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Cutting Edge: Chemotactic Activity of Soluble Fas Ligand Against Phagocytes

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A recombinant soluble form of human Fas ligand (sFasL) was tested for its chemotactic activity against human and mouse polymorphonuclear neutrophils (PMN) by the Boyden chamber method. sFasL exhibited a potent chemotactic activity against both human and mouse PMN and HL-60 cells when differentiated into neutrophils or monocytes. A neutralizing anti-FasL mAb abolished the chemotactic activity, while control mAb did not. Ligation of Fas by either IgM- or IgG-type anti-Fas mAb also induced PMN migration. PMN derived from lpr mouse that express few Fas molecules did not respond to sFasL. In contrast, those derived from lpr mice that express Fas molecules with a mutated death domain normally responded to sFasL. These results directly indicated a chemotactic activity of sFasL against PMN and suggest a novel signaling function of Fas, which appears to be independent of the death domain-mediated apoptosis. The Journal of Immunology, 1998, 161: 4484–4488.

The Fas ligand (FasL) belongs to the TNF family, which includes TNF, lymphotoxin, TNF-related apoptosis-inducing ligand (TRAIL), CD40 ligand, CD27 ligand, CD30 ligand, and OX40 ligand (1). Most members of the TNF family, except for lymphotoxin-α, are type II membrane proteins. However, a soluble form of FasL (sFasL) is naturally produced by metalloproteinase-mediated processing such as TNF-α (2, 3). The physiologic roles of the shedding of TNF family members have not been well characterized.

FasL induces apoptotic cell death by binding to its receptor, Fas (also called APO-1 or CD95), which is a member of the TNF receptor family (4). FasL is predominantly expressed in activated T cells and NK cells, while Fas is ubiquitously expressed on various cells. FasL-mediated cell death is involved in the T or NK cell-mediated cytotoxicity, some pathologic tissue damages, and the regulation of lymphocyte homeostasis (4). FasL is also expressed in the testis (5), eye (6), and some malignant tumor cells (7, 8), which has been proposed to contribute to their immune-privileged status. FasL expressed in such immune-privileged tissues may eliminate infiltrating immune cells. By applying this concept, Lau et al. reported that syngeneic myoblasts expressing FasL protected allogeneic pancreatic islets from immune rejection when cotransplanted under the kidney capsule (9).

In contrast, we and others have found that enforced FasL expression in tumor cells or islets elicited neutrophilic inflammation and destruction of the graft. When implanted s.c. or i.p., various tumor cells expressing FasL induced neutrophil infiltration and rapid rejection (10). Similarly, expression of functional FasL in the pancreatic islets of FasL-transgenic mice induced granulocytic infiltrates and damage of the islets (11, 12). In this context, we here verified the chemotactic activity of FasL against polymorphonuclear neutrophils (PMN). A novel signaling function of Fas, which appears to be independent of the functional death domain, was noted.

Materials and Methods

Preparation of sFasL

A recombinant soluble form of human FasL (sFasL) was produced by using the baculovirus expression system as described previously (13). Briefly, the full-length FasL cDNA was subcloned into PVL1393 (PharMingen, San Diego, CA) and transfected into Spodoptera frugiperda (SPF) cells according to the manufacturer’s instruction. The culture supernatant was applied to an affinity column of protein A-Sepharose A conjugated with an anti-FasL (NOX-1) mAb (2). The bound sFasL was eluted with 0.1 M glycine-HCl buffer (pH 4.0). The concentration of sFasL was determined by sandwich ELISA as described previously (2).

