THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 15, Issue of April 11, pp. 12613–12617, 2003

Printed in U.S.A.

FEEL-1 and FEEL-2 Are Endocytic Receptors for Advanced Glycation End Products*

Received for publication, October 4, 2002, and in revised form, December 2, 2002
Published, JBC Papers in Press, December 6, 2002, DOI 10.1074/jbc.M210211200

Yoshiaki Tamura‡§, Hideki Adachi§¶, Jun-ichi Osuga‡, Ken Ohashi‡, Naoya Yahagi‡, Motohiro Sekiya‡, Hiroaki Okazaki‡, Sachiko Tomita‡, Yoko Iizuka‡, Hitoshi Shimano‖, Ryozo Nagai, Satoshi Kimura‡‡, Masafumi Tsujimoto¶, and Shun Ishibashi‡§§¶¶**

*From the ‡Departments of Metabolic Diseases, **Cardiovascular Medicine, and ‡‡Infectious Diseases, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655 Japan; the ¶Laboratory of Cellular Biochemistry, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-0198 Japan; the ‖Departments of Metabolism, Endocrinology, and Atherosclerosis, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8575 Japan; and the §§Department of Internal Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi, 329-0498 Japan*

Advanced glycation end products (AGEs) are nonenzymatically glycosylated proteins, which accumulate in vascular tissues in aging and diabetes. Receptors for AGEs include scavenger receptors, which recognize acetylated low density lipoproteins (Ac-LDL) such as scavenger receptor class AI/AII (SR-A), cell surface glycoprotein CD36, scavenger receptor class B type I (SR-BI), and lectin-like oxidized low density lipoprotein receptor-1. The broad ligand repertoire of these receptors as well as the diversity of the receptors for AGEs have prompted us to examine whether AGEs are also recognized by the novel scavenger receptors, which we have recently isolated from a cDNA library prepared from human umbilical vein endothelial cells, such as the scavenger receptor expressed by endothelial cells-I (SREC-I); the fasciclin EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1 (FEEL-1); and its paralogous protein, FEEL-2. At 4 °C, ^{125}I-AGE-bovine serum albumin (BSA) exhibited high affinity specific binding to Chinese hamster ovary (CHO) cells overexpressing FEEL-1 (CHO-FEEL-1) and FEEL-2 (CHO-FEEL-2) with K_d of 2.55 and 1.68 μg/ml, respectively, but not to CHO cells expressing SREC (CHO-SREC) and parent CHO cells. At 37 °C, ^{125}I-AGE-BSA was taken up and degraded by CHO-FEEL-1 and CHO-FEEL-2 cells but not by CHO-SREC and parent CHO cells. Thus, the ability to bind Ac-LDL is not necessarily a prerequisite to bind AGEs. The ^{125}I-AGE-BSA binding to CHO-FEEL-1 and CHO-FEEL-2 cells was effectively inhibited by Ac-LDL and polyanionic SR-A inhibitors such as fucoidan, polyinosinic acids, and dextran sulfate but not by native LDL, oxidized LDL, or HDL. FEEL-1, which is expressed by the liver and vascular tissues, may recognize AGEs, thereby contributing to the development of diabetic vascular complications and atherosclerosis.

Advanced glycation end products (AGEs)[1] are generated by nonenzymatic glycosylation of proteins or lipids after prolonged exposure to glucose (1). AGEs elicit a wide variety of cellular responses including induction of growth factors and cytokines (2), adhesion molecules (3), oxidant stress (4), and chemotaxis (5). These proinflammatory responses are implicated to contribute to the development of pathologies associated with aging, diabetes mellitus, and Alzheimer's disease (6). Indeed, AGEs were shown to be present in atherosclerotic lesions (7) and diabetic kidney (8).

The AGE-elicited proinflammatory reactions are mediated by its receptors or binding proteins, which include the receptor for advanced glycation end product (RAGE) (9, 10), OST-48 (ARE-R1)/80K-H (AGE-R2)/galectin-3 (AGE-R3) (11), scavenger receptor class AI/AII (SR-A) (12), scavenger receptor class B type I (SR-BI) (13), cell surface glycoprotein CD36 (14), lectin-like oxidized low density lipoprotein receptor-1 (15), lactoferin (16), and lysozyme (16).

The broad ligand repertories of these AGE-binding proteins as well as the diversity of receptors for AGEs have prompted us to examine whether AGEs are recognized by novel members of scavenger receptors that Adachi *et al.* (17, 18) have recently cloned from a cDNA library prepared from human umbilical vein endothelial cells as receptors for acetylated low density lipoproteins (Ac-LDL), such as the scavenger receptor expressed by endothelial cells-I (SREC-I) (17) and the fasciclin EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1 (FEEL-1) (18). These receptors are structurally unrelated to other scavenger receptors. SREC is a protein of 830 amino acids with five epidermal growth factor-like cysteine pattern signatures. FEEL-1 is a protein of 2570 amino acids including 7 fasciclins, 16 EGF-like, 2 laminin-type EGF-like, and 1 link domain near the transmembrane region. FEEL-2 is a paralogous gene of FEEL-1 whose amino acid sequence is ~40% identical to FEEL-1. Quantitative PCR analyses showed that both FEEL-1 and FEEL-2 are expressed in

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Both authors contributed equally to this work.

