Methods and Results

Prediction to personalize strategies for individual patients. There is, therefore, a need for better risk stratification. Small aneurysms may rupture between successive scheduled imaging sessions, whereas some large but relatively stable aneurysms are operated on, exposing patients to unnecessary risks. The decision to intervene surgically depends on the size and location of the aneurysm: a rapidly increasing size or a diameter above 5.5 cm is considered an indication for intervention. If lower diameters are encountered, repeated monitoring with anatomic imaging is recommended. The timing of therapy and imaging is difficult but crucial, because both invasive repair and progressive disease carry significant risks. Smaller aneurysms may rupture between successive scheduled imaging sessions, whereas some large but relatively stable aneurysms are operated on, exposing patients to unnecessary risks. There is, therefore, a need for better risk prediction to personalize strategies for individual patients.

Objective—Current management of aortic aneurysms (AAs) relies primarily on size criteria to determine whether invasive repair is indicated to preempt rupture. We hypothesized that emerging molecular imaging tools could be used to more sensitively gauge local inflammation. Because macrophages are key effector cells that destabilize the extracellular matrix in the arterial wall, it seemed likely that they would represent suitable imaging targets. We here aimed to develop and validate macrophage-targeted nanoparticles labeled with fluorine-18 ($^{18}$F) for positron emission tomography–computed tomography (PET-CT) detection of inflammation in AAs.

Methods and Results—Aneurysms were induced in apolipoprotein E/−/− mice via systemic administration of angiotensin II. Mice were imaged using PET-CT and a monocyte/macrophage–targeted nanoparticle. AAs were detected by contrast-enhanced micro-CT and had a mean diameter of 1.85±0.08 mm, whereas normal aortas measured 1.07±0.03 (P<0.05). The in vivo PET signal was significantly higher in aneurysms (standard uptake value, 2.46±0.48) compared with wild-type aorta (0.82±0.05, P<0.05). Validation with scintillation counting, autoradiography, fluorescence, and immunoreactive histology and flow cytometry demonstrated that nanoparticles localized predominantly to monocytes and macrophages within the aneurysmatic wall.

Conclusion—PET-CT imaging with $^{18}$F-labeled nanoparticles allows quantitation of macrophage content in a mouse model of AA. (Arterioscler Thromb Vasc Biol. 2011;31:750-757.)

Key Words: aneurysms ■ imaging agents ■ macrophages ■ positron emission tomography ■ nanoparticle

The prevalence of aortic aneurysms (AAs) in the elderly population is approximately 5%. Up to 50% of larger aneurysms rupture, an event that carries a mortality rate of around 50%. The current management strategy for patients with aneurysms includes a combination of anatomic imaging, watchful waiting, and surgical intervention to preempt deadly ruptures. The decision to intervene surgically depends on the size and location of the aneurysm: a rapidly increasing size or a diameter above 5.5 cm is considered an indication for intervention. If lower diameters are encountered, repeated monitoring with anatomic imaging is recommended. The timing of therapy and imaging is difficult but crucial, because both invasive repair and progressive disease carry significant risks. Smaller aneurysms may rupture between successive scheduled imaging sessions, whereas some large but relatively stable aneurysms are operated on, exposing patients to unnecessary risks. There is, therefore, a need for better risk prediction to personalize strategies for individual patients.

See accompanying article on page 723

Insight into the pathobiology of aneurysms is evolving quickly, and increasing evidence points to an important role for innate immune cells. Monocytes/macrophages infiltrate the vessel wall and release proteases, among them elastase and metalloproteinases, that compromise the integrity of the vascular wall through degradation of the extracellular matrix. Monocytes/macrophages also secrete inflammatory cytokines in the media and adventitia of aneurysmatic vessels, such as tumor necrosis factor, interferon-γ and interleukin-6. These inflammatory processes precede the increase in vessel diameter and are involved in aneurysm growth and rupture in animal models. Therefore, imaging of macrophage presence and function in a vascular domain may report on the propensity of an aneurysm to rupture earlier and with greater specificity than methods that focus on anatomy. In this investigation, we hypothesized that nanoparticles targeted to macrophages could be used to detect inflammation in AAs using imaging methods.

We used nanoparticle-based positron emission tomography (PET) reporters that can be detected at trace concentrations and that are analogous to carbohydrate coated nanoparticles recently approved by the FDA (Feraheme). The coating and size of the nanoparticles in this study were optimized to target
monocytes/macrophages. In addition, recently developed PET isotope labeling strategies using “click” chemistry\(^\text{12}\) allowed for rapid labeling of nanoparticles with the widely used clinical PET tracer fluorine-18 \(^{18}\text{F}\). PET–computed tomography (CT) is a preeminent choice of modality for this application because it combines the superior sensitivity of quantitative PET imaging with high-resolution anatomic information derived from CT. Nanoparticles used in this study were colabeled with fluorochromes to facilitate the validation of agent location with optical imaging techniques.

