Increased In-Stent Stenosis in ApoE Knockout Mice
Insights from a Novel Mouse Model of Balloon Angioplasty and Stenting

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Objective—We aimed to develop and validate a model of angioplasty and stenting in mice that would allow investigation of the response to stent injury using genetically modified mouse strains.

Methods and Results—Aortic segments from either C57BL/6 wild-type or atherosclerotic ApoE-KO mice underwent balloon angioplasty alone or balloon angioplasty and stenting with a 1.25×2.5 mm stainless steel stent. Vessels were carotid-interposition grafted into genetically identical littermate recipients and harvested at 1, 7, 14, or 28 days. In wild-type mice, stenting generated an inflammatory vascular injury response between days 1 to 7, leading to the development of neointimal hyperplasia by day 14, which further increased in area by day 28 leading to the development of in-stent stenosis. Uninjured vessels and vessels injured by balloon angioplasty alone developed minimal neointimal hyperplasia. In stented ApoE-KO mice, neointimal area at 28 days was 30% greater compared with wild-type mice.

Conclusions—By reproducing important features of human stenting in atherosclerotic mice, we provide the potential to investigate molecular pathways and evaluate novel therapeutic targets for stent injury and restenosis. (Arterioscler Thromb Vasc Biol. 2007;27:833-840.)

Key Words: vascular biology ■ genetically modified mice ■ angioplasty ■ stents ■ restenosis

Percutaneous coronary intervention (PCI) is now the most commonly performed method of revascularization for coronary artery disease. The use of drug-eluting stents (DES), which target excessive smooth muscle cell proliferation and neointimal hyperplasia, has reduced clinical restenosis rates. However, current agents used for DES do not target inflammation or thrombosis, and may inhibit reendothelialization, potentially increasing late thrombosis risk within the stented vessel. Accordingly, there remains a pressing need to identify more rational, specific, and effective pharmacological targets in the vascular injury response to angioplasty and stenting.

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Various experimental models have been used in the preclinical development of stenting, including rabbit, rat, and pig models. Although these systems reproduce some aspects of the human vascular response to PCI, there are limitations in replicating the typical features of human atherosclerosis. In addition, constraints in genetic manipulation limit existing large-animal models to pharmacological and physical interventions, rather than dissection of relevant biological pathways by genomic approaches. In contrast, genetically-modified mice have provided important insights into the role of specific pathways in the pathophysiology of atherosclerosis and other vascular disease states. Mouse models also provide refinements in laboratory animal use, are cost-effective, and have a favorable generation time for cross-breeding experiments. Although mouse models would provide powerful approaches to investigate molecular mechanisms relevant to angioplasty and stenting, technical challenges have restricted such studies in mice to surrogate models of vascular injury, such as wire-denudation of endothelium or perivascular polyethylene cuff placement. These models have significant limitations in their ability to address potentially important aspects of balloon angioplasty and stenting.

Accordingly, we aimed to develop a mouse model of balloon angioplasty and stenting that would reproduce important mechanical aspects of clinical PCI, enable studies in genetically modified mice relevant to atherosclerosis, and allow studies of both local and systemic aspects of the vascular response to stent injury.

Developing a mouse model of arterial stenting poses unique challenges. The small animal size leads to technical difficulties in stent design, deployment techniques, and availability of suitable target vessels. Furthermore, the importance of stent geometry in the response to stent injury in humans...
requires careful stent bioengineering to replicate these properties in mice. Based on a series of preliminary designs and stent deployment studies, we developed a 2.5 mm-length stainless steel slotted tube stent to generate medial angulation and stretch, inter-strut distance, and strut surface area in mouse aorta analogous to human coronary stenting. We deployed the stent in thoracic aortas of donor mice using a clinical 1.25 mm-diameter balloon angioplasty catheter, after which the stented segment was explanted and grafted into the arterial circulation of littermate recipient mice. We evaluated the vascular injury responses to both balloon angioplasty and stenting in C57BL/6 (wild-type) mice, and then compared these with the responses in the genetically modified atherosclerotic apolipoprotein E–knockout (ApoE-KO) mouse model of atherosclerosis.10–12