¶¶ To whom correspondence should be addressed: Dept. of Internal Medicine, Division of Endocrinology and Metabolism, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan. Tel.: 81-285-58-7355; Fax: 81-285-40-6035; E-mail: ishibash@jichi.ac.jp.

[1] The abbreviations used are: AGEs, advanced glycation end products; LDL, low density lipoprotein; Ac-LDL, acetylated low density lipoprotein; Ox-LDL, oxidized low density lipoprotein; HDL, high density lipoprotein; SR-A, scavenger receptor class AI/AII; SR-BI, scavenger receptor class B type I; SREC, scavenger receptor expressed by endothelial cells; EGF, epidermal growth receptor; FEEL-1, fasciclin EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1; RAGE, receptor for advanced glycation end product; BSA, bovine serum albumin; poly I, polyinosinic acid; CHO, Chinese hamster ovary; HUVECs, human umbilical endothelial cells; PBS, phosphate-buffered saline.

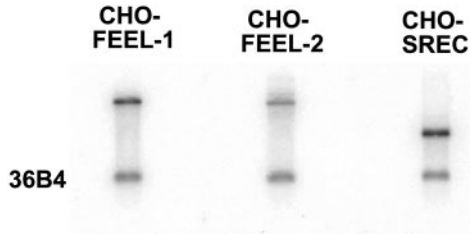

FIG. 1. **Northern blot analysis of FEEL-1, FEEL-2, and SREC in transfected CHO cells.** 3 μg of poly(A)$^+$ RNA was subjected to Northern blot analysis with cDNA probe for FEEL-1, FEEL-2, and SREC. 36B4 was used as a molecular reference.

the spleen and lymph node, whereas only FEEL-1 is detectable in CD14$^+$-mononuclear cells and vascular endothelial cell lines (18).

Here we show that FEEL-1 and FEEL-2, but not SREC, are endocytic receptors for AGEs. Because FEEL-1 is expressed by the liver, macrophages, and endothelial cells in an amount comparable with other receptors for AGEs, FEEL-1 may play a significant role in the elimination of AGEs from the circulation as well as in the development of diabetic vascular complications and atherosclerosis.

EXPERIMENTAL PROCEDURES

Materials—Ham's F-12 medium, Dulbecco's modified Eagle medium (DMEM), penicillin G, streptomycin sulfate, G418, and TriZOL were purchased from Invitrogen. Bovine serum albumin (BSA), MOPC21 (mouse IgG), fucoidan, polyinosinic acid (poly I), and dextran sulfate were purchased from Sigma. Heparin sodium salt was purchased from Mitsubishi Pharma (Osaka, Japan). Glucose 6-phosphate was purchased from Oriental Yeast (Tokyo, Japan). Endothelial cell growth supplement was purchased from BD Biosciences. Oligotex-dT30™ was purchased from Roche Molecular Biochemicals. Hybond N was purchased from Amersham Biosciences. Na-^{125}I was purchased from Daiichi Chemical (Osaka, Japan). Iodogen™ and BCA protein assay reagent kit were purchased from Pierce. Human umbilical vein endothelial cells (HUVECs) were purchased from Kurabo (Tokyo, Japan). A murine monoclonal antibody against human FEEL-1 (FE-1-1) was described previously (18). cDNA probes for murine FEEL-1, FEEL-2 SREC, RAGE, galectin-3, SR-A, CD36, and SR-B1 were prepared by reverse transcriptase-PCR using primers designed based on the reported nucleotide sequences.

Ligand Preparation and Radiolabeling—AGE-BSA was prepared as described previously (19). 600 mg of BSA was incubated with 50 mM glucose 6-phosphate in 10 ml of sterile sodium phosphate buffer or phosphate-buffered saline (PBS) for 10 weeks at 37 °C and dialyzed overnight against PBS. AGE-specific fluorescence was measured at 450 nm after excitation at 360 nm at a concentration of 1 mg/ml with a fluorescence spectrometer (Biolumin, Amersham Biosciences). The AGE-BSA exhibited ~38-fold higher fluorescence intensity than BSA. AGE-BSA was labeled with ^{125}I by using Iodogen according to the manufacturer's instruction. Protein concentrations were determined by BCA protein assay reagent kit. LDL (d = 1.019–1.063 g/ml) and high density lipoprotein (HDL) (d = 1.063–1.21 g/ml) were prepared by stepwise ultracentrifugation from plasma obtained from healthy volunteers. LDL was acetylated with acetic anhydrate as described previously (20) and oxidized by incubation in a buffer containing 5 μM CuSO4 for 16 h at 37 °C (21). The lipoproteins were dialyzed against a buffer containing 10 mM sodium phosphate, pH 7.4, and 150 mM NaCl.

Cell Culture—CHO cells overexpressing human FEEL-1 (CHO-FEEL-1), human FEEL-2 (CHO-FEEL-2), and human SREC (CHO-SREC) were obtained as described previously (17, 18). These cells were maintained at 37 °C with 5% (v/v) CO_2 in medium A (Ham's F-12 supplemented with 100 units/ml penicillin and 100 units/ml streptomycin) containing 10% (v/v) fetal calf serum and 0.2 mg/ml G418 (medium B). Untransfected CHO cells designated as CHO-Wild were maintained in medium B without G418. The four lines of CHO cells were cultured for 2 days to confluence in 12-well plates and used for the following experiments. Thioglycolate-elicited mouse peritoneal macrophages were prepared as described previously (22) and maintained in DMEM containing 10% (v/v) fetal calf serum. HUVECs were cultured with DMEM containing 20% fetal calf serum, 30 μg/ml endothelial cell growth supplement, and 10 IU/ml heparin. Confluent dishes were used for the studies within the 15 passages.

