FEEL-1, a Novel Scavenger Receptor with in Vitro Bacteria-binding and Angiogenesis-modulating Activities*

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Hideki Adachi‡ and Masafumi Tsujimoto
From the Laboratory of Cellular Biochemistry, RIKEN (The Institute of Physical and Chemical Research), Saitama 351-0198, Japan

Employing the expression cloning technique, we cloned a novel scavenger receptor that is structurally unrelated to other scavenger receptors. The cloned receptor contained fasciclin (Fas-1), epidermal growth factor (EGF)-like, laminin-type EGF-like, and link domains. Based on the domain structures, we temporarily named it FEEL-1 (fasciclin, EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1). A data base search suggested the presence of a paralogous gene of FEEL-1, the full-length cDNA of this gene was also cloned, and its nucleotide sequence was determined. The deduced amino acid sequence of the clone indicated that its domain organization is similar to FEEL-1, and we named this clone FEEL-2. The effect of monoclonal antibodies against FEEL-1 indicated that FEEL-1 is the major receptor for 1,1-diiodoacetyl-3,3',3'-tetramethylindocarbo-cyanine perchlorate (DiI)-labeled acetylated low density lipoprotein (DiI-Ac-LDL) in human umbilical vein endothelial cells. Reverse transcription and PCR analysis revealed that both FEEL-1 and FEEL-2 were expressed in several tissues and expressed highly in the spleen and lymph node. On the other hand, only FEEL-1 was expressed in mononuclear cells, particularly resting CD14+ cells. The transient expression of FEEL-1 and FEEL-2 in Chinese hamster ovary cells demonstrated that both FEELs could bind to DiI-Ac-LDL. Both receptors were also found to bind to Gram-negative and Gram-positive bacteria. These results suggest that FEELs play important roles in the defense mechanisms against bacterial infection. Finally, the phenotypic effect of the inhibition of FEEL-1 on vascular remodeling was tested in vitro using the Matrigel tube formation assay, and we found a marked reduction in the degree of cell-cell interaction in anti-FEEL-1 monoclonal antibody-treated cells, suggesting the role of this receptor in angiogenesis.

Scavenger receptors mediate the endocytosis of chemically modified lipoproteins, such as acetylated low density lipoprotein (Ac-LDL)1 and oxidized LDL (Ox-LDL), and have been implicated in the pathogenesis of atherosclerosis. The scavenger receptor gene family comprises a series of unlinked genes encoding membrane proteins with multiple ligand binding activity (1–5). We have cloned a subgroup of this family, the class F (6, 7) receptor termed SREC (scavenger receptor expressed by endothelial cell), from a cDNA library prepared from human umbilical vein endothelial cells (HUVECs). SREC mediates the binding and degradation of Ac-LDL. On the other hand, several scavenger receptors other than SREC have been reported to be expressed in endothelial cells (8, 9). In this study, we cloned a novel scavenger receptor termed FEEL-1, which is expressed in endothelial cells and is structurally unrelated to other scavenger receptors by expression cloning. The receptor comprises unique domain structures and has binding activity to both Gram-positive and Gram-negative bacteria. Moreover, an in vitro tube formation assay suggested that the receptor might play a role in angiogenesis. Initial characterization of this novel scavenger receptor together with the subsequently cloned related receptor, FEEL-2, is reported.

EXPERIMENTAL PROCEDURES

Cell Culture—The parental CHO-K1 cell was obtained from the RIKEN Cell Bank and cultured in Ham's F-12 medium with 10% heat-inactivated fetal calf serum. Human coronary smooth muscle cells, human coronary arterial endothelial cells, and human umbilical vein endothelial cells (HUVECs) were purchased from Clonetics and maintained in SmGM2, EGM-MV, or EGM medium according to the manufacturer's instructions. Human brain microvascular endothelial cells were purchased from Applied Cell Biology Research Institute and maintained according to the manufacturer's instructions. SDS-polyacrylamide gel electrophoresis and Western blot analysis were as described previously (10) using the ECL Plus Western blotting Detection Reagents (Amersham Biosciences).