**Methods**

**Animals**

Experimental C57BL/6 and ApoE-KO mice were originally sourced from Jackson Laboratories. ApoE-KO mice were backcrossed more than 9 generations into the C57BL/6 strain. Eighteen- to 22-week-old male and female mice were maintained in temperature-controlled (20°C to 22°C) cages with 12-hour light–dark cycle, and received free access to sterilized water and standard rodent chow (Rodent diet BK002P, B&K Ltd). To determine the time course of the vascular injury response to stenting, stented vessels of C57BL/6 mice were harvested 1 day, 7 days, 14 days, or 28 days postoperatively (n = 6 to 9). To investigate the biological utility of this model, the response to stent injury was compared in C57BL/6 mice and ApoE-KO mice (in a C57BL/6 strain background) using 3 experimental groups (n = 9 per group): uninjured aorta alone, aorta after balloon angioplasty, and aorta after stenting.

**Arterial Stent-Graft Procedure**

All animal procedures were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. All operative procedures involved grafting a thoracic aortic segment from a female donor mouse to the carotid artery of a male littermate recipient, of identical genotype. One week before operation, mice received aspirin 10 mg/kg/d in drinking water, and this was continued throughout the experimental period. Mice were anesthetized using a combination of subcutaneous Hypnorm (25 mg/kg, Bayer) and Hypnoval (25 mg/kg, Roche).

In the donor (female) mouse, the anterior thoracic cage was retracted, and the stented segment is removed and vigorous pulsations are seen within the stent graft. B, OCT imaging of stented vessel and 3D reconstruction demonstrating morphometric changes from carotid artery through to stented aortic graft. Upper panels, Ex vivo OCT image files of a paraformaldehyde fixed stented vessel harvested 14 days postoperatively. Middle panels, The image files were segmented by computerized image analysis into carotid-cuff-aorta, stented media, stent struts, stented intima, aortic media, aortic intima, and carotid artery. Lower panel, The segmented images were stacked to create a 3D reconstruction of the stented vessel to show morphometric changes from carotid artery through to the unstented and stented aorta (n = 1 vessel).

**Results**

**A** Angioplasty and stenting in wild-type and ApoE-KO mouse aorta. Operative procedure: (1) In the donor mouse the stent is crimped onto a 1.25 mm balloon angioplasty catheter and the catheter advanced retrograde up the thoracic aorta. (2) The balloon is inflated to 8 atmospheres pressure for 30 seconds. (3) The balloon catheter is retracted, and the stented segment is removed by electrocautery of the intercostal branches. (4) In the recipient mouse the carotid artery is ligated at its midpoint, divided between ties, and cuffs placed over the ends. The vessel and cuff handle are fixed using microhemostatic clamps, the free ends of the artery everted over the cuffs and secured onto the cuff using 8-0 silk sutures. The harvested stented vessel is grafted by slewing the ends over the artery cuffs and securing them with 8-0 silk sutures. The hemostatic clamps are removed and vigorous pulsations are seen within the stent graft. B, OCT imaging of stented vessel and 3D reconstruction demonstrating morphometric changes from carotid artery through to stented aortic graft. Upper panels, Ex vivo OCT image files of a paraformaldehyde fixed stented vessel harvested 14 days postoperatively. Middle panels, The image files were segmented by computerized image analysis into carotid-cuff-aorta, stented media, stent struts, stented intima, aortic media, aortic intima, and carotid artery. Lower panel, The segmented images were stacked to create a 3D reconstruction of the stented vessel to show morphometric changes from carotid artery through to the unstented and stented aorta (n = 1 vessel).
heparinized PBS, maintaining an ischemic time of 15 minutes for all vessels (whether un-injured aorta, balloon-injured aorta, or stented aorta).