**Methods**

**Animal Models**

In this investigation, apolipoprotein E (apoE)\(^\text{−/−}\) mice were systemically administered angiotensin II (AT-II) over a prolonged period. This model system was chosen because of the presence of atherosclerosis, a shared common risk factor with human patients. Even more importantly, this model resembles the clinical situation in that aneurysm rupture is a documented complication.\(^\text{16}\)

For aneurysm induction, apoE\(^\text{−/−}\) mice were subcutaneously implanted with osmotic minipumps (Alzet, 2004) releasing 1\(\mu\)g/min per kg AT-II (Bachem) for 28 days (\(n=19\)). Minipumps were preincubated in PBS for 4 hours to ensure immediate delivery of the agent after implantation. At the time of implantation, apoE\(^\text{−/−}\) mice were 6 months old and had been kept on a high-cholesterol diet (Harlan). Female apoE\(^\text{−/−}\) mice and C57BL/6 controls (\(n=14\)) were purchased from the Jackson Laboratory.

In 1 subgroup of mice, the splenic monocyte reservoir was surgically removed to modulate the supply of monocytes and consequently the number of their lineage descendant macrophages in the aortic wall. In previous work, it had been shown that the spleen contains a large population of undifferentiated monocytes and that these cells are released into the blood pool on AT-II administration. It was subsequently shown that splenic monocytes can contribute up to \(\approx 50\%\) of the myeloid cells in inflamed tissue.\(^\text{17,18}\) To surgically remove the spleen, animals were anesthetized in an isoflurane chamber at 2 L/min O\(_2\) at a concentration of 2\%. Under anesthesia, the abdominal cavity of the mice was opened in the left upper quadrant. The spleen was gently exteriorized, and the splenic artery and vein were cauterized. The spleen was then removed, and the peritoneum and skin were then closed using Ethicon 6-0 Ethilon suture.

**Nanoparticle Preparation**

Dextran-coated iron oxide nanoparticles (CLIO, Center for Systems Biology, Boston, MA) served as the starting materials. The dextran coating of the nanoparticle was cross-linked with epichlorohydrin, aminated, and labeled with a near-infrared fluorochrome (a method we have used previously; VT680, PerkinElmer, Boston, MA), to render particles fluorescent.\(^\text{13}\) The ratio of VT680 per nanoparticle was approximately 5 fluorochromes/particle. The copper-catalyzed azide/alkyne click labeling strategy was used for \(^{18}\text{F}\) labeling of nanoparticles.\(^\text{14}\) Briefly, cross-linked iron oxide (CLIO) nanoparticles were derivatized with azide, and the click partner azide was attached to an \(^{18}\text{F}\)-labeled polyethylene glycol \((^{18}\text{F}-\text{PEG}_\text{N}_\text{A})\). Amination of derivated CLIO with N-succinimidyl 4-pentynoate was performed as described previously.\(^\text{10}\) Following size exclusion chromatography, the concentration of the alkylene-modified CLIO solutions was 1 mg/100 \(\mu\)L. 1-Azido-2-(2-(2-\(^{18}\text{F}\)-fluoroethoxy)ethoxy)ethane \((^{18}\text{F}-\text{PEG}_\text{N}_\text{A})\) was prepared from 2-(2-azidoethoxy)ethoxyethyl p-toluenesulfonate in 51 \(\pm\) 5\% (\(n=15\)) average decay-corrected radiochemical yield. Analytic HPLC demonstrated \(>99\%\) radiochemical purity of \((^{18}\text{F}-\text{PEG}_\text{N}_\text{A})\). Finally, \((^{18}\text{F}-\text{PEG}_\text{N}_\text{A})\) was conjugated to alkylene-modified CLIO nanoparticles using click chemistry.\(^\text{11}\) Following size exclusion chromatography using a PD-10 column (GE Biosciences, Piscataway, NJ), the final solution of \(^{18}\text{F}\)-CLIO was 0.25 mg/100 \(\mu\)L in 1\(\times\) PBS, and HPLC analysis demonstrated that \(^{18}\text{F}\)-CLIO was \(>98\%\) radiochemically pure. The mean specific activity was 677.1 \(\pm\) 81.4 MBq/\(\mu\)g Fe.