Expression Cloning of Novel Scavenger Receptors—A cDNA library was constructed using the pEAK8 vector (Edge BioSystems) and SuperScript® plasmid system for cDNA synthesis and plasmid cloning (Invitrogen) with poly(A)+ RNA isolated from HUVECs. The resultant transformants were divided into small pools (~3,000 clones/pool), and the plasmid DNA was purified using the Plasmid Mini kit (Qiagen). The plasmid DNA was transfected into COS-1 cells using LipofectAMINE Reagent (Invitrogen), and the transiently transfected cells were screened visually for endocytosis of fluorescent DiI-Ac-LDL in the presence of high density lipoprotein (HDL) (100 µg/ml) and the monoclonal antibody against SREC (30 µg/ml). A positive pool was serially subdivided and retested to permit the purification of a single positive plasmid (7). Sequencing was performed using an automated

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The nucleotide sequence(s) reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession number(s) AB052956, AB052957, and AB052958.

‡ To whom correspondence should be addressed: Laboratory of Cellular Biochemistry, RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan. Tel.: 81-48-467-9372; Fax: 81-48-462-4670; E-mail: adachihi@postman.riken.go.jp.

1 The abbreviations used are: LDL, low density lipoprotein; Ac-LDL, acetylated LDL; Ox-LDL, oxidized LDL; HDL, high density lipoprotein; DiI, 1,1-diiodoacetyl-3,3',3'-tetramethylindocarbo-cyanine perchlorate; SREC, scavenger receptor expressed by endothelial cells; CHO, Chinese hamster ovary; HUVECs, human umbilical vein endothelial cells; EGF, epidermal growth factor; RT, reverse transcription; FEEL, fasciclin, EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor; SR-BI, scavenger receptor class B type-1.

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Cloning of FEEL-2 Full-length cDNA Clone—A human spleen cDNA library (Invitrogen) was screened in combination with PCR and hybridization (11) employing two oppositely oriented primers from the 5’ region of FEEL-2 (5’-GCCAAGGCTGACTGTAAGAG-3’ and 5’-TGTTTGGGTCTGTTCTGTT-3’).

Transient Expression of FEEL-1 or FEEL-2—Plasmids carrying the FEEL-1 or FEEL-2 cDNA under control of an EF-1α promoter (in the pEAK8 vector) or mock pEAK8 vector were transiently expressed in CHO-K1 cells using LipofectAMINE reagent (Invitrogen) (7). Transient expression of gene activity was tested at 48 h after the start of transfection.

Isolation of CHO-K1 Clones Stably Expressing FEEL-1 or FEEL-2—Stable expression of FEEL-1 or FEEL-2 cDNAs was obtained by transfecting pCMV-neo-based constructs (containing the neomycin resistance gene) into parental CHO-K1 cells as described (7). In this case, 72 h after transfection, cells were passaged 1:50 into selective medium containing 0.2 mg/ml G418. Cells resistant to G418 were subcloned by limiting dilution. Expression of FEEL-1 or FEEL-2 by individual clones of cells was determined by cellular association of DiI-Ac-LDL and by measuring fluorescence intensity.

FIG. 1. Primary structures of FEEL-1 and FEEL-2. A, alignment of the deduced amino acid (AA) sequences. The deduced amino acid sequences are aligned by the ClustalW program. Asterisks indicate the identical amino acids between FEEL-1 and FEEL-2. EGF-like (EGF_1, C-x-C-x (5)-G-x (2)-C, EGF_2, C-x-C-x (2)-[GP]-[FYW]-x (4, 8)-C in Prosite data base) and laminin-type EGF-like domains are shown in boxes. The fasciclin domains are indicated by red, and the link domain is indicated by blue. B, schematic representation of domain structures of FEEL-1 and FEEL-2. The alternatively transcribed variant that encodes the soluble form of FEEL-1 is also shown. The sequences of FEEL-1, FEEL-2, and the soluble form of FEEL-1 have been deposited in the DDBJ/GenBankTM/EBI Data Bank under accession numbers AB052956, AB052958, and AB052957, respectively.
Cloning of a Novel Endothelial Scavenger Receptor

Antibody Production and Purification—Murine monoclonal antibodies were produced using either CHO cells expressing SREC (CHO-SREC) or CHO cells expressing FEEL-1 (CHO-FEEL-1) as an immunizer. Approximately 2 × 10⁵ cells were resuspended in 200 µl of saline and injected into female BALB/c mice intraperitoneally. Two days after the final injection, the spleen was removed for fusion with P3X63-Ag8.653 cells using polyethylene glycol 1500 (Roche Molecular Biochemicals). Spent media of the primary cultures were screened by multiple plate reader (CytoFluorII, PerSeptive Biosystems) according to the manufacturer’s instructions. After solubilization with 0.1% Triton X-100, cellular association of DiI-Ac-LDL or BODIPY Staphylococcus aureus BODIPY FL conjugate (Molecular Probes) according to the manufacturer’s instructions. After solubilization with 0.1% Triton X-100, cellular association of DiI-Ac-LDL or BODIPY fluorescent bacteria was measured by its fluorescence intensity at 530/25 nm employing a fluorescence multiple plate reader (CytoFluoII, PerSeptive Biosystems).