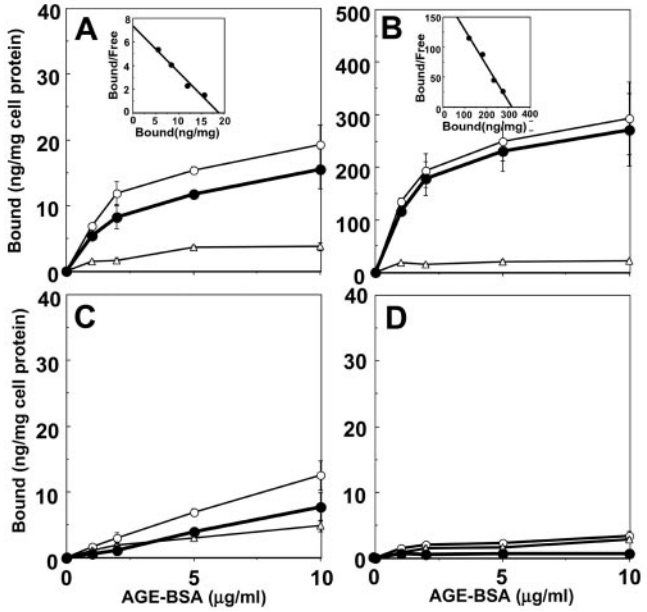

FIG. 2. **Binding of ^{125}I-AGE-BSA to CHO-FEEL-1 (*A*), CHO-FEEL-2 (*B*), CHO-SREC (*C*), and parent CHO cells (*D*).** Cells were incubated with the indicated concentrations of ^{125}I-AGE-BSA for 2 h at 4 °C in the presence (*open triangle*) or absence (*open circles*) of 20-fold excess amounts of unlabeled AGE-BSA in triplicate wells. Specific binding (*closed circle*) was determined by subtracting nonspecific binding from total binding. *Error bars* represent mean ± S.D. *Insets* show the Scatchard analysis of the specific binding curve.

Northern Blot Analysis—Total RNA was prepared by TriZOL. Poly(A)$^+$ RNA was purified using Oligotex-dT30 and subjected to 1% (w/v) agarose gel electrophoresis in the presence of formalin. The fractionated RNA was transferred to Hybond N, hybridized to ^{32}P-labeled cDNA probes, and analyzed by BAS2000 (FUJI XEROX, Tokyo, Japan). The expression levels of each gene were compared between various organs after adjusting to the expression level of 36B4.

Binding of ^{125}I-AGE-BSA at 4 °C—After washing twice with PBS, the confluent cells were incubated with 0.5 ml of medium C (medium A supplemented with 3% (w/v) BSA) containing the indicated concentrations of iodinated AGE-BSA with or without 20-fold excess of unlabeled AGE-BSA for 2 h at 4 °C. After washing three times with ice-cold PBS containing 2 mg/ml BSA and three times further with PBS, the cells were dissolved with 0.1 N NaOH and the cell-bound radioactivity and cellular proteins were determined.

Uptake and Degradation of ^{125}I-AGE-BSA—One hour prior to the study, the media of the confluent cells were changed to medium C. The media were replaced with medium C containing the indicated concentrations of ^{125}I-AGE-BSA with or without 20-fold excess of unlabeled AGE-BSA and incubated for 6 h at 37 °C. The amounts of ^{125}I-AGE-BSA either degraded by or associated with the cells were measured using trichloroacetic acid and AgNO3 as described previously (22). The differences of the means were compared by Student's t test.

RESULTS

Expression of FEEL-1, FEEL-2, and SREC in Transfected CHO Cells—Northern blot analyses were performed to compare the mRNA expression of FEEL-1, FEEL-2, and SREC in CHO-FEEL-1, CHO-FEEL-2, and CHO-SREC cells, respectively (Fig. 1). CHO-FEEL-1 and CHO-SREC expressed comparable amounts of the respective mRNA, which was 2-fold higher than the mRNA of FEEL-2 mRNA in CHO-FEEL-2 cells.

Binding of ^{125}I-AGE-BSA to CHO-FEEL-1, CHO-FEEL-2, and CHO-SREC Cells—^{125}I-AGE-BSA bound to both CHO-FEEL-1 and CHO-FEEL-2 cells in a saturable manner at 4 °C (Fig. 2, *A* and *B*). Scatchard analysis (*insets*) showed the presence of single binding site for AGE-BSA with an apparent K_d of 2.55 and 1.68 μg/ml for FEEL-1 and FEEL-2, respectively.