**PET-CT**

Mice were imaged with PET-CT using an Inveon small animal scanner (Siemens). Initially, 4 dynamic scans were performed over several hours to determine the best imaging time point after intravenous nanoparticle injection. A high-resolution Fourier rebinning algorithm was used to refine sinograms, followed by a filtered back-projection algorithm to reconstruct 3-dimensional images without any attenuation correction. Isotropic mage voxel size was 0.796 \(\times\) 0.861 \(\times\) 0.861 mm, for a total of 128 \(\times\) 128 \(\times\) 159 voxels. Peak sensitivity of the Inveon accounts for 11\% of positron emission, with a mean resolution of 1.65 mm.\(^\text{19}\) More than 100 counts were acquired per pixel, and the average signal-to-noise ratio was greater than 20. Calibration of the PET signal with a cylindrical phantom containing \(^{18}\text{F}\) was performed before all scans. Data are expressed as mean standard uptake values (SUV), which normalizes activity for body weight and injected activity. Target-to-background ratios were calculated in regions of interest to account for contribution of blood signal. Mean activity was 77.7 \(\pm\) 5.6 MBq in 60 \(\pm\) 10 \(\mu\)L was injected through the tail vein in mice with an average weight of 22.8 \(\pm\) 0.83 g.

CT images were reconstructed from 360 cone-beam x-ray projections with a power of 80 keV and 500 mA. The isotropic resolution of the CT images was 60 \(\mu\)m. During CT acquisition, iodine contrast was infused into the tail vein at a rate of 35 \(\mu\)L/min to enhance intravascular contrast. Projections were acquired at end expiration using a BioVet gating system (M2 mol/L Imaging, Cleveland, OH), and the CT acquisition time was \(\approx 10\) minutes. Reconstruction of data sets, PET-CT fusion, and image analysis were done using IRW software (Siemens). Three-dimensional visualizations were produced using the DICOM viewer OsiriX (The OsiriX Foundation, Geneva, Switzerland).

**Ex Vivo Tissue Imaging**

All mice were placed in a well counter (CRC-127R, Capintec, Ramsey, NJ) after injection and again before dissection to record total corporeal activity. Aortas were then excised using a surgical microscope and microdissection tools, and radioactivity was measured using a gamma counter (1480 Wizard 3\(\text{\textsuperscript{\textregistered}}\), PerkinElmer, Boston, MA). Finally, tissues were exposed overnight for digital autoradiography, and plates were analyzed using a Typhoon scanner (GE). Six aortas were also imaged with a surface reflectance fluorescence microscope (OV-100, Olympus) to detect signal from the fluorochrome on the nanoparticles.

**Histology**

Aorta histology was assessed in mice with aneurysms, which had been euthanized 28 days after minipump (delivering AT-II) implantation and 24 hours after injection of a cold version of the nanoparticle. Aortas were excised, rinsed in PBS, and embedded in OCT compound (Sakura Finetek). Fresh-frozen 6-\(\mu\)m-thick serial sections were stained with hematoxylin/eosin, as well as immunohistochemically stained for detection of macrophages. The primary antibody was MAC-3 (BD Pharmingen), followed by a secondary antibody (Vector Laboratories), and avidin-biotin peroxidase. The reaction was visually stained for detection of macrophages. The primary antibody was MAC-3 (BD Pharmingen), followed by a secondary antibody (Vector Laboratories), and avidin-biotin peroxidase. The reaction was visualized using a 3-amino-9-ethyl-carbazol substrate (Dako). On adjacent sections, we analyzed microscopic nanoparticle distribution with a Nikon 80i upright fluorescence scope equipped with a charge-coupled device camera connected to a Macintosh workstation.