Detection of FEEL-1 or FEEL-2 mRNA by Reverse Transcription (RT)-PCR from Various Human Tissues—RT-PCR analyses were carried out according to the manufacturer’s instructions using MTC probes (human I, human II, human immune, and human blood fractions, CLONTECH) or cDNAs derived from poly(A)+ RNA prepared from cultured human primary cells employing the SuperScript first-strand synthesis system for RT-PCR (Invitrogen). As FEEL-1-specific primers, 5’-CACTTGCCTGAGCTGCT-3’ and 5’-CACCCGGCCTGCTGCAACC-3’ were employed, and as FEEL-2-specific primers, 5’-TTTTTCTTTTCTGAAGGC-3’ and 5’-CATCCGGGCGACTTGACTC-3’ were employed.

Modulation of Endothelial Cell Tube Formation on Matrigel by Monoclonal Antibody against FEEL-1—The phenotypic effect of the inhibition of FEEL-1 on vascular remodeling was tested by in vitro tube formation assay using the Matrigel basement membrane matrix (BD

Fig. 3. Inhibition of DiI-Ac-LDL binding to HUVECs, CHO-FEEL-1, or CHO-SREC cells by various compounds. HUVECs, CHO-FEEL-1, or CHO-SREC cells were incubated with 2 µg/ml DiI Ac-LDL at 4 °C for 2 h in the presence of various inhibitors at 200 µg/ml. Inhibitors used were Ac-LDL, LDL, HDL, Ox-LDL, Maleylbovine serum albumin (BSA), and dextran sulfate.

DiI Ac-LDL at 4 °C for 2 h in the presence of various inhibitors at 200 µg/ml LDL (d = 1.019–1.063 g/ml) was prepared as described previously (7). HDL was obtained from INTRACEL. Acetylation of LDL was achieved by the addition of acetic anhydride, whereas oxidation was carried out by incubating at 200 µg/ml LDL in 5 mM CuSO₄ for 24 h at 37 °C according to a previously described method (7). To quantify the amount of DiI-Ac-LDL, cells were washed twice with Ham’s F-12 medium, washed with 10% fetal calf serum in PBS, and then washed twice with PBS and solubilized with 0.1% Triton X-100, and the fluorescence intensity was measured at 590/35 nm with the excitation wavelength set at 530/25 nm employing a fluorescence multipurpose reader (CytoFluoII, PerSeptive Biosystems).

Binding of BioParticles® Fluorescent Bacteria—CHO cells transiently transfected with either FEEL-1 or FEEL-2 cDNA in the pEAK8 vector were incubated for 2 h at 37 °C with 2 µg/ml DiI-Ac-LDL or 30 µg/ml BioParticles® Escherichia coli BODIPY® FL conjugate or BioParticles® Staphylococcus aureus BODIPY® FL conjugate (Molecular Probes) according to the manufacturer’s instructions. After solubilization with 0.1% Triton X-100, cellular association of DiI-Ac-LDL or BioParticles® fluorescent bacteria was measured by its fluorescence intensity at 530/25 nm with the excitation wavelength set at 485/20 nm (for BioParticles® fluorescent bacteria) employing a fluorescence multiple plate reader (CytoFluoII, PerSeptive Biosystems).

Inhibition of DiI-Ac-LDL Binding to CHO-SREC Cells by Various Compounds—The CHO-FEEL-1 cells were incubated with 2 µg/ml Ac-LDL and subtracted from the data.