In the recipient (male) mouse, the right common carotid artery was isolated and mobilized from the thoracic inlet to its bifurcation, ligated, and divided between ties at its midpoint. Polyethylene cuffs (0.65 mm diameter, Portex Ltd) were placed over the ends and anchored by microhemostatic clamps (Aesculap). Sutures at the ends of the artery were removed, and the artery everted over the cuffs and secured with 8-0 silk sutures. The stented aorta from the donor mouse was interposition grafted by sleeving the ends of the aorta over the 2 ends of the carotid artery and ligating them with 8-0 silk sutures. Vigorous pulsation in the conduit vessel confirmed successful engraftment. The skin was closed using a continuous 5/0 suture. The median total procedure time was approximately 60 minutes. The operative procedure is summarized in Figure 1A.

**Tissue Preparation, Histology, and Lesion Quantification**

Vessels were harvested 28 days after surgery and perfusion fixed in situ with 4% phosphate-buffered parafomaldehyde. Vessels were excised, fixed in parafomaldehyde overnight, and subsequently embedded into gel methacrylate resin (Technovit 8100, TAAB Laboratories) for histomorphometry, or methyl methacrylate resin (Technovit 9100, TAAB Laboratories) for immunohistochemistry, according to the manufacturer’s instructions. Four transverse sections were cut through each stent using an Isomet 5000 diamond-coated rotary saw and polished (to 5 to 10 μm) using a Metaserv 2000 Polisher (Buehler).

For histomorphometric analysis, sections were stained with hematoxylin and eosin. Lesions were quantified for total vessel area (area inside the external elastic lamina), neointimal area (area inside the internal elastic lamina, minus stent struts, minus lumen), lumen, and stent expansion (area inside a polygon connecting the midpoint of each stent strut). In some vessels, measurements of neointimal thickness were performed by measuring the perpendicular distance from lumen to internal elastic lamina at the mid point between stent struts on each section (4 to 6 measurements per section; 4 sections per vessel to generate n=1).

**Immunohistochemistry**

Immunohistochemistry was performed by dissolving the methyl methacrylate resin according to the manufacturer’s instructions. Vessel sections were stained for platelets (adsorbed rabbit anti-mouse thrombocyte, 1:20 000, Clone 1A4, Inter-Cell Technologies), macrophage/monocytes (monoclonal rat anti-mouse macrophage/monocytes MAC-3, 1:50, Clone M3/84, Pharmingen), proliferating cells (monoclonal rat anti-mouse Ki67, Clone TEC-1), and SMCs (α-smooth muscle actin, 1:50, Clone 1A4, Sigma).

Image analysis was performed using Image Pro Plus software (Media Cybernetics). Vessel cell density and surface-adherent leukocytes were measured at x200 light magnification by manual cell counting. For quantification of immunohistochemistry (platelets, macrophages, cell proliferation, SMCs, and collagen), the total stained area was determined by computerized threshold segmentation of colors. Using this technique, stained area is isolated according to a standardized color threshold set based on staining in appropriate positive controls. Positive staining is presented as a percentage of the total neointima. Four sections spanning the entire length of the vessel were combined as n=1 for all quantifications. All histological analysis was performed in a blinded fashion.

**Injury and Inflammation Scores**

Arterial injury and inflammation after stenting was determined by the anatomic structures penetrated by each stent strut using two previously described methods. For the Schwartz13 injury score a numeric value was assigned to each stent strut in every section as follows: 0=no injury, 1=perforation in internal elastic lamina, 2=medial perforation, 3=perforation in external elastic lamina. For inflammation score14 a numeric value was assigned to each stent strut as follows: 0=no inflammatory cells surrounding the strut; 1=light, noncircumferential lymphohistocytic infiltrate surrounding the strut; 2=localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially; and 3=circumferential dense lymphohistiocytic cell infiltration of the strut.

**Optical Coherence Tomography**

Optical coherence tomography (OCT) is an imaging technique able to capture high resolution cross-sectional images in real time. OCT images were acquired using an Imagewire (TM) (Lightlab Imaging Inc) at 8.2 frames per second with a center wavelength of 1310 nm and a bandwidth of 70 nm, providing an axial resolution of approximately 10 μm and a transverse resolution of approximately 25 μm. Imaging of stented vessels was performed ex vivo. Before imaging, the stented vessel was placed into a petri dish and immersed in PBS. Under the light microscope, the stented vessel was cannulated and the imaging catheter carefully advanced beyond the stented segment. The vessel was then imaged by automated pullback at 0.5 mm/second. For 3D reconstruction of the whole vascular segment, individual cross-sectional images from a stented vessel harvested 14 days postoperatively were converted to 2D TIFF files, and segmented into vascular regions of interest: (carotid-cuff-aorta, stented media, stent struts, stented intima, aortic media, aortic intima,