**Flow Cytometry**

Flow cytometry experiments were conducted on cell suspensions retrieved from excised aortas to assess the cellular probe distribution and the number of infiltrating leukocytes in different groups.\(^\text{20}\) A total of 8 mice were used: 2 apoE\(^\text{−/−}\) mice with splenectomy and 2 apoE\(^\text{−/−}\) mice without splenectomy, 7 days after minipump implantation and 24 hours after injection of 5 mg/kg bodyweight of a cold version of the nanoparticle preparation; 2
wild-type mice 24 hours after probe injection; and 2 wild-type mice without probe injection as a background/autofluorescence control. The entire aorta from each mouse was harvested; minced with fine scissors; placed into a cocktail of collagenase I, collagenase XI, DNase I, and hyaluronidase (Sigma-Aldrich); and shaken at 37°C for 1 hour. Cells were then triturated through a nylon mesh and centrifuged (15 minutes, 500 g, 4°C). Total cell numbers were determined with trypan blue (Mediatech, Inc.). The following antibodies were used to stain the cell suspensions retrieved from the aortas: anti–CD90-PE, 53-2.1 (BD Biosciences, San Jose, CA); anti–B220-PE, RA3-6B2 (BD Biosciences); anti–CD49b-PE, DX5 (BD Biosciences); anti–NK1.1-PE, PK136 (BD Biosciences); anti–Ly-6G-PE; anti-Ly6C-FITC; anti-CD11b-APC-Cy7 (BD Biosciences); and F4/80-PE-Cy7 (eBiosience, San Diego, CA). Monocytes/macrophages were identified as CD11bhi (CD90/B220/CD49b/NK1.1/Ly-6G)lo. Neutrophils were identified as CD11bhi (CD90/B220/CD49b/NK1.1/Ly-6G)hi. Data were acquired on an LSRII instrument (BD Biosciences) with 670/LP and a 695/40 filter configuration to detect VT-680 on nanoparticles. The relative contribution of signal was calculated by multiplying the proportion of cells in the living cell gate by the mean fluorescent intensity. Data were analyzed using FlowJo, version 8.5.2 (Tree Star, Inc.).

Statistics
Results are expressed as means±SEM. Statistical comparisons between 2 groups were evaluated using the Mann-Whitney test, and ANOVA with the Bonferroni post test was used for multiple comparisons. A value of P<0.05 was considered to indicate statistical significance.

Results
Micro-CT Imaging Determines Incidence, Location, and Dimension of AAs
The dimensions of AAs were initially evaluated in wild-type mice and in apoE−/− mice after AT-II administration, using contrast enhanced micro-CT. Supplemental Table I (available online at http://atvb.ahajournals.org) shows the intra- and interobserver variability of the used CT angiography method. Aneurysm size was found to vary, ranging from 1.4 to 2.9 mm in diameter, with a mean of 1.8 mm (Table and Figure 1). Aneurysms were commonly observed in the ascending aorta and in the abdominal aorta. In 2 animals, more than 1 AA was found. Six mice died during the 28-day period of AT-II administration, and autopsy confirmed that aneurysm rupture was the cause of death. All but 1 mouse in this cohort developed aneurysms. The normal aortic diameter in the ascending aorta of wild-type mice was 1.1 ± 0.03 mm, which was comparable to the diameter of nondilated aortic sections in apoE−/− mice (Table). These sizes, location, and incidence of rupture are consistent with prior work.15,16

Table. Model Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AA-Related Deaths</th>
<th>Deaths (%)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aneurysm</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>6</td>
<td>37.5</td>
<td>1.85±0.08*</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>1</td>
<td>17</td>
<td>1.64±0.08*</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Shown are the number of animals used, mortality, and aortic diameter (assessed by micro-CT imaging). The spatial resolution of CT was 60 μm isotropically. Data are presented as mean±standard error. NA indicates not applicable.

*P<0.01 vs wild-type and nonaneurysmatic sections.

Figure 1. CT angiography in experimental groups. Arrows indicate the short axis view of the aorta. Diameter data are listed in the Table. AsA, ascending aorta; TA, thoracic aorta; AbA, abdominal aorta.
Dynamic PET-CT Imaging to Determine Injection-Imaging Sequence

To determine the best imaging time point, a factor that is dependent on the in vivo biodistribution of the nanoparticle and on the decay of the PET reporter, we first performed dynamic PET imaging in 4 mice over several hours. Imaging was initially done between 2.5 and 6.5 hours following injection of the probe. During this time, the signal in blood was found to be consistently higher than in the target region within the aortic wall. We therefore imaged from 9 to 13 hours after injection of the nanoparticle. Here, the blood pool signal dropped progressively. The 10- to 12-hour time point showed an optimal target-to-blood activity ratio, and activity of the PET isotope was still sufficient to provide a high number of counts. These in vivo findings were in line with the blood half-life of 18F-CLIO, which was determined by serial bleeds in a cohort of 4 wild-type mice (Supplemental Figure I). The mean blood half-life estimated by monoexponential decay was 192 ± 110 minutes. Therefore, all further imaging was done at 10 to 12 hours after injection of nanomaterials.

Macrophage-Targeted PET Imaging Detects Inflammation in AA

Nanoparticle uptake into the aortic wall was then quantified with PET, using CT images to guide placement of PET regions of interest. Significantly higher activities were found in the aneurysmal aortic sections (SUV are shown in Figure 2A and 2B). The target-to-blood background ratio in aneurysm was 2.4 ± 0.4, significantly higher than what was found in wild-type aorta (0.7 ± 0.1, P < 0.05).