FIG. 2. Characterization of FEEL-1 and FEEL-2 as scavenger receptor. A, binding of DiI-Ac-LDL by CHO expressing either FEEL-1 or FEEL-2. CHO-FEEL-1 or CHO-FEEL-2 cells grown to confluency in 24-well plates were incubated with 1.25–20 µg/ml DiI-Ac-LDL at 4 °C for 2 h. Nonspecific binding was measured in the presence of 200 µg/ml Ac-LDL and subtracted from the data. The inset shows the Scatchard analysis of the data. B, Western blot analysis of CHO and CHO-FEEL1 cells by the anti-FEEL-1 monoclonal antibody (FE-1-1). C, effect of monoclonal antibodies on FEEL-1-mediated association of DiI-Ac-LDL to CHO-FEEL-1 cells and HUVECs. Cellular associations of DiI-Ac-LDL were measured in the presence of purified monoclonal antibodies to FEEL-1 (FE-1-1 and FE-1-2) at 30 µg/ml for 2 h at 37 °C. Results are expressed as means ± S.D. from four independent experiments.

Specific Binding of DiI-Ac-LDL to FEEL-expressing CHO Cells—CHO-FEEL-1 or CHO-FEEL-2 cells grown to confluency in 24-well plates were incubated with 1.25–20 µg/ml DiI-Ac-LDL at 4 °C for 2 h. Nonspecific binding was measured in the presence of 200 µg/ml Ac-LDL and subtracted from the data.

Inhibition of DiI-Ac-LDL Binding to CHO-SREC Cells by Various Compounds—The CHO-FEEL-1 cells were incubated with 2 µg/ml

FIG. 3. Inhibition of DiI-Ac-LDL binding to HUVECs, CHO-FEEL-1, or CHO-SREC cells by various compounds. HUVECs, CHO-FEEL-1, or CHO-SREC cells were incubated with 2 µg/ml DiI Ac-LDL at 4 °C for 2 h in the presence of various inhibitors at 200 µg/ml. Inhibitors used were Ac-LDL, LDL, HDL, Ox-LDL, Maleylbovine serum albumin (BSA), and dextran sulfate.
Labware). HUVECs were trypsinized and counted, and then 2.5 × 10^4 cells were plated onto a 96-well plate with each well containing 100 µl of the Matrigel basement membrane matrix in the presence of 30 µg/ml anti-FEEL-1 monoclonal antibody, anti-SREC monoclonal antibody, or isotype control IgG1 (Sigma) and cultured for 16 h. To quantify the extent of capillary-like tube formation, photographs were taken on a Zeiss Axiovert 25 inverted phase microscope equipped with a cooled CCD camera (C5810, Hamamatsu color chilled 3 CCD camera, Japan). Images were captured using Adobe PhotoShop, and areas surrounded by tube network were quantified using Image Gauge software (Fujifilm, Tokyo, Japan).

RESULTS AND DISCUSSION

To determine the contribution of SREC (7) to the activity of scavenger receptor in endothelial cells, the effect of monoclonal antibodies against SREC (SR-4 and SR-15) on the receptor-mediated uptake of DiI-Ac-LDL was initially examined. Although the antibodies employed significantly inhibited the uptake of DiI-Ac-LDL in CHO-SREC, only slight inhibition was observed in HUVECs (data not shown). These results suggest the presence of another scavenger receptor in endothelial cells. The expression cloning of another scavenger receptor was then conducted in the presence of monoclonal antibody against SREC. As a result, several cDNA clones were identified, and nucleotide sequencing revealed that they were all CD36 and LIMPII Analogous-1/scavenger receptor class B type-I (CLA-1/SR-BI) cDNA (12, 13). Since CLA-1/SR-BI is also known as an HDL receptor, we examined the effect of HDL on the scavenger receptor activity in HUVECs but found no significant inhibition of the activity (data not shown). We again attempted to clone a novel scavenger receptor in the presence of both monoclonal antibodies against SREC and HDL to reduce the possibility of encountering SREC and CLA-1/SR-BI cDNA clones during expression cloning. Finally, only one pool was obtained, and we cloned a single plasmid DNA. Sequence analysis of the clone indicated that it encodes a novel type I membrane protein (2570 amino acids) (Fig. 1A) that has a unique domain structure organization. It has 7 fasciclin (Fas-1), 16 EGF-like, 2 laminin-type EGF-like, and 1 link domain near the transmembrane region (Fig. 1B). Based on these domain structures, we temporarily named it FEEL-1 (fasciclin, EGF-like-, laminin-type EGF-like, and link domain-containing scavenger receptor-1). The data base search revealed a match with functionally uncharacterized human cDNA, KIAA0246 from KG-1 cells (GenBank™ accession number D87433) (14), and stabilin-1 cDNA (GenBank™ accession number AJ275213) and sequence from the human genome sequencing projects, chromosome 3p21.1-9 (GenBank™ accession number AC006208).