![Figure 2. Time course of vascular response to stent injury. Histomorphometric analysis of stented vessels at 1, 7, 14, and 28 days after implantation in C57BL/6 (wild-type) mice. Upper row, One day after the stenting procedure, balloon deployment provided symmetrical stent expansion and uniform stretching of the vessel media around the stent struts, with adherent leukocytes on the luminal surface of the neointima. Second row, After 7 days, organizing thrombus with inflammatory cell infiltrate was observed. Third row, After 14 days there was a modest organized neointima. Fourth row, The neointima increased in area progressively over the 28-day time period. (n=6 to 9 mice per group).](http://artvah.angiojournals.org/ by guest on November 9, 2017)
Implantation in wild-type C57BL/6 mice. To determine the biological response in mouse vessels to stenting, we performed a systematic time-course evaluation of histomorphometry at 1, 7, 14, and 28 days post-implantation in wild-type C57BL/6 mice.

Histomorphometry
One day after the stenting procedure, balloon deployment provided symmetrical stent expansion and uniform stretching of the vessel media around the stent struts (Figure 2). Stent expansion increased the total vessel wall area by approximately 50% compared with reference aorta. Neointimal hyperplasia increased progressively over the 28 day time period as evidenced by measures of both neointimal thickness and neointimal area (P<0.0001) (Figure 2 and Table 1). Neointimal thickness also increased during the 28 day period, following a similar pattern to the measurements of neointimal area (Table 1). Because total medial area remained consistent through the 4 time points, a progressive increase in the neointima to media ratio over time was also observed (P<0.0001) (Table 1). Consistent with these findings, luminal area significantly decreased between day 1 and day 28 (P<0.02), equating to in-stent stenosis of 1.3±0.2, 10.8±1.5, 15.6±1.4, and 32.9±2.0% on days 1, 3, 7, and 28 respectively (P<0.0001) (Table 1). Importantly, stent expansion was not different between the various time points, indicating that in-stent stenosis was the result of neointimal hyperplasia (Table 1). Furthermore, injury scores were not different among the time intervals (Table 1).

Histological analyses of stented vessels harvested at the 4 time points are shown in Figure 3 and Table 2. One day after stent implantation, the luminal surface of the stented vessel was covered with a monolayer of leukocytes (Figure 3A and Table 2), and a thin adherent monolayer which stained positively with a monoclonal antibody against mouse platelets (Figure 3C and Table 2). Staining for proliferating cells identified small clusters of cells within the media. Monocyte/macrophage staining was negative. By 7 days, mural thrombi surrounded the stent struts (Figure 2 and Table 2). Leukocytes (Figure 3B) and platelets (Figure 3D) were present on the stented surface and lined the organizing thrombus/neointima, which also contained some macrophages (Figure 3E) and a high proportion of proliferating cells (Figure 3G and Table 2). At 14 days, the localized thrombus was no longer present, and

### Table 1. Histomorphometric Characteristics of Neointimal Response to Stenting in C57BL/6 Mice (time course)

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of mice per group</th>
<th>Total vessel area, mm²</th>
<th>Neointimal Thickness, μm</th>
<th>Neointimal area, mm²</th>
<th>Medial Area, mm²</th>
<th>N/M ratio</th>
<th>% Stenosis</th>
<th>Inflammation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>6</td>
<td>1.01±0.06</td>
<td>5.6±1.8</td>
<td>0.01±0.00</td>
<td>0.12±0.02</td>
<td>0.09±0.02</td>
<td>1.3±0.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Day 7</td>
<td>8</td>
<td>0.95±0.04</td>
<td>31±8.5*</td>
<td>0.07±0.01</td>
<td>0.13±0.01</td>
<td>0.58±0.10</td>
<td>10.8±1.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Day 14</td>
<td>8</td>
<td>0.97±0.02</td>
<td>48±10</td>
<td>0.10±0.01</td>
<td>0.11±0.01</td>
<td>0.93±0.10</td>
<td>15.6±1.4</td>
<td>2.41*†</td>
</tr>
<tr>
<td>Day 28</td>
<td>9</td>
<td>1.08±0.03</td>
<td>110±4.1†</td>
<td>0.26±0.01†</td>
<td>0.18±0.01</td>
<td>1.52±0.11†</td>
<td>32.9±2.0†</td>
<td>1.00†</td>
</tr>
</tbody>
</table>

