Development of Receptor for Advanced Glycation End Products–Directed Imaging of Atherosclerotic Plaque in a Murine Model of Spontaneous Atherosclerosis

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Background—The receptor for advanced glycation end products (RAGE) is implicated in the development and progression of atherosclerosis. We tested the hypothesis that 99mTc-labeled anti-RAGE F(ab’)_2 can be used as a noninvasive tool to image atherosclerotic lesions in apolipoprotein E–deficient (apoE/−/−) mice.

Methods and Results—A sequence in the V-type Ig extracellular domain of RAGE was used to develop a peptide injected into rabbits; serum was retrieved, IgG prepared and affinity-purified, and pepsin-digested into F(ab’)_2. Thirteen 6-week apoE/−/− mice were fed a Western diet. At 20 weeks, 6 were injected with 15.2±1.9 MBq (350 to 411 µCi) 99mTc-labeled anti-RAGE F(ab’)_2, 6 were injected with 99mTc-labeled control nonspecific IgG F(ab’)_2, and 1 was injected with dual-labeled 99mTc and rhodamine anti-RAGE F(ab’)_2. Four 20-week C57BL/6 mice were injected with 99mTc-labeled anti-RAGE F(ab’)_2. All mice were imaged on a high resolution mini-γ camera 4 hours after injection and euthanized. The aortic tree was dissected and photographed, and the proximal aorta was sectioned for staining after scintillation counting. All 6 apoE/−/− mice injected with 99mTc-labeled anti-RAGE F(ab’)_2 fragments showed focal tracer uptake in the proximal aorta (mean %ID/g, 1.98%). Disease and antibody controls showed no focal tracer uptake (%ID/g, 1.0%). Histological sections of the proximal aorta showed American Heart Association class III lesions with lipid laden macrophages, smooth muscle cells, and positive staining for RAGE. On immunofluorescence, RAGE colocalized with macrophages.

Conclusion—These data show the feasibility of noninvasively imaging RAGE in atherosclerotic lesions in a murine model and confirm levels of RAGE expression sufficient to allow detection on in vivo imaging. (Circ Cardiovasc Imaging. 2008;1:212-219.)

Key Words: atherosclerosis ■ imaging ■ RAGE

Information available from discoveries in vascular biology and from human autopsy data has elucidated in detail the process of plaque development from a molecular to anatomic level. This information has been used to develop probes targeting a variety of molecular species for nuclear imaging and MRI platforms. An advantage of nuclear imaging is that the targeting probes are in nanomolar concentrations, have no biological effect, and the small size allows the peptide or antibody fragment to access the extravascular space and bind to sites on the cell membrane or intracellular sites. Radionuclide probes have been developed to target molecules expressed during all stages of the atherosclerotic plaque development from fatty streak to American Heart Association class IV lesions. The clinical impetus for this research is the potential value of a tool to noninvasively assess total plaque burden and individual plaque vulnerability to rupture. Developing probes to new targets is therefore an important endeavor. Such a target is the receptor for advanced glycation end products (RAGE).

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RAGE expression plays a key role in initiation and acceleration of atherosclerosis in both diabetics and nondiabetics. RAGE is a member of the immunoglobulin superfamily expressed at low levels in adult tissues in homeostasis but highly expressed at sites of vascular pathology.1–3 Expression of RAGE and its inflammatory ligands is a consistent observation in human and animal models of diabetes and atherosclerosis.4,5 Administration of RAGE antagonists to rats or mice, both with and without diabetes, attenuates...
vascular injury and greatly attenuates the initiation and acceleration of atherosclerosis.6,7 These findings support key roles for RAGE in atherosclerosis.