To compare the PET signal of aneurysm with that of atherosclerotic plaque, which also contains macrophages that may be targeted by 18F-CLIO, we imaged a cohort of age-matched apoE−/− mice that were not implanted with AT-II delivering minipumps. Here, we found intermediate PET signal in aortic areas that are typically laden with atherosclerotic plaque. However, the uptake was significantly lower than in aneurysmal aorta (SUV aneurysm, 2.5 ± 0.5, versus atherosclerotic plaque, 1.2 ± 0.1, P < 0.05). Finally, these differences were also found when data were expressed as the target-to-blood ratio (1.3 ± 0.1, P < 0.05 versus aneurysm).

We also examined whether the aortic diameter measured by CT correlated with PET activity in the same vascular segment. Data pairs from aneurysms and nonaneurysmatic areas in apoE−/− mice were pooled and showed a weak correlation (Figure 2C, R = 0.45, P < 0.05).

Ex Vivo Scintillation Counting and Autoradiography Confirm In Vivo PET

To validate in vivo PET data, animals were euthanized immediately after imaging, and activity within excised aortas was assessed by scintillation counting and autoradiographic exposure. In comparison to wild-type aortas, the percentage of injected radioactive dose per gram tissue increased in aortas with aneurysms (aneurysm, 1.59 ± 0.18; nonaneurysmal aorta, 0.87 ± 0.15; wild-type aorta, 0.52 ± 0.05; P < 0.05; Figure 3A). Autoradiography showed that activity reached peak levels within the aneurysms, with some uptake also...
occurring in atherosclerotic plaques in the nonaneurysmatic vessel wall of apoE\(^{-/-}\) mice (Figure 3B). By focusing on the fluorescent reporter on the nanoparticle, we also imaged aortas using a fluorescence reflectance microscope (Figure 3C) and found that fluorescence colocalized with the \(^{18}\text{F}\) signal. This demonstrated that the nuclear reporter and fluorochrome were still conjugated to the nanoparticle.

Microscopic Nanoparticle Signal Colocalizes With Macrophages in the Aneurysm Wall

Next, we studied the uptake of nanoparticles using microscopy (Figure 4). By comparing fluorescence microscopy images to immunohistochemistry staining for the macrophage antigen MAC-3 in adjacent sections, we found colocalization of macrophages with nanoparticles in inflamed areas of the aneurysmatic wall. Macrophage presence, as well as nanoparticle uptake, was found in the intimal, medial, and adventitial layers of the aorta. Imaging of sections in the lower-wavelength fluoresceinthiocarbamyl channel confirmed that the observed signal was specific to nanoparticles and was not caused by autofluorescence (Figure 4), and it also demonstrated the disintegration of the tunica media, typically seen in aneurysms.

Cellular Signal Distribution by Flow Cytometry

We were also interested in quantitating uptake of nanoparticles by leukocyte populations (monocytes, macrophages, neutrophils, and lymphocytes). To do this, we began by digesting aneurysmatic aortas following injection of the probe. We then studied the fluorescence signal derived from VT680 attached to the nanoparticles in cell suspensions that were stained for specific surface antigens of leukocytes. Using this method, we found that the majority of nanoparticles were taken up by monocytes/macrophages. Therefore, \(\sim 90\%\) of the PET signal in aneurysms was caused by uptake of nanoparticles by these cells (Figure 5). Some, but much lower, uptake was also observed in neutrophils and lymphocytes (Figure 5). Administration of AT-II or mouse genotype did not change the uptake profile (Supplemental Figure II).

Macrophage PET in Nascent Aneurysms

Our overall goal was to develop an imaging strategy that could detect inflammatory aneurysms before they rupture. If
macrophage-targeted PET imaging is to be useful in the clinical setting, it would need to be able to detect inflammation in younger, evolving aneurysms. Therefore, we studied apoE$^{-/-}$ mice that had been exposed to AT-II administration for only 7 days. In these mice, we found that the PET signal was already significantly elevated (Figure 6A and 6B). The mean SUV of the aneurysms in these apoE$^{-/-}$ mice was $3.0 \pm 0.4$, compared with $0.82 \pm 0.05$ in wild-type and $2.46 \pm 0.48$ in the more mature aneurysms of apoE$^{-/-}$ mice that were imaged after 28 days of systemic AT-II exposure ($P<0.0001$ versus wild-type). These imaging findings correlated well with the number of monocytes/macrophages in the atherosclerotic wall, as quantified by flow cytometry (Figure 6C). Compared with wild-type, the monocyte/macrophage number in the aneurysmatic aortic arches of apoE$^{-/-}$ mice was increased 23-fold ($14.1 \times 10^4 \pm 5.5 \times 10^4$ versus $0.6 \times 10^4 \pm 0.4 \times 10^4$). The profile of myeloid cells in the vessel also changed profoundly. In wild-type mice, $80\%$ of myeloid cells in the vessel wall were resident macrophages. However, in the wall of aortas with aneurysms, inflammatory Ly6C<sup>high</sup> monocytes dominated this population (Figure 6C).