Preparation of PMN

C3H/He wild-type and lpr mice were purchased from SLC (Shizuoka, Japan). CBA lpr mice, which have a loss-of-function mutation in the death domain of the Fas molecule (14), had been backcrossed 12 times to C3H mice, and the C3H lpr mice were maintained in the animal facilities of Tokyo University and used for the experiments. Mouse PMN were isolated...
from peritoneal exudates 4–5 h after an i.p. injection of 3 ml 4% thigly-
collate (Difco, Detroit, MI) as described previously (15). Mouse PMN
preparations contained >87% Gr-1+ cells as estimated by flow cytometry.
Human PMN were isolated from citrate-anticoagulated peripheral blood of
healthy donors by Polymorphoprep (Nycomed Pharma, Oslo, Norway)
centrifugation techniques as described previously (16). The purity of hu-
man PMN was >95% as estimated by Wright-Giemsa stain. PMN were
suspended in PBS containing 1 mM CaCl2 and 1 mM MgSO4.

Cell culture

Human promyelocytic leukemia HL-60 cells were maintained in RPMI
1640 medium (Nissui, Tokyo, Japan) containing 10% FBS, 2 mM glu-
tamine, 100 µg/ml streptomycin, and 100 U/ml penicillin. To differentiate
HL-60 cells into neutrophilic or monocytic lineage, the cells were cultured
with 1.3% DMSO or 5 nM PMA for 4 days, respectively (17, 18), and then
used for the chemotaxis assay. The differentiation was confirmed by
Wright-Giemsa stain of cytospin preparations and expression of cell sur-
face CD11b (17, 18).

Chemotaxis assay

Cell migration was assayed by employing a modified Boyden chamber
with cellulose nitrate filter, as described previously (19). We used a 3-µm
pore size filter for PMN and untreated or DMSO-treated HL-60 cells and
a 5-µm pore size filter for PMN-treated HL-60 cells. Varying concentra-
tions of purified sFasL, anti-Fas mAb (CH-11 (IgM, Medical Biologic
Laboratories, Nagoya, Japan) or DX-2 (IgG1, Pharmingen)), or control
mAb (mouse IgM or IgG1, Pharmingen) in PBS supplemented with 1 mM
Ca2+, Mg2+, and 1 mg/ml BSA (Fraction V, Sigma, St. Louis, MO) were
added to the upper or lower wells of the chamber. One million cells in 250
µl PBS supplemented with 1 mM Ca2+, Mg2+, and 1 mg/ml BSA were
placed into the upper wells. The entire apparatus was incubated at 37°C for
45 min for PMN and untreated or DMSO-treated HL-60 cells, and 90 min
for PMA-treated HL-60 cells. The membrane was then fixed with neutral
buffered formalin for 30 min and stained with hematoxylin, hematoxylin
and eosin, or Wright-Giemsa. With a ×40 objective, the distance (µm) from
the top of the filter to the furthest two cells at the same focal plane was
measured microscopically. This measure was taken for 10 fields across
the filter. In each assay, human IL-8 (Genzyme, Cambridge, MA) or FMLP
was used as a positive control. For a blocking experiment, anti-Fas, mAb
(NOK-1) (2) or control mAb (mouse IgG1, Pharmingen) at a concentration
of 10 µg/ml was added to both upper and lower chambers.

Statistical analysis

The statistical significance of differences from control was evaluated by
Student’s t test. Values of p < 0.05 were considered significant.

Results

We first evaluated the ability of sFasL to induce human PMN
migration across the cellulose nitrate filters. The mean data from three
individual experiments is shown in Fig. 1A. Significant PMN
migration for sFasL ranging from 0.1 to 10 nM was observed,
although the positive chemotactrant IL-8 was more active. sFasL
as well as IL-8 showed a bell-shaped chemotactic activity. When the
same concentration of sFasL was added to both upper and lower
chambers, the PMN migration was not observed, indicating that
chemokinetic movement of PMN was not enhanced. A neu-
tralizing mAb to FasL, but not control mAb (data not shown),
abolished the FasL-induced migration, indicating that the PMN
migration was indeed induced by FasL but not by some contam-
inant in the rFasL preparation.

Because it was possible that Fas/FasL interaction might trigger
the production of some chemotactic factor responsible for the che-
motaxis, we obtained supernatant of human PMN that had under-
gone chemotaxis to sFasL and tested its chemotactic activity
against PMN in the presence of anti-FasL mAb. However, the
supernatant failed to induce chemotaxis (data not shown), exclud-
ing this possibility.