Methods

Development of Anti-RAGE Antibody

We developed a novel antibody in rabbits against the V-domain of RAGE designed to display immunoreactivity in mice, pigs, and human. Based on Genbank sequences of human, murine, and porcine RAGE, the following sequence alignment was determined and peptide prepared:�

- Human: 103-NRRGKETKSNYRVRVYQIP-121
- Murine: 102-NRRGKEVKSNYRVRVYQIP-120
- Peptide: 1-NRRGKEVKSNYRVRVYQIC-19

This peptide was injected into rabbits and one rabbit displayed optimal titer of antibody; serum was retrieved, IgG prepared and then affinity-purified. Western blotting performed on lung extract from mouse and human revealed that this antibody recognized human, murine, and porcine RAGE.9

Preparation of F(abˆ′)2 Fragments and Radiolabeling

Purified antibodies were subjected to digestion with immobilized pepsin beads using a kit from Pierce Chemical Co (Rockford, Ill) to produce F(abˆ′)2 fragments. These fragments have more antigen binding sites available than Fab, and faster blood pool and renal clearance compared with whole antibody. Direct coupling of anti-RAGE F(abˆ′)2 antibodies to diethyleneetriaminepentaacetic acid (DTPA) (Sigma Chemical Co, St Louis, Mo) for 99mTc labeling was performed as described.10 The immunoreactivity of DTPA modified antibody was tested by ELISA using soluble RAGE antigen-coated microtiter plates. Binding of the anti-RAGE F(ab)2, to the receptor was compared with that of unmodified anti-RAGE IgG using horseradish peroxidase (HRP)-conjugated secondary antirabbit IgG. The antibody concentration, which gave 50% of maximum binding concentration of unmodified anti-RAGE IgG was 0.8 μg/mL, which is equivalent to 8 × 10^{-9} moles/L or apparent affinity of 0.11 × 10^{10} L/mole. The 50% of maximum binding concentration of unmodified anti-RAGE IgG was 0.8 μg/mL, which is equivalent to 8 × 10^{-9} moles/L or apparent affinity of 0.12 × 10^{10} L/mole.

For radiolabeling, an aliquot of modified anti-RAGE F(abˆ′)2 (1 to 2 mg) was reacted with 5-fold molar excess of bicyclic anhydride of rhodamine-labeled DTPA-anti-RAGE F(abˆ′)2. Two 20-week-old C57BL/6 mice were anesthetized with inhaled isoflurane (1.5% isoflurane at a flow of 0.5 L/min oxygen per mouse) and injected with 99mTc-labeled antibody fragments. Blood samples (2 mL) were collected in capillary tubes via the tail vein at 2, 10, 30, 60, 120, 180, 320, 600, 1440 minutes and radioactivity counted in a γ counter (Wallac Wizard 1470, PerkinElmer, Waltham, Mass).

In Vivo and Ex Vivo Imaging

Male apolipoprotein E–deficient (apoE−/−) mice (backcrossed >10 generations in the C57BL/6 background) were purchased from the Jackson Laboratories (Bar Harbor, Me). At age 6 weeks, 13 apoE−/− mice were placed on Western-type diet (21%, w/w, fat [polysaturated/saturated ratio, 0.07]) and 0.15%, w/w, cholesterol (Harlan Teklad, Madison, Wis) for 14 weeks. Corresponding wild-type male C57BL6 mice (n = 4) on normal chow were used as controls. All animal studies were performed in accordance with the approval of the Institutional Animal Care and Use Committee of Columbia University.

At 20 weeks of age, 13 apoE−/− mice were anesthetized with inhaled isoflurane and 6 were injected with 99mTc-anti-RAGE F(abˆ′)2 antibody fragments, 1 injected with dual rhodamine labeled antibody fragments and the remaining 6 mice were injected with 99mTc-labeled control nonspecific IgG F(abˆ′)2 (radiotracer control). Four C57BL6 mice were also injected with 99mTc-anti-RAGE F(abˆ′)2 and similarly imaged (disease control). Four hours later, the animals were reanesthetized and serial whole body planar γ images in the anteroposterior and lateral views were acquired each for 10 minutes on a high spatial resolution high sensitivity dedicated small animal camera with parallel-hole collimator (provided by Jefferson Laboratory, Newport News, Va). The camera is based on a 5” Hamamatsu position sensitive photomultiplier type R3292 with an active field-of-view of 9~5 mm diameter. The scintillator sensor is 1.6 mm step 6-mm thick pixellated NaI (Tl) scintillator array. The photo peak was set at 140 keV with a 15% energy window.