**PET Signal Is Decreased When Inflammation Is Blunted**

Next, the development of aneurysms and related PET signals were investigated in a group of mice that had undergone splenectomy at the time of AT-II minipump implantation. In a recent study, it was reported that the spleen contains a large reservoir of monocytes that significantly contribute to inflammation and that release of this reservoir is triggered by AT-II signaling. Because monocytes give rise to macrophages in tissue, we hypothesized that the splenic monocyte reservoir would contribute macrophages in our model of AA, and that the removal of the splenic monocyte reservoir would decrease the number of macrophages in the aortic wall, reflected by a change in the PET signal. As expected, our results did indeed confirm this hypothesis and demonstrated that splenectomized mice had decreased PET signals in the aortic wall. The SUV was reduced to $0.81 \pm 0.11$ ($P<0.05$ versus the signal from nonsplenectomized apoE$^{-/-}$ mice). Importantly, these mice also displayed attenuated development of aneurysms under CT (only 1 aneurysm evolved; see also Table). Lastly, using flow cytometry, the number of monocytes/macrophages in the aorta of splenectomized mice was assessed and found to have a 3.8-fold reduction in number.

**Predictive Value of PET Signal**

Finally, we were interested in determining whether macrophage-targeted PET-CT had predictive potential. To this end, PET-CT imaging was performed on day 7 after implantation of AT-II minipumps, and mice were followed up by weekly serial CT angiography while the AT-II treatment was continued up to 4 weeks later. Of a total of 8 mice that underwent initial PET-CT, 3 mice died before completion of the study because of aneurysm rupture. In these mice and in mice with aneurysms that increased over time, the PET signal was increased compared with aneurysms that did not increase in diameter on CT angiography follow-up (target-to-background ratio, $2.1 \pm 0.3$ versus $1.5\pm0.1$; $P<0.05$, Figure 6D).

**Discussion**

The abundant presence and central role of macrophages in aneurysm formation make them an interesting and clinically relevant imaging target. Experimental and clinical studies have shown that macrophages infiltrate the vessel wall, differentiate into macrophages, and secrete inflammatory cytokines. In our study, using flow cytometry, we found an 18.6-fold increase in the population of macrophages present in aneurysms as compared with the aortic wall of control mice. Importantly, monocytes/macrophages are a major source for metalloproteinases and elastase, enzymes that degrade the media and thus reduce the tensile strength of the vascular wall. These cells not only function as a major source of destabilizing enzymes but also are key instigators of further inflammation. Because aneurysm formation and progression is dependent on the strategic position of monocytes/macrophages, targeting these cells with imaging may offer early insight into the course of disease.

In this investigation, we used nanoparticles that are avidly internalized by phagocytic cells. The derivatization of these nanoparticles with $^{18}$F allowed us to use PET imaging for in vivo macrophage detection. PET is a clinically attractive modality on account of its quantitative capabilities and its unsurpassed sensitivity. Indeed, this approach has enabled us to reduce the nanoparticle dose to clinically used levels (4.5 mg of Fe/kg body weight, which is lower than the approved...
longer half-lives, such as copper-64 (64Cu) or zirconium-89 (89Zr), could be used to image at later time points. These findings suggest that the use of macrophage-targeted imaging could be a valuable tool for assessing disease activity in nascent AAs, supported by initial reports of 18F-Fluorodeoxyglucose-PET in patients with aortic disease.