*P<0.0001 across all time points (ANOVA); †P<0.01 across all time points (ANOVA); *P<0.05 compared with previous time point (Bonferroni Multiple Comparison Test).

Histological analyses of stented vessels harvested at the 4 time points are shown in Figure 3 and Table 2. One day after stent implantation, the luminal surface of the stented vessel was covered with a monolayer of leukocytes (Figure 3A and Table 2), and a thin adherent monolayer which stained positively with a monoclonal antibody against mouse platelets (Figure 3C and Table 2). Staining for proliferating cells identified small clusters of cells within the media. Monocyte/macrophage staining was negative. By 7 days, mural thrombi surrounded the stent struts (Figure 2 and Table 2). Leukocytes (Figure 3B) and platelets (Figure 3D) were present on the stented surface and lined the organizing thrombus/neointima, which also contained some macrophages (Figure 3E) and a high proportion of proliferating cells (Figure 3G and Table 2). At 14 days, the localized thrombus was no longer present, and
A defined neointima (Figure 2), which stained positively for SMC \(\alpha\)-actin (Figure 3I), was seen. Masson trichrome staining revealed that a proportion of the neointima was composed of collagen and elastin (Figure 3K and Table 2). Monocyte/macrophage staining increased and was predominant in the peri-strut regions (Figure 3F and Table 2). Furthermore, the total number of proliferating cells at 14 days was similar to 7 days, yet as a proportion of the neointima markedly decreased (Table 2). Platelet staining was absent. Twenty-eight days after the procedure a marked neointima was present, leading to moderate in-stent stenosis (Figure 2). The proportion of the neointima which stained positive with \(\alpha\)-actin increased (Figure 3J and Table 2), as did the collagen and elastin content of the neointima (Figure 3L and Table 2). Monocyte/macrophage and cell proliferation staining both decreased compared with 7 and 14 days.

Comparison of Balloon Angioplasty and Stenting in C57BL/6 and ApoE-KO Mice

We next sought to determine how the vascular response to stent injury differed in genetically modified ApoE-KO mice compared with C57BL/6 mice, and aimed to establish the statistical power of this model for comparing the neointimal hyperplasia response at 28 days between mouse strains.

Cholesterol levels in ApoE-KO mice were significantly higher compared with wild-type mice (431 ± 1100 mg/dL versus 20 ± 50 mg/dL).

Table 2. Cellular Composition of Neointimal Response to Stenting in C57BL/6 Mice (time course)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice per group</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Vessel cell density (\times 100/\text{mm}^2)</td>
<td>3.90 ± 0.37</td>
<td>26.1 ± 4.54*</td>
<td>41.7 ± 2.36*</td>
<td>55.24 ± 9.67†</td>
</tr>
<tr>
<td>Surface-adherent leucocytes, cells per (\times 200) high power field</td>
<td>14.4 ± 1.74</td>
<td>32.8 ± 5.27*</td>
<td>7.60 ± 2.37*</td>
<td>0.40 ± 0.25†</td>
</tr>
<tr>
<td>Platelets, % lumen circumference</td>
<td>100 ± 0.00</td>
<td>100 ± 0.00</td>
<td>0*</td>
<td>0†</td>
</tr>
<tr>
<td>Macrophage, % neointima</td>
<td>0</td>
<td>22.6 ± 6.32*</td>
<td>39.3 ± 8.93*</td>
<td>8.8 ± 1.2*†</td>
</tr>
<tr>
<td>Cell proliferation, % neointima</td>
<td>0</td>
<td>27.4 ± 8.68*</td>
<td>8.89 ± 3.84*</td>
<td>2.13 ± 0.84†</td>
</tr>
<tr>
<td>SMC, % neointima</td>
<td>0</td>
<td>14.2 ± 4.34*</td>
<td>44.2 ± 8.33*</td>
<td>73.2 ± 9.43†</td>
</tr>
<tr>
<td>Collagen, % neointima</td>
<td>0</td>
<td>0</td>
<td>3.34 ± 2.12*</td>
<td>12.3 ± 4.62†</td>
</tr>
</tbody>
</table>