Image Analysis and Ex Vivo Counting

At the end of imaging, mice were euthanized by intraperitoneal injection of pentobarbital (100 mg/kg). The aortic tree was dissected and photographed. Biodistribution studies were performed 5 to 6 hours after injection of the 99mTc-anti-RAGE F(abˆ′)2 or nonspecific IgG F(abˆ′)2 (radiotracer control). Four C57BL6 mice were also injected with 99mTc-anti-RAGE F(abˆ′)2 and similarly imaged (disease control). Four hours later, the animals were reanesthetized and serial whole body planar γ images in the anteroposterior and lateral views were acquired each for 10 minutes on a high spatial resolution high sensitivity dedicated small animal camera with parallel-hole collimator (provided by Jefferson Laboratory, Newport News, Va). The camera is based on a 5” Hamamatsu position sensitive photomultiplier type R3292 with an active field-of-view of 9~5 mm diameter. The scintillator sensor is 1.6 mm step 6-mm thick pixellated NaI (Tl) scintillator array. The photo peak was set at 140 keV with a 15% energy window.

Histopathology and Quantitative Morphometry

The heart and aorta were harvested by perfusion fixation for 10 minutes at physiological pressure with formalin (10%). Tissues were fixed for 24 hours in formalin (10%), followed by paraffin embedding. A 400-μm section of the proximal aorta from the aortic valve leaflets was excised. Serial 5-μm-thick sections were stained with

Preparation of Rhodamine-Labeled DTPA-anti-RAGE F(abˆ′)2

To localize the in vivo antibody uptake by histology, DTPA labeled anti-RAGE F(abˆ′)2 was conjugated to rhodamine isothiocyanate (Pierce Chemical Co) and purified as previously reported.11 The rhodamine-labeled DTPA-anti-RAGE F(abˆ′)2 was radioiodinated as described above.

Blood Clearance of 99mTc-Labeled Anti-RAGE F(abˆ′)2

Blood pool clearance study in mice was performed to determine the optimal time for imaging after injection of the 99mTc-labeled anti-RAGE F(abˆ′)2. Two 20-week-old C57BL/6 mice were anesthetized with inhaled isoflurane (1.5% isoflurane at a flow of 0.5 L/min oxygen per mouse) and injected with 99mTc-labeled anti-RAGE F(abˆ′)2 antibody fragments. Blood samples (2 mL) were collected in capillary tubes via the tail vein at 2, 10, 30, 60, 120, 180, 320, 600, 1440 minutes and radioactivity counted in a γ counter (Wallac Wizard 1470, PerkinElmer, Waltham, Mass).

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hematoxylin-eosin (H&E) for morphology and for immunohistochemistry. Morphometric analyses of the arterial segments were performed using a Nikon microscope and image analysis system (Media Cybernetics Inc, Silver Spring, Md). The amount of aortic lesion formation in each animal was measured as percent lesion area per total area of the aorta.13

For cellular characterization of atherosclerotic lesions, adjacent sections were deparaffinized in xylene, and treated with 0.3% hydrogen peroxide for 20 minutes to inactivate endogenous peroxidase. Tissue sections were then incubated in protein-free block (Dako, Carpinteria, Calif) for 10 minutes to inhibit the nonspecific binding of primary antibody. Staining for RAGE was performed using polyclonal antibody to RAGE (50 μg/mL). For identification of endothelial cells von Willebrand factor (vWF) (1:200; Dako) was used. Macrophages were identified using the marker Mac-3 (1:20; BD Pharmingen, San Diego, Calif). Smooth muscle cells (SMCs) were identified using a primary antibody HHF-35 against α-actin (1:250; Sigma). Control immunostaining was performed using the respective nonspecific IgG. Detection was performed with HRP-conjugated goat antirabbit IgG (for RAGE) (Sigma), and mouse antirat IgG (for macrophages) (Serotec), and goat antimouse IgG (for SMC), followed by diaminobenzidine (DAB substrate kit for peroxidase, Vector Laboratories, Burlingame, Calif) and counterstaining with Gill hematoxylin solution.