To characterize the cells undergoing chemotaxis to sFasL, we
stained the filters with hematoxylin and eosin or Wright-Giemsa.
The migrating cells had no eosinophilic or basophilic granules (not
shown), indicating that the cells undergoing chemotaxis were neutrophils.

Next, we examined whether ligation of Fas by anti-Fas mAb
might also induce PMN migration. We used IgM (CH-11)- or IgG
(DX-2)-type anti-Fas mAbs, which are cytotoxic or noncytotoxic
in solution, respectively. As indicated in Fig. 1B, not only cyto-
xic CH-11 but also noncytotoxic DX-2 induced PMN migration,
suggesting that the signal transduction for the chemotaxis may be
different from that for apoptosis.

It has been well characterized that a human promyelocytic leu-
kenia cell line HL-60 can be differentiated into neutrophils or monocytes
in the presence of DMSO or PMA, respectively (17, 18). Untreated, DMSO-treated, and PMA-treated HL-60 cells ex-
pressed Fas at a comparable level as estimated by FACS analysis
(data not shown). Then, we examined the ability of sFasL to in-
duce migration of the HL-60 cells that were differentiated into
neutrophilic or monocytic lineage. As shown in Fig. 2, A and B,
both untreated and DMSO-treated HL-60 cells significantly re-
sponded to sFasL. Similarly, PMA-treated HL-60 cells also re-
sponded to sFasL (Fig. 2C). These results suggest that sFasL has
a chemotactic activity against not only neutrophils but also monocytes.

Further, we evaluated the ability of sFasL to induce mouse PMN
migration. As shown in Fig. 3A, sFasL ranging from 0.1 to 10 nM
induced as potent a migration as did IL-8 when PMN were ob-
tained from wild-type C3H mice. The chemokinetic movement
of neutrophils was not enhanced as in Fig. 1A. The anti-FasL mAb,
but not control mAb (data not shown), abolished the FasL-induced
migration. The chemotactic activity of sFasL was not observed
against PMN obtained from the lpr/mice, which lack Fas (Fig. 3B),
indicating that the FasL-induced PMN migration is mediated
through Fas on PMN. In contrast, as shown in Fig. 3C, PMN
obtained from the lpr/mice responded to sFasL almost compar-
ably to those from wild-type mice, suggesting that the signal
transduction for cell migration may not need a functional death
domain in the cytoplasmic region of Fas.

Discussion

In the present study, we demonstrated that sFasL acts as a che-
motactrant against human and mouse PMN in vitro. This
conclusion is consistent with our in vivo observation that FasL trans-
fectants injected in syngeneic mice induced neutrophil infiltration
and rejection of the cells (10). The FasL-induced rejection ap-
peared to be dependent upon the expression of Fas on the host
leukocytes, because wild-type→lpr, but not lpr→wild-type, bone
marrow chimeras rejected the FasL transfectants with neutrophilic
infiltrates (10). Therefore, it has been speculated that FasL might
directly act on infiltrating cells that were mainly composed of neu-
traphils. Although the present study does not exclude a possible
contribution of other indirect mechanisms, it is strongly suggested
that the chemotactic action of sFasL is directly involved in the
FasL-induced inflammation in vivo.

We used human sFasL in this study. We and other groups dem-
onstrated that mouse FasL also induced a similar inflammation
(10–12). Although mouse sFasL has been shown to have little
cytotoxic activity (3), it may have another function such as che-
motaxis, which remains to be determined in further studies. Re-
cently, several groups demonstrated a greatly reduced cytotoxic
activity of sFasL, as compared with membrane-bound FasL (20–
22), arguing against a systemic cytotoxic role of sFasL. Consis-
tently, the sFasL preparation used in this study did not enhance the
apoptosis of Jurkat cells (13) and PMN (data not shown) after
several hours of incubation. Our present results suggest that sFasL
can act proinflammatory by recruiting PMN rather than proapoptotically in certain pathophysiologic conditions.