In clinical management of AAs, doctors and patients are often faced with the important decision of whether to perform invasive repair or to manage the condition conservatively. The mortality rate associated with reparative surgery has been reported to be as high as 5.5%, but this is far exceeded by the mortality rate associated with aneurysm rupture. Current guidelines recommend endovascular repair or surgery if the aortic diameter exceeds 5.5 cm and the performance of anatomic imaging every 3 to 6 months for aneurysms above 4 cm. If growth is observed to exceed 1 cm per year in smaller aneurysms, surgical repair is also recommended. However, individual risk is also influenced by gender, age, smoking, and comorbidities, but its assessment is rarely easy because reliable data regarding how best to evaluate these variables are not readily available. This situation often leaves one with a difficult decision, and many patients are unnecessarily exposed to the risks of reparative surgery when their aneurysm might never have ruptured if left untreated. An imaging approach that targets a key component of the biology underlying aneurysm rupture may help to more accurately determine individual risk as well as population-based risk factors. It is possible that a numeric cut-off SUV, determined by PET imaging, could be used as a decisive indicator for invasive repair, because underlying macrophage activity would be weakening the aneurysm wall. However, prospective outcome studies are needed, first in other preclinical models of AA and eventually, after completion of toxicology studies, in patients. These trials will determine whether an increased macrophage PET signal, despite the limited spatial resolution of PET, can indeed predict aneurysm rupture.

Ultimately, we envision that imaging approaches, which target vascular biology, would be capable of identifying patients with rupture-prone aneurysms and who require surgical intervention. We also hope to accurately distinguish these patients early from patients with aneurysms who would be best served by watchful waiting strategies.

Acknowledgments
We gratefully acknowledge Dr Peter Libby for discussions, and the help of Rostic Gorbatov, Yoshiko Iwamoto, Aleksey Chudnovskiy, Brenne Sena, Dr Won Woo Lee, and Dr Nikolay Sergeyev.

Sources of Funding
This work was funded in parts by grants from the NIH Translational Program of Excellence in Nanotechnology Grants U01- HL080731/ HHSN268201000044C, R24-C A292782 (to R.W.), R01-HL095629 and R01-HL096576 (to M.N.), and R01-HL095612 (to F.K.S.); an American Heart Association Established Investigator Award (to R.E.G.); and Deutsche Herzstiftung e.V. (to F.L.).
Disclosures
None.

References
Detection of Macrophages in Aortic Aneurysms by Nanoparticle Positron Emission Tomography–Computed Tomography
Matthias Nahrendorf, Edmund Keliher, Brett Marinelli, Florian Leuschner, Clinton S. Robbins, Robert E. Gerszten, Mikael J. Pittet, Filip K. Swirski and Ralph Weissleder

Arterioscler Thromb Vasc Biol. 2011;31:750-757; originally published online January 20, 2011; doi: 10.1161/ATVBAHA.110.221499
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/4/750

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2011/01/20/ATVBAHA.110.221499.DC1
http://atvb.ahajournals.org/content/suppl/2011/12/20/ATVBAHA.110.221499.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Blood half life of $^{18}$F-CLIO was determined in a cohort of 4 wild type mice by serial bleeding. Figure shows blood activity corrected for decay and individual fits of a mono-exponential decay ($T_{1/2} = 192 \pm 14\text{ min}; R^2 = 0.97 - 0.99$).
Supplementary Figure II: Uptake of nanoparticles does not depend on AT-II treatment

Representative histogram shows comparison of probe uptake in digested aortas of untreated wild type mice without probe injection (red), untreated wild type injected with probe (blue), and AT-II treated apoE\(^{-/-}\) (green). No significant differences of nanoparticle-uptake were seen between AT-II treated and untreated animals.
### Supplementary Table I: Intra- and interobserver variability of microCT measurements

<table>
<thead>
<tr>
<th>group</th>
<th>intraobserver variability</th>
<th>interobserver variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>value (μm)</td>
<td>%</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;−/−&lt;/sup&gt; aneurysm (n = 10)</td>
<td>17 ± 12</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;−/−&lt;/sup&gt; no aneurysm (n = 13)</td>
<td>17 ± 10</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>wild type (n = 9)</td>
<td>33 ± 10</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;−/−&lt;/sup&gt; splenectomy (n = 9)</td>
<td>34 ± 11</td>
<td>2.9 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.
나노파티클을 이용한 PET-CT는 대동맥류의 대식세포를 발견할 수 있다

김용주 교수
고려대학교 구로병원 소화기내과

Summary

목적
대동맥류는 그 크기에 따라 파열예방을 위한 침습적 치료가 필요를 결정한다. 작자들은 동맥류의 세포의 기질을 밝혀내기 위한 혁신적인 대식세포 분석 방법으로는 본인형을 이용한 CT를 이용하여 방사선학적 동맥류의 분화를 목적으로 하였다. 본 연구는 대동맥류의 영상을 찾아내는 PET-CT용 fluorine-18(18F)가 표준화된 대식세포 표적 나노파티클의 개발과 검증을 목적으로 하였다.