Data base analysis also suggested the presence of a paralogous gene (15) of FEEL-1 (GenBank™ accession number AL133021, cDNA DKFZp434E0321). Then a full-length cDNA of this paralogous gene was cloned, and its nucleotide sequence was determined. The deduced amino acid sequence indicated that it contained a domain organization similar to that of FEEL-1, and we named it FEEL-2 (Fig. 1B). It has 7 fasciclin (Fas-1), 15 EGF-like, 2 laminin-type EGF-like, and 1 link domain (Fig. 1B). The overall amino acid sequence identity of FEEL-2 with FEEL-1 is 39.8%, and 1023 amino acids are identical between FEEL-1 and FEEL-2 (Fig. 1A).

The fasciclin (Fas-1) domain is originally found in fasciclin I that is expressed on subsets of axon pathways during neuronal development in the grasshopper (16), and Fas-1-containing molecules such as βig-h3 and peristin (17) have been reported to function as adhesion molecules. It was recently shown that the Fas-1 domain of the transforming growth factor-β-induced
gene product, β1γ-h3, also contributed to corneal epithelial cell adhesion through interaction with α3β1 integrin (18). The link domain is a hyaluronan-binding region found in vertebrate proteins that are involved in the assembly of the extracellular matrix, cell adhesion, and migration (19, 20). The EGF-like domain includes six cysteine residues that have been shown (in

Fig. 5. Binding of BioParticles E. coli BODIPY® FL conjugate or BioParticles S. aureus BODIPY® FL conjugate by CHO cells expressing FEELs. CHO cells transiently expressing either FEEL-1 or FEEL-2 were incubated for 2 h at 37 °C with 2 μg/ml of DiI-Ac-LDL or 30 μg/ml BioParticles® E. coli BODIPY® FL conjugate or BioParticles® S. aureus BODIPY® FL conjugate according to the manufacturer’s instructions. To confirm the direct interaction between FEEL-1 and bacteria, the effect of the FE-1-1 antibody was examined after the addition of antibody at 30 μg/ml in the binding of S. aureus to the CHO cells transiently expressing FEEL-1. A, fluorescence photomicrographs showing cellular association of DiI-Ac-LDL or bacterial BioParticles®. B, quantitative data of S. aureus binding to CHO cells.
EGF) to be involved in disulfide bond formation. One-half of the amino acid sequence of laminin contains consecutive repeats of about 60 amino acids that include eight conserved cysteine residues (21). The N-terminal of this domain (laminin-type EGF-like (LE) domain) is remotely similar to that of the EGF-like domain. In addition to laminins, the domain is also found in agrin, perlecan, netrins, and the EGF receptor (21–24).

Although the functional significance of these EGF-like domains is still obscure, a certain EGF-like domain was shown to mediate homophilic or heterophilic protein-protein interaction. Therefore, it is conceivable that FEEL-1 can interact with various proteins through its domains. To elucidate the physiological or pathological functions of FEEL-1, it is necessary to identify and characterize the proteins that interact with FEEL-1.

As for the intracellular domain, it should be mentioned here that intracellular amino acid sequences, namely NPVF and NPLY, found in FEEL-1 and FEEL-2, respectively, serve as an endocytosis signal of DiI-Ac-LDL. The role of these motifs in the endocytosis of DiI-Ac-LDL will be reported elsewhere.

After the screening of the human spleen cDNA library, an alternatively spliced variant that encodes the soluble form of FEEL-1 was cloned, and its exon and intron junction were determined by comparison with genomic DNA sequence (GenBank™ accession number AC006208). Whether this alternatively spliced form of FEEL-1 is expressed in tissues is not yet known. However, it is conceivable that such a receptor in soluble form modulates the pathophysiological function of FEEL-1 by competing with the ligands (25–28).