†P < 0.01 across all time points (ANOVA); *P < 0.05 compared with previous time point (Bonferroni Multiple Comparison Test).
51 ± 4 mg/dL; *P < 0.001) (supplemental Table I). To confirm that ApoE-KO mice developed the expected vascular phenotype of atherosclerosis, we evaluated histological sections of aortas using the Masson-Goldner stain. Aortas from 22-week-old ApoE-KO mice indeed demonstrated lipid deposition and typical atherosclerotic lesions, which were not observed in wild-type mice of the same age (supplemental Figure II).

**Histomorphometry**

Twenty-eight days after operation, uninjured arterial grafts in both wild-type mice and ApoE-KO mice had appearances similar to control noninstrumented mouse aorta (Figure 4A), indicating that the surgical procedure itself did not induce a significant vascular injury response. Despite acute stretching of the vessel wall during inflation, balloon angioplasty did not increase total vessel wall area at 28 days in either genotype compared with uninjured controls. However, balloon angioplasty resulted in neointimal hyperplasia that was 3-fold (*P < 0.01) greater in ApoE-KO mice compared with wild-type mice (Figure 4A). In ApoE-KO mice, neointimal hyperplasia caused significant luminal narrowing compared with both wild-type balloon-injured and uninjured control vessels (Figure 4A and 4B).

Stenting significantly increased neointimal hyperplasia compared with balloon angioplasty alone (Figure 4A and 4B). In atherosclerotic ApoE-KO mice neointimal hyperplasia in stented vessels was further increased by 30%, compared with wild-type animals. Stenting also increased total vessel area by >50% compared with balloon angioplasty (equivalent to a vessel diameter expansion ratio of 1.2:1), resulting in an overall increase in lumen area in stented vessels. However, lumen area was significantly reduced in ApoE-KO mice compared with wild-type mice (Figure 4A and 4B).

Based on the mean and standard deviation of the neointimal area measurements at 28 days in ApoE-KO mice, a power calculation (using 2-tailed statistical testing) indicated that this model would be able to distinguish a relative neointimal area difference of 20% using 8 mice per group, assuming a conventional alpha value of 0.05 and a statistical power of 80%.

**Stent Injury and Inflammation Scores**

The extent of vascular injury13 and inflammation14 caused by stent deployment is an important determinant of neointimal hyperplasia. Because our stent was deployed using an identical angioplasty balloon inflated to a consistent pressure in all animals, there were no differences between wild-type and ApoE-KO stented vessels in either stent expansion (0.80 ± 0.02 versus 0.86 ± 0.08 mm², *P = 0.47) or mean Schwartz vessel injury score13 (1.3 ± 0.3 versus 1.4 ± 0.4; *P = 0.54). Despite this, inflammation scores14 were significantly higher in ApoE-KO stented vessels (2.1 ± 0.1 versus 1.0 ± 0.2; *P < 0.001) compared with wild-type vessels.

**Immunohistochemistry**

Immunohistochemical analysis of the cellular composition of the neointima of stented vessels revealed smooth muscle cells
and macrophages, identified by α-actin and MAC-3 immunostaining, respectively. Importantly, we observed a significant increase in both the absolute and relative content of MAC-3-positive macrophages within the neointima of stented ApoE-KO vessels compared with stented wild-type vessels (supplemental Figure III). Absolute levels of α-actin-positive cells were higher in stented ApoE-KO vessels in keeping with increased neointimal hyperplasia. However, there was no difference in the proportion of smooth muscle cells to vessel area between groups.