To determine the cell types expressing RAGE in atherosclerotic aortic sections dual-label confocal microscopy was performed. Sections were subjected to immunofluorescence colocalization of RAGE with endothelial cells, macrophages and SMCs by overnight incubation at 4°C with the respective antisera as described above in immunoperoxidase section. Sections were then incubated with conjugated fluorescent secondary antibodies (Texas Red antirabbit, fluorescein isothiocyanate antimouse and antirat; Vector Laboratories) at 1:200 at 4°C for 2 hours. When the Texas Red and fluorescein isothiocyanate signals colocalize, a yellow signal is seen. Aortic sections from the animal that was injected with dual rhodamine-labeled antibody fragments were also examined under confocal fluorescent microscopy. Adjacent sections were subjected to immunoperoxidase staining for RAGE as described above.

Statistical Analysis
Because of the small sample sizes used in the study, nonparametric statistical methods were used. All continuous data are presented in terms of median and range. Comparison of radioactivity uptake between thoracic organs was performed using the Friedman test, with post hoc pairwise significance of differences assessed using Nemenyi test. Comparisons of aortic radioactivity uptake between apoE−/− mice receiving 99mTc anti-RAGE F(ab′)2, apoE−/− mice receiving nonspecific IgG F(ab′)2, and control C57BL/6 mice receiving anti-RAGE F(ab′)2 were performed using the Kruskal-Wallis test, with post hoc pairwise significance of differences assessed using Dunn test. All tests of significance were 2-tailed, and differences between groups were considered significant at a value of P<0.05, except for multiple comparison tests where adjusted probability values were used. All statistical analyses were performed using Stata 10.1 (StataCorp, College Station, Tex; commands: friedman and kwallis2), with the exception of Nemenyi test, for which calculations were performed with Excel 2003 (Microsoft, Redmond, Wash). The authors had full access to the data and took responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Blood Clearance of 99mTc-anti-RAGE F(ab′)2
Blood pool clearance showed a biexponential curve. The t1/2 for the first component was 15 minutes and for the second component 7 hours.

In Vivo Scans

All seven atherosclerotic apoE−/− mice injected with 99mTc-labeled anti-RAGE F(ab′)2 showed focal tracer uptake in the thorax corresponding to the location of the proximal aorta and correlating with atherosclerotic lesions seen at necropsy. An example from one experiment is shown in Figure 1A. Atherosclerotic apoE−/− mice injected with 99mTc-labeled nonspecific IgG F(ab′)2 showed no tracer uptake in the thorax although in situ dissection of the aortic arch showed extensive atherosclerotic plaque (Figure 1B). Control C57BL/6 mice injected with 99mTc-labeled anti-RAGE F(ab′)2 also showed no localization of the radiotracer at the target and gross examination of the aorta revealed no lesions (Figure 1C).

Quantitative 99mTc-anti-RAGE F(ab′)2 Uptake

The localization of focal uptake to the proximal aorta was confirmed by ex vivo counting of thoracic organs, namely the lungs, heart, and aorta. In apoE−/− mice injected with 99mTc anti-RAGE F(ab′)2, there was a significant difference in RAGE uptake between thoracic organs (Friedman χ2=8; approximate P=0.0183) In post hoc pairwise comparisons, the difference in uptake was statistically significant between aortic segments (median uptake, 1.76, range, 1.43 to 2.96% ID/g) and the heart (median, 0.50; range, 0.05 to 0.87% ID/g), whereas comparisons of aorta and heart with the lungs (median, 0.90; range, 0.71 to 1.03% ID/g) did not attain significance in this small sample(Figure 2A). The radiotracer uptake in the proximal aorta differed significantly among the 3 groups of mice (χ2=9.613; P=0.0082). Aortic uptake in apoE−/− mice receiving 99mTc anti-RAGE F(ab′)2 was significantly greater (P=0.001) than aortic uptake in apoE−/− mice receiving nonspecific IgG F(ab′)2 (median uptake, 0.13; range, 0.06 to 0.19% ID/g). Comparison of aortic uptake between each of these groups of mice and control C57BL/6 mice receiving anti-RAGE F(ab′)2 (median, 0.39; range, 0.17 to 0.86% ID/g) did not attain statistical significance (P=0.05 and P=0.08, respectively, adjusted probability value required for significance 0.0083; Figure 2B).