방법 및 결과
대동맥류를 apo E 결손마우스에 안정성을 입혀하여 유도하여 외부의 혈관에, 단핵구/대식세포 표적 나노파티클을 이용한 PET-CT로 형광화하였다. 후후 조영증강 마이크로 CT로 발견된 대동맥류의 평균 직경은 1.85±0.08mm보다 얇으며, 정상 대동맥의 직경은 1.07±0.08mm(p<0.05)였다. PET 신호는 일반 마우스의 대동맥 (standard uptake value, 0.82±0.05)에 비해 동맥류 (2.46±0.48, p<0.05)에서 유의하게 높았다. 세균계수(scintillation counting).
Commentary

노령인구가 계속 증가하는 추세임을 감안하면, 노인 인구에서 유병률이 5%에 이르는 대동맥류 또한 절대 반도가 더 증가할 것임을 예측할 수 있다. 대동맥류는 직경이 큰 경우 과열 핵들이 50%까지도 보고되고 있고, 과열된 경우 치유가 몇 위험한 점이다. 나이, 수술 관련 치사율도 5.5%로 적지 않으므로 의심이 환자 모두 대동맥류에 대한 최적의 치료법을 결정하는 것은 어려운 문제이다. 현재의 치료 지침에서는 대동맥류의 직경이 5.5cm를 넘거나 1년에 1cm 이상 직경이 커지는 경우 치료적 지하(수술 또는 스타트 삽입)를 시행하고, 4cm이 넘는 경우에는 3~5개월마다 검사할 것을 권고하고 있다. 하지만 전반에 걸쳐 진단과 치료에 적절한 크기에서 파열이도 완전히 기존 이상의 직경이라도 안정적으로 유지되는 경우가 있어, 치료의 적절성을 놓치거나 혹은 필요로 하지 않은 경우에 노출되는 경우가 있다. 따라서, 만성적 재발성 고혈압에서 간과되는 세포의 질환과 연관, 그 동맥류 구조를 약화시키는 단백질분해효소의 분비와 같은 대동맥류의 확장이 관여하는 병리과정을 비침습적 영상으로 알아낼 수 있다면, 치료와 예후 판단에 활발 유용할 것이다. 이런 점에서 대동맥류/대식세포는 메타의학적 분자영상의 표지를 찾을 수 있다. 이에 PET을 통해 동맥류의 복합경화바로써 발견할 수 있음을 볼 때, 검증된 바 있으며, 단백질/대식세포는 동맥류의 질환에 elastase, metalloproteinase와 같은 간백분해효소를 분비함으로써, 대동맥류의 형성 및 악화에 결정적인 역할을 하는 것으로 알려져 있기 때문이다. 따라서, 비록 동료 실험일지라도 과학의/대식세포를 표현으로 한 나노파티를 PET-CT로 대동맥류를 기능적으로 조사한 본 연구는 몇 가지 전문에서 매우 의미있고 생각한다. 첫째, 안드로스신 파괴를 통한 동맥류의 영증을 매우 조기에(7일제) 발견할 수 있었고, 이는 동맥의 확장과 대형의 질변을 확인하였다. 즉, 대동맥류 발생의 진단법은 매우 조기에 발견할 수 있을 가능성을 제시하는 결과이다. 둘째, 같은 대동맥류에도 지속 직경이 커지거나 파열되는 경우는 직경이 계속 안정적인 경우에 비해, 적절조사 중 초기부터 내내 PET 신호가 높았다. 즉, 예후의 중요성에서 많은 대동맥류가 계속 커지거나 파열할 가능성이 높음을 강조함으로써, 지표적 개입 여부 및 시기가 좀 더 정확히 판단하는 데 도움이 될 것으로 생각한다. 셋째, 단백질의 저작권 비건을 제거함으로써 대식세포의 과정을 찾을 수 있다. 대동맥류의 대식세포의 조작학적 감소하는 것과 비례하여 PET 신호도 감소하였으며, CT상 대동맥류의 성장도 악화되었다. 즉, 치료에 대한 반응 평가에도 이용될 수 있음을 보여주는 결과이다. 골격적으로 사람에게 영양시킬 위해기도 하기 위해서는 같은 시간의 추가검증을 거쳐야겠지만, 단백질/대식세포를 표현적으로 한 나노파티를 PET-CT 검사법은 대동맥류의 예방 및 치료 및 예후 개선에 매우 유용한 검사법으로 기대된다.

REFERENCES

3. Chakof EL, Brewster DC, Dalman RL, Makaroun MS, Big EA.