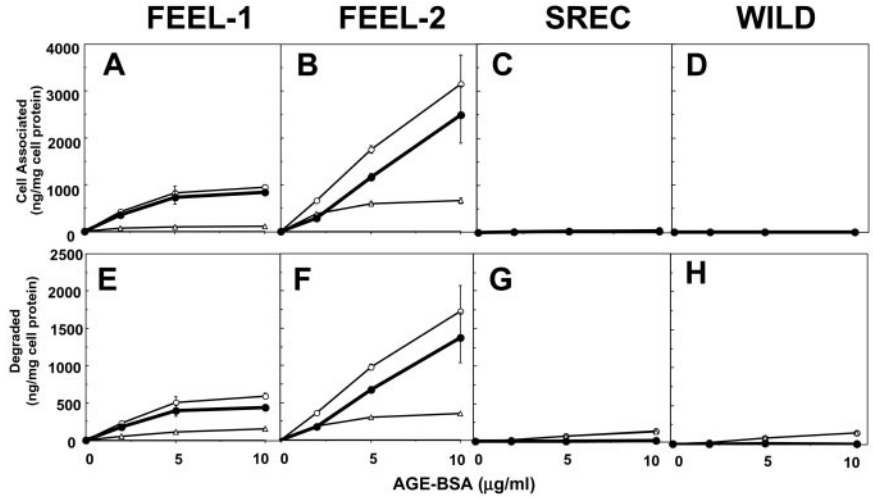

FIG. 3. **Cellular uptake (*A–D*) and degradation (*E–H*) of ^{125}I-AGE-BSA by CHO-FEEL-1 (*A* and *E*), CHO-FEEL-2 (*B* and *F*), CHO-SREC (*C* and *G*), and parent CHO cells (*D* and *H*).** Cells were incubated with the indicated concentrations of ^{125}I-AGE-BSA for 6 h at 37 °C in the presence (*open triangles*) or absence (*open circles*) of excess amounts of unlabeled AGE-BSA in triplicate wells. Cellular uptake (*A–D*) and degradation (*E–H*) of ^{125}I-AGE-BSA were determined. Specific uptake or degradation (*closed circles*) was determined by subtracting nonspecific values from total values. *Error bars* represent mean ± S.D.

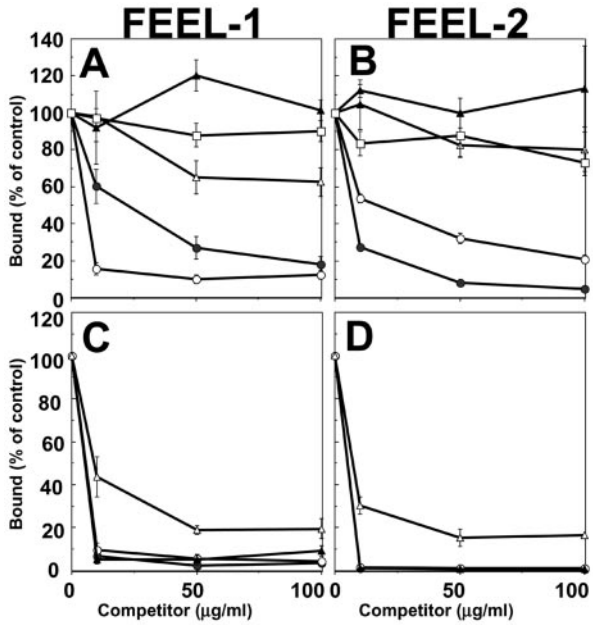

FIG. 4. **Competitive inhibition of the ^{125}I-AGE-BSA binding to CHO-FEEL-1 (*A* and *C*) or CHO-FEEL-2 cells (*B* and *D*).** *A* and *B*, cells were incubated with 2 μg/ml ^{125}I-AGE-BSA for 2 h at 4 °C in the presence of indicated dose of AGE-BSA (*closed circles*), LDL (*closed triangles*), Ac-LDL (*open circles*), Ox-LDL (*open triangles*), and HDL (*open squares*) in triplicate wells. *C* and *D*, cells were incubated with 2 μg/ml ^{125}I-AGE-BSA for 2 h at 4 °C in the presence of indicated dose of fucoidan (*closed circles*), poly I (*closed triangles*), dextran sulfate (*open circles*), and heparin (*open triangles*) in triplicate wells. *Error bars* represent mean ± S.D.

CHO-FEEL-2 cells bound ~17 times larger amounts of ^{125}I-AGE-BSA than CHO-FEEL-1 cells (B_{max} = 315.5 *versus* 18.9 ng/mg). On the other hand, the specific ^{125}I-AGE-BSA binding to CHO-SREC cells was 30% of that to CHO-FEEL-1 cells with lower affinity (Fig. 2*C*). Untransfected CHO cells bound negligible amounts ^{125}I-AGE-BSA (Fig. 2*D*).

Cellular Uptake and Degradation of ^{125}I-AGE-BSA by CHO-FEEL-1, CHO-FEEL-2, and CHO-SREC Cells—To examine whether the binding is associated with cellular uptake and degradation of ^{125}I-AGE-BSA, we incubated the cells with ^{125}I-AGE-BSA at 37 °C and determined the amounts of ^{125}I-AGE-BSA bound (Fig. 3, *A–D*) and degraded by the cells (Fig. 3, *E–H*). Significant amounts of ^{125}I-AGE-BSA were taken up and degraded by both CHO-FEEL-1 and CHO-FEEL-2 cells but not by CHO-SREC and untransfected CHO cells. The activity was larger in CHO-FEEL-2 cells than in CHO-FEEL-1 cells. These results indicate that FEEL-1 and FEEL-2 but not SREC are endocytic receptors for AGE-BSA.