There was also a significant difference between the 3 groups of mice in terms of average tracer uptake in ROIs drawn on the scans relative to the whole body (χ2=12.83; P=0.0016). Average uptake relative to the whole body was 1.03% (range, 0.71 to 1.54%) for ROIs drawn on the scans around the focal uptake of 99mTc-labeled anti-RAGE F(ab′)2 in the thorax in the experimental apoE−/− mice. Tracer uptake in ROIs drawn in corresponding locations in the thorax of the apoE−/− mice receiving nonspecific IgG F(ab′)2 was 0.17 (range, 0.11 to 0.22) (P=0.0002 versus 99mTc-labeled anti-RAGE F(ab′)2) and was 0.31 (range, 0.22 to 0.39) in control C57BL/6 mice receiving anti-RAGE F(ab′)2 (P=0.04 versus apoE−/− mice, adjusted probability value for significance 0.0083).

Biodistribution of radiolabeled anti-RAGE F(ab′)2 and nonspecific IgG F(ab′)2 in nontarget organs performed by well counting of harvested tissues are shown in Figure 3. In both groups, the highest uptake was in the liver as was noted on the in vivo scans. Although the largest % ID/g is in the subdiaphragmatic organs, this activity is anatomically removed from the target (aortic root and arch) and T/B ratio for the chest is high.
Histological Characterization of Atherosclerotic Lesions

Histological sections through the proximal aorta in the apoE<sup>−/−</sup> mice showed American Heart Association class III lesions. The mean cross-sectional area of the proximal aortic lesions, expressed as percent lesion area of total aortic area, in apoE<sup>−/−</sup> mice injected with 99mTc-labeled anti-RAGE F(ab)'<sub>2</sub> (33.1%; range, 30.1 to 38.9%) was similar to apoE<sup>−/−</sup> mice injected with 99mTc-labeled control antibody (34.5%; range, 31.2 to 37.6%). The control C57BL/6 mice showed normal aortas without lesions. Histological sections through the proximal aorta of apoE<sup>−/−</sup> mice injected with dual rhodamine and 99mTc labeled F(ab')<sub>2</sub> showed co-localization of fluorescence with RAGE staining (Figure 4). This confirmed that the dual-labeled antibody fragments were taken up in the proximal aortic lesions expressing RAGE.

Immunohistochemical staining of the apoE<sup>−/−</sup> aortas identified endothelial cells, macrophages, and smooth muscle cells and showed positive staining for RAGE in the neointima.

Figure 1. A, Planar γ images of the anteroposterior (AP) and lateral (LAT) views of a 20-week apoE<sup>−/−</sup> mouse on Western-type diet with atherosclerotic lesion 4 hours after intravenous injection of 99mTc-labeled anti-RAGE F(ab)'<sub>2</sub>, corresponding to the location of the atherosclerotic lesions shown on the photograph. B, Planar γ images of the anteroposterior and lateral views of a 20-week apoE<sup>−/−</sup> mouse on Western-type diet with atherosclerotic lesion 4 hours after intravenous injection of 99mTc-labeled nonimmune rabbit IgG F(ab')<sub>2</sub> show no tracer uptake in the thorax, although the in situ dissection of the aortic arch showed extensive atherosclerotic plaque. C, Anteroposterior planar γ image of a wild-type control C57BL/6 mouse 4 hours after intravenous injection of 99mTc-labeled anti-RAGE F(ab')<sub>2</sub> and gross examination of the aorta revealed no lesion.

Figure 2. A, Bar graph shows uptake of radiotracer in thoracic organs expressed as % ID/g in apoE<sup>−/−</sup> mice injected with 99mTc anti-RAGE F(ab')<sub>2</sub>. There is significantly higher uptake in the proximal aorta as compared with the uptake in the heart or the lungs. B, Graph shows uptake of radiotracer in the proximal aorta expressed as % ID/g for apoE<sup>−/−</sup> mice injected with 99mTc anti-RAGE F(ab')<sub>2</sub> (striped bar), apoE<sup>−/−</sup> mice injected with 99mTc-labeled nonimmune IgG (black bar), and C57BL/6 mice injected with 99mTc anti-RAGE F(ab')<sub>2</sub> (gray bar).
(Figure 5a and 5b). Using confocal imaging and double-immunofluorescence labeling, RAGE is shown to colocalize predominantly with macrophages.