Recently, signaling pathways for Fas-mediated apoptosis have been extensively characterized. Recruitment of FADD/Mort-1 and activation of caspase-8 mediate apoptosis through the activation of down-stream caspases (23). Fas can also induce apoptosis through RIP and RAIDD followed by caspase-2 activation (24), or through Daxx followed by jun kinase activation (25). In contrast, signaling for Fas-mediated inflammation has not been characterized. So far, it has been demonstrated that crosslinking of Fas by Ab could induce activation of the transcription factor NF-κB (26, 27) and secretion of IL-8 (28) in certain cell lines. In this study, both IgM- and IgG-type anti-Fas mAbs induced PMN migration (Fig. 1B).

Although it is generally believed that trimerization of Fas is needed for the induction of apoptosis, these results suggest that trimerization may not be necessary for the induction of chemotaxis, but dimerization of Fas may be sufficient. Furthermore, in the present study the chemotaxis was normally induced in PMN

**FIGURE 1.** sFasL and anti-Fas mAbs induce human PMN migration. A. Effect of sFasL on human PMN migration. IL-8 and sFasL (0.001–1000 nM) were assessed for chemotactic activity against human peripheral blood PMN by the Boyden chamber method. The column designated as 1/1 represents the presence of sFasL (1 nM) in both upper and lower chambers. For blocking, anti-FasL mAb or control mAb (10 μg/ml) was added to both upper and lower chambers. B. Effect of anti-Fas mAbs on human PMN migration. Control mouse IgM and IgG1 mAbs (1 μg/ml) and anti-Fas mAbs (CH-11 (IgM) and DX-2 (IgG1) 0.01–10 μg/ml) were assessed for chemotactic activity against human PMN. *, p < 0.05; **, p < 0.01 compared with negative control (cont.). ns, Not significant. Data are shown as mean ± SD of three independent experiments using different donors.

**FIGURE 2.** Effect of sFasL on migration of undifferentiated or differentiated HL-60. FMLP (10 nM) and sFasL (0.1–10 nM) were assessed for chemotactic activity against untreated (A), DMSO-treated (B), and PMA-treated (C) HL-60 cells. *, p < 0.05; **, p < 0.01 compared with negative control (cont.). ns, Not significant. Data are shown as mean ± SD of three independent experiments.
obtained from lpr\(^{g}\) mice (Fig. 3C) whose Fas molecules have a point mutation in the death domain and cannot induce apoptosis due to the inability to recruit FADD or RIP (14). These data suggest that some other signaling components may be recruited to the Fas molecules independently of the known death domain-interacting molecules for the chemotactic activity, which remained to be identified in further studies.

It has been shown that soluble human TNF-\(\alpha\) has a chemotactic activity against human PMN in vitro (29, 30). The chemotactic activity of sFasL demonstrated in this study appears to be comparable to that of TNF-\(\alpha\) described in these studies. Thus, it will be interesting to see whether other members in the TNF family have similar function. It has been reported that low concentrations of TNF-\(\alpha\) act synergistically with IL-1 to induce neutrophil infiltration in vivo (31). Similarly, it is possible that low concentrations of sFasL may enhance proinflammatory function of other cytokines.

Actually, sFasL has been detected in the sera of patients suffering from some inflammatory diseases such as systemic lupus erythematosus (32), rheumatoid arthritis (32), Sjogren’s syndrome (32), leukemia (33), lymphohistiocytosis (34), myocarditis (35), and alcoholic liver disease (36). Recently, Hashimoto et al. (37) reported that sFasL was detected in the joints of patients with rheumatoid arthritis, where neutrophil influx is often observed. The sFasL was produced by lymphocytes in the synovial fluid, and cleaved sFasL accumulated in the inflamed joints. They also reported that the concentration of sFasL was remarkably higher in patients with severe rheumatoid arthritis than in patients with its mild form. Further clinical studies may elucidate the pathophysiologic role of sFasL at a local site of inflammation in other diseases.

References