Ligand Specificity of ^{125}I-AGE-BSA Binding to CHO-FEEL-1 or CHO-FEEL-2 Cells—To characterize the ligand specificity for the AGE-BSA binding site of the FEEL-1 and FEEL-2, we performed a competition study. First, we examined whether the binding of ^{125}I-AGE-BSA is inhibited by lipoproteins (Fig. 4, *A* and *B*). The binding of ^{125}I-AGE-BSA to CHO-FEEL-1 (Fig. 4*A*) and CHO-FEEL-2 (Fig. 4*B*) cells was inhibited by Ac-LDL but not by LDL and HDL. It is noteworthy that Ac-LDL inhibited the binding more potently than AGE-BSA itself in CHO-FEEL-1 cells, whereas AGE-BSA inhibited the binding more potently than Ac-LDL in CHO-FEEL-2 cells. Table I shows concentrations required to inhibit the binding by 50% (IC_{50}). Ox-LDL partially inhibited the binding only in CHO-FEEL-1 cells (~40% reduction at 100 μg/ml). We next examined the inhibitory effect of various materials known as SR-A inhibitors (Fig. 4, *C* and *D*). Fucoidan, poly I, and dextran sulfate inhibited the ^{125}I-AGE-BSA binding almost completely in both CHO-FEEL-1 (Fig. 4*C*) and CHO-FEEL-2 cells (Fig. 4*D*). Heparin was also effective in suppressing the binding, but the effect was weaker than the other three compounds (Table I).

Inhibition of Uptake and Degradation of ^{125}I-AGE-BSA by CHO-FEEL-1 Cells by Anti-FEEL-1 Antibody—We determined the effects of anti-FEEL-1 monoclonal antibody (FE-1-1) as well as other compounds on the endocytic uptake and degradation of ^{125}I-AGE-BSA by CHO-FEEL-1 cells (Fig. 5). We have previously reported that FE-1-1 effectively suppressed the cellular uptake of Ac-LDL in CHO-FEEL-1 cells (18). AGE-BSA and Ac-LDL effectively inhibited the cellular uptake and degradation of ^{125}I-AGE-BSA by CHO-FEEL-1 cells. No inhibitory effect was observed with 100 μg/ml native LDL, Ox-LDL, HDL, and 30 μg/ml control IgG. FE-1-1 inhibited both cellular uptake and degradation of ^{125}I-AGE-BSA by 72 and 48%, respectively. The extent of inhibition by the antibody was slightly smaller than that by AGE-BSA or Ac-LDL but was almost the same as observed in inhibition study for cellular uptake of Ac-LDL (18).

TABLE I
Concentrations of various lipoproteins and SR-A inhibitors to inhibit the binding of ^{125}I-AGE-BSA to CHO-FEEL-1 and CHO-FEEL-2 cells (IC_{50})

CHO-FEEL-1 and CHO-FEEL-2 cells were incubated with 2 μg/ml ^{125}I-AGE-BSA for 2 h at 4 °C with AGE-BSA, LDL, Ac-LDL, Ox-LDL, HDL, fucoidan, polyinosinic acid (poly I), dextran sulfate, or heparin at the concentrations of 0, 10, 50, or 100 μg/ml. The IC_{50} value were calculated and shown for competitors that inhibited the binding of ^{125}I-AGE-BSA >50% at 100 μg/ml. Values in parentheses are IC_{50} in molar concentrations, which were calculated based on the assumption that molecular masses of AGE-BSA, AcLDL, fucoidan, poly I, dextran sulfate, and heparin are 66, 549, 66.4, 200~500, 500, and 5~20 kDa, respectively.

Competitors	CHO-FEEL-1	CHO-FEEL-2
	μg/ml (nM)	
AGE-BSA	22.2 (337)	<6.9 (104)
AcLDL	<5.9 (11)	16.8 (31)
Fucoidan	<5.4 (81)	<5.0 (76)
Polyinosinic acid	<5.3 (11~26)	<5.0 (10~25)
Dextran sulfate	<5.5 (11)	<5.1 (10)
Heparin	8.9 (443~1,770)	7.2 (358~1,434)

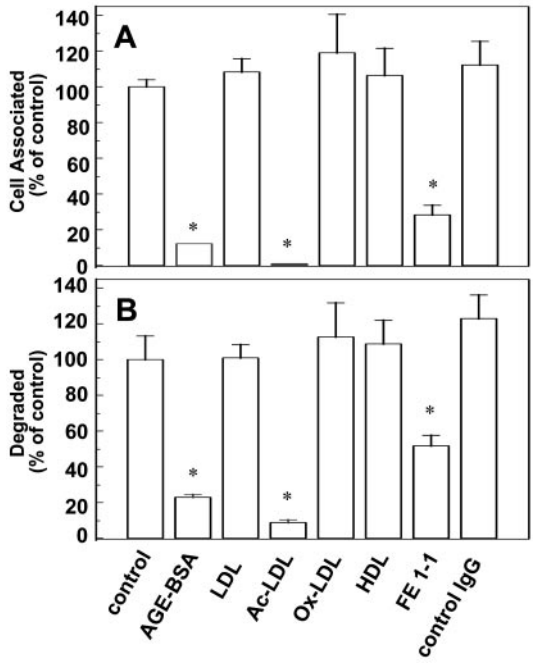

FIG. 5. **Competitive inhibition of the uptake (*A*) and degradation (*B*) of ^{125}I-AGE-BSA by CHO-FEEL-1 cells.** Cells were incubated with 2 μg/ml ^{125}I-AGE-BSA for 6 h at 37 °C in the presence of 100 μg/ml AGE-BSA, LDL, Ac-LDL, Ox-LDL, HDL, or 30 μg/ml FE-1-1 antibody or control IgG in triplicate wells. Cellular uptake (*A*) and degradation (*B*) of ^{125}I-AGE-BSA were determined. *Error bars* represent mean ± S.D. *, $p < 0.05$ *versus* control values.