**Discussion**

The results of this study provide proof of concept that RAGE can be imaged in vivo using a radiolabeled antibody. Focal tracer uptake in the thorax with good target to background activity ratios corresponding to the location of the proximal aortic atheroma both by gross dissection and by biodistribution was visible by 4 to 5 hours after administration of the radiolabeled antibody.

Advances in vascular biology have elucidated a number of targets for noninvasive imaging of the atherosclerotic plaque. Clinical importance of this imaging includes identification of the vulnerable plaque and quantitative assessment of total plaque burden as a surrogate end point for evaluation of novel drugs to stabilize and/or regress atherosclerosis. An important component of the vulnerable plaque is inflammation and a primary player in plaque inflammation is the macrophage. A number of radiotracer approaches have been reported that target the macrophage through either increased metabolism (F-18 FDG) or increased receptor expression (metalloproteinases) or programmed cell death.\(^\text{14–16}\) RAGE is another important marker for atherosclerotic plaque inflammation.

The predominant ligand for RAGE are advanced glycation end products formed by the nonenzymatic linkage of glucose to proteins and their formation is a direct consequence of prolonged levels of hyperglycemia in diabetes. Dr Ann Marie Schmidt and coinvestigators were the first to describe endothelial cell surface-associated proteins that mediate the interaction of AGEs with endothelium and characterized the binding site.\(^\text{1,9,17,18}\)

RAGE participates in plaque inflammation by also binding non-AGE–related proinflammatory markers including S100/calgranulins, High Mobility Group Box-1, EN-RAGE.\(^\text{19}\) Because of this latter broader function, these receptors are implicated in progression of atherosclerosis in nondiabetics. Dr Schmidt and her collaborators developed a soluble form of RAGE (s-RAGE) that includes the extracellular ligand-binding domain. This group has shown that treatment of diabetic mice with accelerated atherosclerosis with s-RAGE reduces AGE-RAGE interaction and suppresses atherosclerosis development.\(^\text{6,7}\) They also showed that s-RAGE suppressed atherosclerosis, although to a lesser degree, in eugly-

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**Figure 3.** Biodistribution of \(^{99m}\)Tc-labeled anti-RAGE F(ab\(^\prime\))\(_2\) (open bar) and nonimmune IgG F(ab\(^\prime\))\(_2\) (black bar) 4 hours after intravenous administration of the radiotracer in nontarget organs of 20-week apoE\(^\text{−}\)/\(^\text{−}\) mice.

**Figure 4.** Epifluorescent micrographs of 5-\(\mu\)m-thick paraffin section of the aortic sinus from an apoE\(^\text{−}\)/\(^\text{−}\) mouse injected with \(^{99m}\)Tc and rhodamine-labeled anti-RAGE F(ab\(^\prime\))\(_2\) (A) and immunohistochemical stained adjacent sections of the aortic sinus with anti-RAGE IgG (B). Fluorescence in the aorta corresponds to localization of RAGE-specific staining in the lesion. (Magnification \(\times 100\).)
Figure 5. A, Immunohistochemical characterization of atherosclerotic lesions in 20-week apoE−/− mouse. Serial sections were stained for endothelial cells (FVIII), SMCs (α-actin), macrophages (Mac-2), and RAGE. The chromagen stains brown. (Magnification ×400.) B, Aortic tissue subjected to immunofluorescence staining to detect RAGE antigen (A, D, and G). Sites of prominent RAGE expression were confirmed to be predominantly macrophages based on colocalization of RAGE expression with Mac-2 (E) in the merged image (F). Colocalization of RAGE with endothelial cells (B) was seen in the merged image (C). RAGE was also expressed in smooth muscle cells, based on colocalization of smooth muscle actin (H) in the merged image (I). Areas in yellow represent colocalization. (Magnification ×100.)
cemic apoE null mice. These findings support the broad function of RAGE to bind other proinflammatory ligands besides AGEs and may have an important role in progression of atherosclerosis in general.

