally.

We also examined the kinetics of the processing in activated T cells by pulse-chase analysis. PMA/ionomycin-activated 4HM1 cells were pulse-labeled with [³⁵S]Cys/Met for 1 h and then chased in the culture medium with or without BB94 (Fig. 5 C). In the absence of BB94, mFasL (p40) in the cell lysate was rapidly lost and, concomitantly, sFasL (p27) was accumulated in the supernatant. In the presence of BB94, the loss of p40 in the lysate and the accumulation of p27 in the supernatant were greatly reduced,

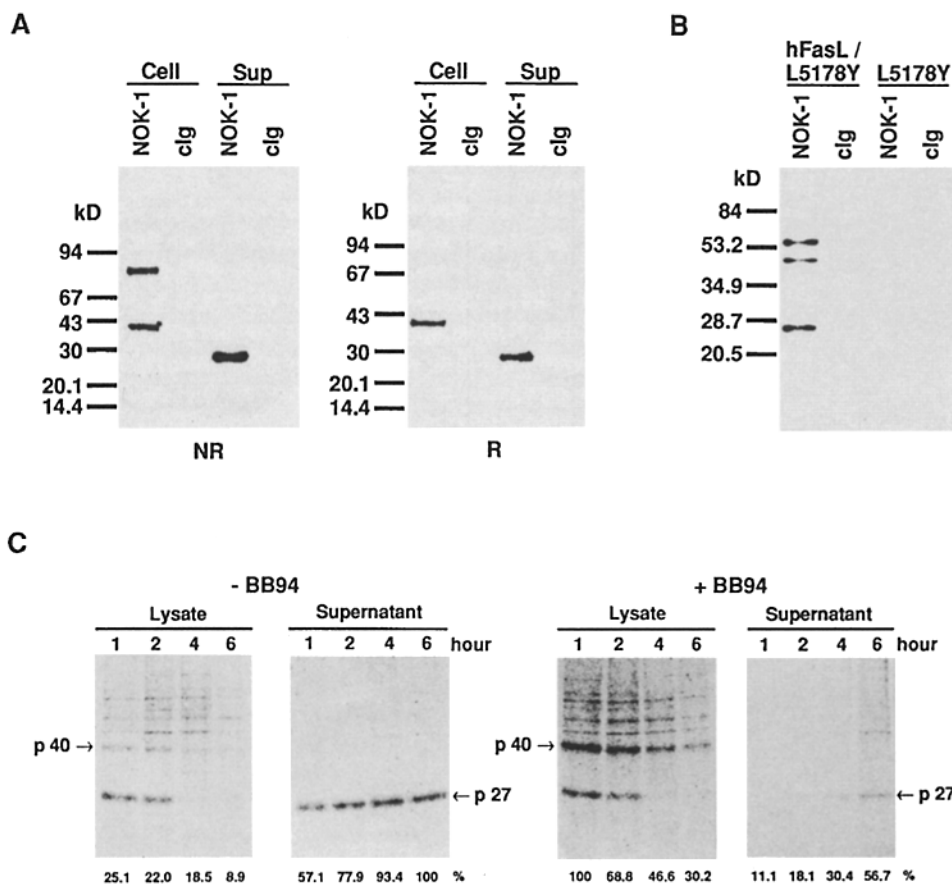


Figure 5. Biochemical characterization of human FasL processing. (A) FasL was immunoprecipitated with NOK-1 or cIg from the cell lysate (Cell) of BB94-treated hFasL/L5178Y or the supernatant (Sup) of hFasL/L5178Y after ^{35}S -Cys/Met labeling. Immunoprecipitates were resolved by SDS-PAGE (12% gel) under nonreducing (NR) or reducing (R) conditions and autoradiography. (B) FasL was immunoprecipitated with NOK-1 or cIg from the cell lysate of BB94-treated hFasL/L5178Y or L5178Y cells after surface biotinylation. Immunoprecipitates were resolved by SDS-PAGE (12% gel) under reducing condition and transferred to nitrocellulose membrane. Biotinylated FasL was detected by ABC and ECL. (C) Human CD4⁺ T cell clone 4HM1 was pulse labeled for 1 h with [^{35}S]Cys/Met in the presence of PMA plus ionomycin. The cells were chased in fresh medium in the presence or absence of 10 μM BB94 for 1–6 h. FasL was immunoprecipitated with NOK-1 from the cell lysates and supernatants, and resolved by SDS-PAGE (12% gel) under reducing condition and autoradiography. The positions of mFasL (p40) and sFasL (p27) are indicated at the left and right, respectively. Values under each lane indicate relative intensity

of p40 in the lysate or p27 in the supernatant when p40 in the BB94-treated cell lysate at 1 h and p27 in the untreated supernatant at 6 h are defined as 100%, respectively.

demonstrating that BB94 is acting at the cell surface to inhibit FasL processing. Comparison of the band intensities revealed that 75% of p40 was processed to p27 and 57% was released into the supernatant within the first 1 h, indicating a high efficiency of FasL processing on the activated T cell surface.

This study indicated that human FasL is released from the T cell surface via processing by some MMP, as was the case with TNF- α . A closely correlated sensitivity to various protease inhibitors raises a possibility that these two type II integral membrane proteins may be processed by the same metalloproteinase. In our preliminary study, activated T cells express a limited repertoire of known MMPs, as reported by others (26). Identification of a candidate enzyme

is now in progress. Recent studies demonstrated that administration of an MMP inhibitor that blocked the TNF processing protected mice from lethal endotoxin shock, which is mainly mediated by macrophage-derived TNF (15). We also observed that KB8301 and BB94 have a similar effect (unpublished data). On the other hand, activated T cell-derived FasL has been implicated in the pathogenesis of virus hepatitis and GVHD (12), in which TNF has been also implicated (27). In our preliminary study, some patients suffering from fulminant hepatitis or GVHD exhibited a high serum sFasL level, as estimated by the ELISA (unpublished data). Therefore, the MMP inhibitors that block both sFasL and TNF release would be clinically useful for the treatment of these diseases.

We thank Drs. Shin Yonehara and Miyuki Azuma for cells and Fumiko Sugino for help preparing the manuscript.

This work was supported by grants from the Ministry of Education, Science, and Culture and the Ministry of Health, Japan.

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Received for publication 5 July 1995 and in revised form 7 August 1995.

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