Tissue Distribution of the Expression of FEEL-1 and FEEL-2—To estimate the contribution of FEEL-1 and FEEL-2 to the endocytosis of AGEs in various tissues in comparison with other receptors for AGEs, we performed Northern blot analyses (Fig. 6). FEEL-1 was expressed in a wide variety of organs in the following order: liver > lung = heart = white adipose tissue > aorta = kidney > spleen. FEEL-2 whose expression was much lower than that of FEEL-1 was detectable only in the liver and spleen. In contrast to organs like lung, spleen, and white adipose tissue in which RAGE, galectin-3, and CD36 were most highly expressed, respectively, organs like heart, liver, and aorta appeared to express significant amounts of FEEL-1 whose expression level was as comparable as that of other receptors for AGEs.

Expression of FEEL-1 and FEEL-2 in Macrophages and Vascular Endothelial Cells—We further performed Northern blot analysis to evaluate the expression of FEEL-1 in mouse peritoneal macrophages and HUVECs (Fig. 7). Mouse peritoneal macrophages and HUVECs expressed FEEL-1 to a degree that was comparable with galectin-3 and SR-A, respectively.

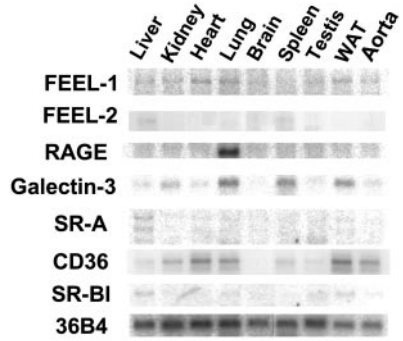

FIG. 6. **Tissue distribution of FEEL-1 and FEEL-2 in comparison with other receptors for AGEs.** 10 μg of total RNA was extracted from liver, kidney, heart, lung, brain, spleen, testis, white adipose tissue (*WAT*), and aorta of mice and subjected to Northern blot analysis with cDNA probe for FEEL-1, FEEL-2, RAGE, galectin-3, SR-A, CD36, and SR-B1. 36B4 was used as a loading control.

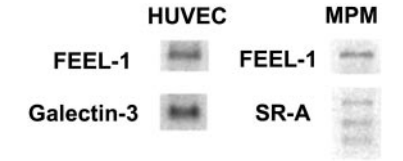

FIG. 7. **Expression of FEEL-1 in HUVEC and mouse peritoneal macrophages.** 10 μg of total RNA from HUVEC and 2 μg of poly(A)$^+$ RNA from mouse peritoneal macrophages were subjected to Northern blot analysis. The expression levels of FEEL-1 were compared with those of galectin-3 in HUVEC and SR-A in mouse peritoneal macrophages.

DISCUSSION

We have recently reported the cloning of novel scavenger receptors expressed on vascular endothelial cells by expression cloning strategy using 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbo-cyanine perchlorate (DiI)-labeled Ac-LDL as the ligand, such as SREC (17), FEEL-1, and its paralogous gene, *FEEL-2* (18). Structures of these receptors are unique and unrelated to other scavenger receptors. Although the precise functions of these receptors are currently unknown, *in vitro* studies have suggested their involvement in cellular interaction and host defense. For example, both FEEL receptors bind to Gram-negative and Gram-positive bacteria (18). Furthermore, anti-FEEL-1 antibody inhibited the *in vitro* vascular tube formation (18). On the other hand, SREC-I and its isoform, SREC-II, showed a strong heterophilic trans-interaction through their extracellular EGF-like repeat domains (23).

This study has first revealed that FEEL-1 and FEEL-2 are endocytic receptors for AGEs, implicating the involvement of these receptors in the pathologies of aging or diabetes. Some of other receptors for AGEs are involved in these pathologies. For example, Park *et al.* (24) have shown that intravenous administration of the soluble extracellular domain of RAGE, which was originally cloned from bovine pulmonary endothelial cells, efficiently suppressed diabetic atherosclerosis in apolipoprotein E-deficient mice. Second, Pugliese *et al.* (25) have shown that diabetic glomerulopathy was rather accelerated in mice lacking galectin-3, a critical component of OST-48/80-K-H/galectin-3 complex (11) that is ubiquitously expressed in mammalian cells, suggesting its protective role. Third, Suzuki *et al.* (26) and Matsumoto *et al.* (27) observed that peritoneal macro-

phages obtained from SR-A knock-out mice had the reduced capacity for endocytic degradation of AGE-BSA, which was 30% of that in wild-type cells. However, liver sinusoidal endothelial cells obtained from SR-A knock-out mice had capacity for endocytic degradation of AGE-BSA, which was indistinguishable from that of wild-type liver sinusoidal endothelial cells (27). These results suggest that SR-A is the primary endocytic receptor for AGE-BSA in macrophages, whereas other receptors mediate the uptake of AGEs in endothelial cells.