The binding of DiI-Ac-LDL was assessed following incubation of the CHO-FEEL-1 or CHO-FEEL-2 cells with DiI-Ac-LDL. Saturation binding of DiI-Ac-LDL was observed at 4 °C (Fig. 2A). Scatchard analysis (Fig. 2A, insets) showed the presence of a single class of receptors, and the calculated $K_d$ was 6.9 and 6.6 μg/ml DiI-Ac-LDL for FEEL-1 and FEEL-2, respectively (~13 nM DiI-Ac-LDL), which is comparable with the value for scavenger receptors on HUVECs (29). It should be noted here that the binding activity of the FEEL-1-expressing cell was rather low when compared with that of FEEL-2 expressing cells. It seems that overexpression of FEEL-1 is not favorable to the isolation of stable transformants of FEEL-1 in CHO cells.

To determine the contribution of FEEL-1 to the cellular association of DiI-Ac-LDL with endothelial cells (HUVECs), the effect of monoclonal antibodies against FEEL-1 was examined. Specificity of the antibodies employed (FE-1-1 and FE-1-2) was confirmed by Western blot analysis. As shown in Fig. 2B, the FE-1-1 monoclonal antibody recognized an ~250-kDa band corresponding to the expected molecular mass of FEEL-1 in CHO-FEEL-1 cells but not in control CHO cells. The same result was obtained by using the FE-1-2 monoclonal antibody (data not shown). As shown in Fig. 2C, both antibodies significantly inhibited the association of DiI-Ac-LDL to CHO-FEEL-1. Treatment of HUVECs with the antibodies also caused a significant reduction in the degree of DiI-Ac-LDL association. These results suggest that FEEL-1 is one of the major receptors of DiI-Ac-LDL in HUVECs.

To further characterize the binding properties of the
FEEL-1, the receptor activity was measured in the presence of various materials known as type I and type II macrophage scavenger receptor inhibitors (Fig. 3). As in the case of macrophage scavenger receptor and SREC examined for comparison, unlabeled Ac-LDL as well as dextran sulfate, but not native lipoproteins such as LDL and HDL, reduced the binding of Di-Ac-LDL on the cells to the basal level. Ox-LDL partially reduced the binding of Di-Ac-LDL on the cells. These results show that the FEEL-1 has a binding specificity similar to those of the scavenger receptors expressed in HUVECs.

RT-PCR analysis employing specific sets of primers revealed that the expressions of FEEL-1 and FEEL-2 in human tissues were rather restricted, and high expression levels were observed in the spleen and lymph node. On the other hand, only FEEL-1 was expressed in mononuclear cells, particularly CD14+ cells (monocytes) (Fig. 4A). Moreover, FEEL-1 is also expressed in endothelial cells such as HUVECs, human coronary arterial endothelial cells, and human brain microvascular endothelial cells, but no expression of FEEL-2 was detected in the endothelial cells tested (Fig. 4B).

Since some scavenger receptors that are expressed in endothelial cells and macrophages are known to bind to Gram-negative and Gram-positive bacteria and play a role in host defense and immune response following bacterial infection (30), we examined the interaction of bacterial particles with FEELs expressed in CHO cells. As shown in Fig. 5A, a fluorescence photomicrograph clearly indicated that both FEELs could bind to Gram-negative and Gram-positive bacteria. Quantitative analysis (Fig. 5B) confirmed the binding of bacteria to both FEELs. To confirm the direct interaction between FEEL-1 and bacteria, the effect of the FE-1-1 antibody was examined, and we found that the addition of the antibody caused a decrease in the binding of S. aureus to the CHO cells that were transiently expressing FEEL-1. These results suggested that they might play a role in the defense against bacterial infection. In this context, FEEL-1 expressed in monocytes may play a role in the antibacterial activity of the cells.

The presence of Fas-1 domains in FEELs reminded us of the interaction of the receptor with some molecules in the extracellular matrix. Therefore, we examined the phenotypic effect of the inhibition of FEEL-1 on in vitro vascular remodeling using the Matrigel tube formation assay. HUVECs were plated onto a 34270 lular matrix. Therefore, we examined the phenotypic effect of the interaction of the receptor with some molecules in the extracel-

Since several investigators reported that the stimulation of scavenger receptors in endothelial cells induces the expression of proteins related to vascular functions (33, 34), determination of the physiological ligand for FEELs will provide a new insight into the pathological function of scavenger receptors.

**Note Added in Proof**—As mentioned in the text, FEEL-1 is identical with stabilin-1 first identified as MS-1 antigen (35, 36). It was reported that the antigen is a specific marker of alternatively activated macrophages with increased angiogenic potential (37).

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