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

Yoshiaki Tamura‡‡‡, Hideki Adachi§§, Jun-ichi Osuga‡, Ken Ohashi‡, Naoya Yahagi‡, Motohiro Sekiya†, Hiroaki Okazaki‡, Sachiko Tomita‡, Yoko Iizuka‡, Hitoshi Shimamoto‡, 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 Yuskujii, 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/II (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, 125I-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 Kd 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, 125I-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 125I-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) 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-elicted 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-R1/90K-H) (AGE-R2)/glectin-3 (AGE-R3) (11), scavenger receptor class AI/II (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), lactoferrin (16), and lysozyme (16). The broad ligand repertoires 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 fasciclin, 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 vascular tissues in aging and diabetes.

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/II; 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.
the spleen and lymph nodes, 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-dT30TM was purchased from Roche Molecular Biochemicals. Hybond N was purchased from Amersham Biosciences. Nac, was purchased from Daiichi Chemical (Osaka, Japan). Iodogen was purchased from Roche Molecular Biochemicals. Hybrid N was purchased from Amersham Biosciences. Sernal, was purchased from Amersham Biosciences. Na, was purchased from Roche Molecular Biochemicals. Iodogen was purchased from Roche Molecular Biochemicals. Hybrid N was purchased from Amersham Biosciences. Sernal, was purchased from Amersham Biosciences.

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 32P-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.

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 and CHO-FEEL-2—[^125]I-AGE-BSA was prepared as described previously (18). cDNA probes for murine FEEL-1, FEEL-2, and SREC were prepared by reverse transcription-PCR using primers designed based on the reported nucleotide sequences.

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cells were incubated with 2 μg/ml 125I-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 circles), and HDL (open squares) in triplicate wells. C and D, cells were incubated with 2 μg/ml 125I-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 125I-AGE-BSA than CHO-FEEL-1 cells (B_{max} = 315.5 versus 18.9 ng/mg). On the other hand, the specific 125I-AGE-BSA binding to CHO-SREC cells was 30% of that to CHO-FEEL-1 cells with lower affinity (Fig. 2C). Untransfected CHO cells bound negligible amounts 125I-AGE-BSA (Fig. 2D).**

**Cellular Uptake and Degradation of 125I-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 125I-AGE-BSA, we incubated the cells with 125I-AGE-BSA at 37 °C and determined the amounts of 125I-AGE-BSA bound (Fig. 3, A–D) and degraded by the cells (Fig. 3, E–H). Significant amounts of 125I-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 125I-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 125I-AGE-BSA is inhibited by lipoproteins (Fig. 4, A and B). The binding of 125I-AGE-BSA to CHO-FEEL-1 (Fig. 4A) and CHO-FEEL-2 (Fig. 4B) 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 125I-AGE-BSA binding almost completely in both CHO-FEEL-1 (Fig. 4C) and CHO-FEEL-2 cells (Fig. 4D). 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 125I-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 125I-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 125I-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 125I-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).
cho-FEEL-1 and cho-FEEL-2 cells were incubated with 2 μg/ml 125I-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 IC50 value was calculated and shown for competitors that inhibited the binding of 125I-AGE-BSA >50% at 100 μg/ml. Values in parentheses are IC50 in molar concentrations, which were calculated based on the assumption that molecular masses of AGE-BSA, Ac-LDL, fucoidan, poly I, dextran sulfate, and heparin are 66, 549, 66.4, 200~500, 500, and 5~20 kDa, respectively.

<table>
<thead>
<tr>
<th>Competitors</th>
<th>CHO-FEEL-1</th>
<th>CHO-FEEL-2</th>
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<tbody>
<tr>
<td>AGE-BSA</td>
<td>22.2 (337)</td>
<td>6.9 (104)</td>
</tr>
<tr>
<td>AcLDL</td>
<td>&lt;5.9 (11)</td>
<td>16.8 (31)</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>&lt;5.4 (81)</td>
<td>&lt;5.0 (76)</td>
</tr>
<tr>
<td>Polyinosinic acid</td>
<td>&lt;5.3 (11~26)</td>
<td>&lt;5.0 (10~25)</td>
</tr>
<tr>
<td>Dextran sulfate</td>
<td>&lt;5.5 (11)</td>
<td>&lt;5.1 (10)</td>
</tr>
<tr>
<td>Heparin</td>
<td>8.9 (443~1,770)</td>
<td>7.2 (558~1,434)</td>
</tr>
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**TABLE I**

Concentrations of various lipoproteins and SR-A inhibitors to inhibit the binding of 125I-AGE-BSA to CHO-FEEL-1 and CHO-FEEL-2 cells (IC50)

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**Fig. 5.** Competitive inhibition of the uptake (A) and degradation (B) of 125I-AGE-BSA by CHO-FEEL-1 cells. Cells were incubated with 2 μg/ml 125I-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 125I-AGE-BSA were determined. Error bars represent mean ± S.D. * p < 0.05 versus control values.

**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-tetramethyl-indocarbo-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-1 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/galectin-3 complex (11) that is ubiquitously expressed in mamalian 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 polyl 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 FEEL-2; 39 nM for FEEL-1; 85 nM for CD36 (14); 100 nM for SR-A (12). The binding activity of CHO-FEEL-1 cells was 

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; HAS-R) first identified as MS-1 antigen (29). AGE uptake by liver sinusoidal endothelial cells is partially inhibited by an antibody against HAS-R (30).

**REFERENCES**