Although FEEL-1 and FEEL-2 are structurally unrelated to SR-A, their ligand specificity is similar to that of SR-A. CHO-FEEL-1 cells bind not only Ac-LDL but also Ox-LDL to a lesser degree (18). The binding was competitively inhibited by SR-A inhibitors, such as maleyl-BSA and dextran sulfate (18). Furthermore, both FEEL-1 and FEEL-2 bind AGE-BSA (Figs. 1 and 2) as does SR-A (12). Ac-LDL and negatively charged compounds such as fucoidan, dextran sulfate, and poly I competitively inhibited the binding of AGE-BSA to CHO-FEEL-1 or CHO-FEEL-2 cells (Figs. 4 and 5) as is the case for SR-A (12). These results suggest that AGE-BSA and Ac-LDL share a same binding site on the receptors. Importantly, the binding affinity of AGE-BSA to FEEL-1 and FEEL-2 cells appeared to exceed that to other receptors for AGEs. Calculated K_d was 25 nM for FFEL-2; 39 nM for FEEL-1; 85 nM for CD36 (14); 100 nM for RAGE (9); 126 nM for SR-BI (13); 148 nM for lectin-like oxidized low density lipoprotein receptor-1 (15); and 350 nM for galectin3 (11), assuming that molecular mass of AGE-BSA is 66 kDa. The binding activity of CHO-FEEL-1 cells was ~20-fold lower than that of CHO-FEEL-2 cells. A similar although less significant difference in the binding activity between the isoforms was observed for Ac-LDL (18). Together with the fact that the mRNA expression of FEEL-2 in CHO-FEEL-2 cells was lower than that of FEEL-1 in CHO-FEEL-1, these results indicate that a significant proportion of the binding sites for AGEs on FEEL-1 was inactive or masked in CHO-FEEL-1 cells. This possibility warrants further investigation.

In many organs, the mRNA expression levels of FEEL-1 were higher than those of FEEL-2 (Fig. 6). Mouse peritoneal macrophages and HUVECs also expressed FEEL-1 to a degree comparable with other receptors for AGEs (Fig. 7). Furthermore, FEEL-2 mRNA was barely detectable in several vascular endothelial cell lines or in monocyte/macrophages at nonstimulated conditions (18). Taken together, we propose that FEEL-1 and possibly FEEL-2 serve as functional endocytic receptors for AGEs *in vivo*. This hypothesis needs to be verified by further studies, such as gene targeting, that are currently in progress in our laboratory. As suggested by the presence in the aorta (Fig. 6), macrophages, and endothelial cells (Fig. 7) (18), FEEL-1 may scavenge AGEs accumulated in vascular tissues in diabetes and aging, thereby directly contributing to the development of diabetic vascular complications and atherosclerosis. Because Smedsrød *et al.* (28) reported that >90% of intravenously injected AGE-BSA was distributed in the liver in rats, it is also possible that FEEL-1, which was expressed in the liver (Fig. 6), may be involved in the elimination of AGEs from the circulation.

In conclusion, we demonstrate that FEEL-1 and FEEL-2, but not SREC-I, function as endocytic receptors for AGEs when overexpressed in CHO cells. Although further studies will be needed to determine the role of these two receptors *in vivo*, they may play a pivotal role in the pathogenesis of diabetic vascular complications and could be the promising target molecules for their prevention.

Addendum—FEEL-1 is identical to stabilin-1 (hyaluronan-scavenger receptor; HA/S-R) first identified as MS-1 antigen (29). AGE uptake by liver sinusoidal endothelial cells is partially inhibited by an antibody against HA/S-R (30).