**Discussion**

In this report we describe the first model of balloon angioplasty and stenting in mice. The procedure can be undertaken efficiently by a single operator, with high procedural success rates. The morphological vessel changes after stenting in mice appear highly reproducible and are similar both to previous animal models and to human vascular remodeling after PCI. Increased intimal hyperplasia after stenting in the atherosclerotic ApoE-KO mouse provides proof-of-principle that genetically modified mice will allow more detailed investigation of the molecular mechanisms underlying neointimal hyperplasia and in-stent restenosis.

The ideal model of in-stent stenosis should use animals that are inexpensive, readily available, convenient to breed, and that develop lesions over relatively short time periods. We have developed and validated a model which fulfills these criteria but also provides the advantage of investigating the role of key genes through transgenic and knockout technology. Importantly, the time course of neointimal hyperplasia in the present study is similar to that found in rat, rabbit, and pig models. In the present study, 1 day after stenting the luminal surface showed a predominance of surface-adherent leukocytes and platelets. By 7 days highly proliferative granulation tissue containing organizing thrombus, inflammatory cells, and SMCs extended between the stent struts. At 14 days there was a mature neointima containing collagen, elastin, and SMCs, with a predominance of macrophages around the stent struts. Finally, by 28 days cellular proliferation was markedly reduced, and a mature neointima predominantly comprising SMCs and connective tissue with some macrophages resulted in moderate in-stent stenosis.

In clinical practice, deployment of a stent reduces restenosis compared with balloon angioplasty alone, and endovascular stenting is now the most commonly performed technique for coronary revascularization. Stent deployment creates a persistent scaffold in the vessel wall, leading to greater acute gain in lumen diameter, so that subsequent neointimal hyperplasia less often results in clinically important restenosis. However, the greater vessel stretch and injury generated by stenting may paradoxically result in more vigorous neointimal hyperplasia. In our model stented animals developed reproducible in-stent stenosis, with significantly greater neointimal area compared with balloon angioplasty. Despite increased neointima in the stented animals, lumen area was maintained. In ApoE-KO vessels neointimal area was 30% greater compared with wild-type. Furthermore, balloon angioplasty in ApoE-KO mice resulted in a 60% reduction in lumen area analogous to the constrictive remodeling and restenosis seen following balloon angioplasty in diseased human coronary arteries.

Inflammation may play a pivotal role linking vascular injury to in-stent restenosis, because increased vascular monocyte content is associated with increased neointimal hyperplasia in both animal models and in humans. In mouse models, targeted deletion of the ApoE gene leads to spontaneous atherosclerosis and increased vascular inflammation. In our model, despite no differences in stent expansion or injury score, inflammation and monocyte/macrophage concentration was significantly higher in ApoE-KO stented vessels compared with wild-type. These results indicate that differences in neointimal hyperplasia and inflammation after stenting were related to genotype rather than mechanical factors. These findings, together with the similarities in cellular composition of the neointima in mouse and human, further validates the clinical relevance of balloon angioplasty and stenting in mice.

In our model, the stent is deployed in the aorta of a donor mouse then grafted into a recipient. This strategy is technically more straightforward than attempting to deploy a stent in situ during recovery anesthesia. Furthermore, grafting a stented vessel from a mouse of one genotype into a mouse of another genotype provides a valuable approach to investigate local versus systemic aspects of the vascular response to stent injury independently, using appropriate crossover experimental designs. This would allow investigation, for example, of the role of endothelial or smooth muscle cell repopulation in the development of neointimal hyperplasia following stenting.

Our findings indicate that hypercholesterolemia, and increased vascular inflammation in the ApoE-KO mouse is associated with increased in-stent stenosis. However, our model is not limited to studies of atherosclerosis, because we observed substantial neointimal hyperplasia after stenting even in wild-type C57BL/6 mice. This finding has important implications for the investigation of other biological mechanisms relevant to restenosis such as thrombus formation, endothelial cell function, and smooth muscle cell proliferation. Furthermore, the conventional stainless steel stent platform used in this model is similar to that used in clinical stents, and is amenable to surface-coating and drug-elution technologies, which would permit preclinical screening of potential antirestenotic stent-based coatings or drugs.