In addition to accelerated progression of atherosclerosis RAGE also mediates inflammatory stresses of the vascular walls after endothelial dysfunction and entry of lipoproteins into the wall that stimulates infiltration of monocyte/macrophages and lymphocytes establishing early atherosclerotic plaque. Binding of AGEs to receptors induces multiple signaling pathways involved in plaque initiation and progression.

RAGE is highly conserved across species and is widely distributed in vascular and lung tissue from nondiabetic animals with close homology to man. Several reports using human material have studied RAGE expression in atherosclerotic plaques. One study used plaques obtained from patients undergoing carotid endarterectomy and the other used coronary arteries from subjects who had sudden cardiac death. Both studies found immunoreactivity for RAGE in atherosclerotic tissue from nondiabetic as well as from diabetic patients. Inflammatory cells (macrophages, T lymphocytes) in the plaques and the cells stained positive for RAGE. The findings in the present study showing predominant colocalization of RAGE with macrophages agree with the human studies.

Summary and Limitations

The results of this pilot study document that RAGE can be imaged in vivo in a relevant murine model of atherosclerosis with American Heart Association class II and class III lesions where RAGE is expressed predominantly in lesion macrophages. The findings justify the performance of further experiments to correlate lesion activity with macrophage density in a wider range of lesion severity in both nondiabetic and diabetic animal models. Ultimately, this approach may have clinical applications to noninvasively identify accelerated atherosclerotic lesions.

The humoral component of the inflammatory process involves circulating immunoglobulins and complement. It is possible that some of the uptake of the anti-RAGE polyclonal antibody fragments into the plaque of the apoE−/− mice were because of this nonspecific immune response. Fischman et al demonstrated uptake of 111In-labeled human nonspecific polyclonal IgG into atherosclerotic lesions of the abdominal aorta of NZW white rabbits after injury and high fat diet. However, when they injected 111In-labeled Fab fragments the uptake was no different from control HSA. Because we used fragments of a specific antibody directed against RAGE, it is unlikely we were seeing a nonspecific effect of immunoglobulins in inflammation.

Monoclonal antibodies have higher specificity for target binding and lower nonspecific binding to nontarget organs and are being produced for further work. Antibody molecules are relatively large compared with peptides and have a longer blood pool clearance time representing a relative drawback for in vivo imaging.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

We report the development of a novel antibody targeting the receptor for advanced glycation end products (RAGE) and pilot results of in vivo nuclear imaging of radiolabeled antibody fragments in apolipoprotein E–deficient mice. RAGE expression plays a key role in initiation and acceleration of atherosclerosis in both diabetic and nondiabetic patients. In this pilot study, we injected $^{99m}$Tc-labeled F(ab')$_2$ fragments of the polyclonal antibody into 24-week apolipoprotein E–deficient mice that were fed a high-fat diet, and we performed in vivo planar imaging using a high-resolution dedicated small animal camera. Planar images showed uptake of radiotracer localized to the proximal aorta, corresponding in location to class III atherosclerotic lesions on pathology; absence of uptake of $^{99m}$Tc-labeled anti-RAGE F(ab')$_2$ in the aortae of C57BL/6 mice; and absence of uptake of radiolabeled nonimmune IgG in atherosclerotic aortae of apolipoprotein E–deficient mice. Dual-label confocal microscopy localized immunofluorescence staining for RAGE expression to macrophages. These results are preliminary, and further work needs to be done to develop a monoclonal antibody and study both diabetic and nondiabetic patients. Nevertheless, these results suggest that RAGE imaging may potentially become an important clinical tool. RAGE binds glycated end products in diabetic patients as well as other inflammatory ligands and plays an important role in atherogenesis. The development of a radiolabeled antibody to noninvasively image RAGE expression in vascular lesions has potential clinical applications to identify and quantify lesional inflammation.
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