REFERENCES

1. Brownlee, M. (1995) *Annu. Rev. Med.* **46,** 223–234
2. Lu, M., Kuroki, M., Amano, S., Tolentino, M., Keough, K., Kim, I., Bucala, R., and Adamis, A. P. (1998) *J. Clin. Invest.* **101,** 1219–1224
3. Schmidt, A. M., Hori, O., Chen, J. X., Li, J. F., Crandall, J., Zhang, J., Cao, R., Yan, S. D., Brett, J., and Stern, D. (1995) *J. Clin. Invest.* **96,** 1395–1403
4. Yan, S. D., Schmidt, A. M., Anderson, G. M., Zhang, J., Brett, J., Zou, Y. S., Pinsky, D., and Stern, D. (1994) *J. Biol. Chem.* **269,** 9889–9897
5. Higashi, T., Sano, H., Saishoji, T., Ikeda, K., Jinnouchi, Y., Kanzaki, T., Morisaki, N., Rauvala, H., Shichiri, M., and Horiuchi, S. (1997) *Diabetes* **46,** 463–472
6. Vlassara, H., and Palace, M. R. (2002) *J. Intern. Med.* **251,** 87–101
7. Nakamura, Y., Horii, Y., Nishino, T., Shiiki, H., Sakaguchi, Y., Kagoshima, T., Dohi, K., Makita, Z., Vlassara, H., and Bucala, R. (1993) *Am. J. Pathol.* **143,** 1649–1656
8. Niwa, T., Katsuzaki, T., Miyazaki, S., Miyazaki, T., Ishizaki, Y., Hayase, F., Tatemichi, N., and Takei, Y. (1997) *J. Clin. Invest.* **99,** 1272–1280
9. Neeper, M., Schmidt, A. M., Brett, J., Yan, S. D., Wang, F., Pan, Y. C., Elliston, K., Stern, D., and Shaw, A. (1992) *J. Biol. Chem.* **267,** 14998–15004
10. Schmidt, A. M., Hasu, M., Popov, D., Zhang, J. H., Chen, J., Yan, S. D., Brett, J., Cao, R., Kuwabara, K., Costache, G., Simonescu, N., Simonescu, M., and Stern, D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 8807–8811
11. Vlassara, H., Li, Y. M., Imani, F., Wojciechowicz, D., Yang, Z., Liu, F. T., and Cerami, A. (1995) *Mol. Med.* **1,** 634–646
12. Araki, N., Higashi, T., Mori, T., Shibayama, R., Kawabe, Y., Kodama, T., Takahashi, K., Shichiri, M., and Horiuchi, S. (1995) *Eur. J. Biochem.* **230,** 408–415
13. Ohgami, N., Nagai, R., Miyazaki, A., Ikemoto, M., Arai, H., Horiuchi, S., and Nakayama, H. (2001) *J. Biol. Chem.* **276,** 13348–13355
14. Ohgami, N., Nagai, R., Ikemoto, M., Arai, H., Kuniyasu, A., Horiuchi, S., and Nakayama, H. (2001) *J. Biol. Chem.* **276,** 3195–3202
15. Jono, T., Miyazaki, A., Nagai, R., Sawamura, T., Kitamura, T., and Horiuchi, S. (2002) *FEBS Lett.* **511,** 170–174
16. Li, Y. M., Tan, A. X., and Vlassara, H. (1995) *Nat. Med.* **1,** 1057–1061
17. Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1997) *J. Biol. Chem.* **272,** 31217–31220
18. Adachi, H., and Tsujimoto, M. (2002) *J. Biol. Chem.* **277,** 34264–34270
19. Ichikawa, K., Yoshinari, M., Iwase, M., Wakisaka, M., Doi, Y., Iino, K., Yamamoto, M., and Fujishima, M. (1998) *Atherosclerosis* **136,** 281–287
20. Ishibashi, S., Inaba, T., Shimano, H., Harada, K., Inoue, I., Mokuno, H., Mori, N., Gotoda, T., Takaku, F., and Yamada, N. (1990) *J. Biol. Chem.* **265,** 14109–14117
21. Steinbrecher, U. P., Witztum, J. L., Parthasarathy, S., and Steinberg, D. (1987) *Arteriosclerosis* **7,** 135–143
22. Perrey, S., Ishibashi, S., Kitamine, T., Osuga, J., Yagyu, H., Chen, Z., Shionoiri, F., Iizuka, Y., Yahagi, N., Tamura, Y., Ohashi, K., Harada, K., Gotoda, T., and Yamada, N. (2001) *Atherosclerosis* **154,** 51–60
23. Ishii, J., Adachi, H., Aoki, J., Koizumi, H., Tomita, S., Suzuki, T., Tsujimoto, M., Inoue, K., and Arai, H. (2002) *J. Biol. Chem.* **277,** 24014–24021
24. Park, L., Raman, K. G., Lee, K. J., Lu, Y., Ferran, L. J., Jr., Chow, W. S., Stern, D., and Schmidt, A. M. (1998) *Nat. Med.* **4,** 1025–1031
25. Pugliese, G., Pricci, F., Iacobini, C., Leto, G., Amadio, L., Barsotti, P., Frigeri, L., Hsu, D. K., Vlassara, H., Liu, F. T., and Di Mario, U. (2001) *FASEB J.* **15,** 2471–2479
26. Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N., Kataoka, M., Jishage, K., Ueda, O., Sakaguchi, H., Higashi, T., Suzuki, T., Takashima, Y., Kawabe, Y., Cynshi, O., Wada, Y., Honda, M., Kurihara, H., Aburatani, H., Doi, T., Matsumoto, A., Azuma, S., Noda, T., Toyoda, Y., Itakura, H., Yazaki, Y., Kodama, T., *et al.* (1997) *Nature* **386, 292–296**
27. Matsumoto, K., Sano, H., Nagai, R., Suzuki, H., Kodama, T., Yoshida, M., Ueda, S., Smedsrød, B., and Horiuchi, S. (2000) *Biochem. J.* **352,** 233–240
28. Smedsrød, B., Melkko, J., Araki, N., Sano, H., and Horiuchi, S. (1997) *Biochem. J.* **322,** 567–573
29. Politz, O., Gratchev, A., McCourt, P. A., Schledzewski, K., Guillot, P., Johansson, S., Svineng, G., Franke, P., Kannicht, C., Kzhyshkowska, J., Longati, P., Velten, F. W., Johansson, S., and Goerdt, S. (2002) *Biochem. J.* **362,** 155–164
30. Hansen, B., Svistounov, D., Olsen, R., Nagai, R., Horiuchi, S., and Smedsrød, B. (2002) *Diabetologia* **45,** 1379–1388