This model has some limitations. The procedure requires an advanced level of microsurgical skill. In our experience, 16% of stented vessels thrombosed despite aspirin therapy. The use of additional antiplatelet agents, such as clopidogrel, may further decrease the thrombosis rate. In terms of the response to injury, because of vessel size considerations, stents are deployed in the mouse aorta, which is a relatively elastic artery, and may respond differently to stenting compared with muscular coronary arteries. Furthermore, it is conceivable that the grafting procedure itself may contribute to the vascular injury response, for example by effects on the adventitia or vasa vasorum. We attempted to control for this possibility by grafting uninjured aortic segments, which did not develop histological changes compared with nongrafted
aorta. Importantly, although we performed stenting in hypercholesterolemic atherosclerotic mice, we did not attempt to stent directly onto existing plaque. Rather, we investigated the biological response to stenting in a mouse known to have hypercholesterolemia, increased vascular inflammation, and high levels of oxidative stress. Future studies aimed at investigating the response to stenting plaque directly may provide important insight into the response to stenting in humans. Finally, additional studies are needed to validate the findings from this model with respect to established preclinical models (reviewed in 4) and results from clinical trials.

In conclusion, we have established a new model of balloon angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of stent angioplasty and stenting in mice which reproduces important features of human PCI and can address mechanisms of 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Disclosures

None.

References

Increased In-Stent Stenosis in ApoE Knockout Mice: Insights from a Novel Mouse Model of Balloon Angioplasty and Stenting
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**Supplementary Table I:** Serum lipid profiles and body weights of mice undergoing operation

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<tr>
<th></th>
<th>Wild Type</th>
<th>ApoE-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>51±4</td>
<td>431±23*</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>17±8</td>
<td>142±23*</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>6.9±2</td>
<td>20±5*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>72±22</td>
<td>1488±33*</td>
</tr>
<tr>
<td>Body Weights (grams)</td>
<td>22.4±1.4</td>
<td>25.3±1.4*</td>
</tr>
</tbody>
</table>

Serum lipid values, measured as described in the methods section, are expressed in mg/dl, and body weights in grams (n=9 per group). ApoE-KO mice had significantly higher Total, HDL, LDL Cholesterols, triglycerides and body weights. * denotes P<0.05 (Mann-Whitney U test)
Supplementary Figure I  Engineering of stent suitable for deployment in mouse aorta.
A, The stent consists of 1 linear unit of 4 radial cells. The stent pattern is cut by YAG laser from 0.635 mm diameter 316 stainless steel hypotube. The overall length is 2.5 mm, and the strut dimensions are 70 mm wide after polishing. B, Pattern of stent strut geometry during the cutting process. C, Stent strut geometry after expansion to an internal diameter of 1.25 mm.
Supplementary Figure II  
ApoE-KO mice develop atherosclerosis. Excised aortas of age matched wild type and ApoE-KO mice. Upper and middle panels show representative images of Masson-Goldner stained low-power (100x magnification) and high power (200x magnification) cross sections of the thoracic aorta. In the wild type aorta normal vessel architecture is seen, whilst atherosclerotic lesions with characteristic fibrous plaques are seen in ApoE-KO mice. Lower panels are representative images of oil red O staining for lipid deposition in descending aortas, showing extensive lipid deposition in the aortic wall of ApoE-KO mice but no lipid deposition in the aorta of wild type mice.
Supplementary Figure III  Immunohistochemistry of stented sections. A, α-actin antibody was used to detect smooth muscle cell area (mm²), and MAC-3 antibody was used to detect total macrophage content (mm²) in the neointima of methyl methacrylate embedded stented vessels. The upper panels show representative images of α-actin staining, and lower panels MAC-3 staining (black arrows) using Vector Red substrate reagent (indicated) 28 days post surgery. Magnification 200x. B, Quantification of α-actin and MAC-3 staining expressed as positive (+ve) staining as a proportion of the neointima. * Denotes significant difference between wild type and ApoE-KO mice, *P< 0.05. (n = 9 mice per group.)