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PRECLINICAL RESEARCH

Apolipoprotein A-I Reduces In-Stent Restenosis and Platelet Activation and Alters Neointimal Cellular Phenotype

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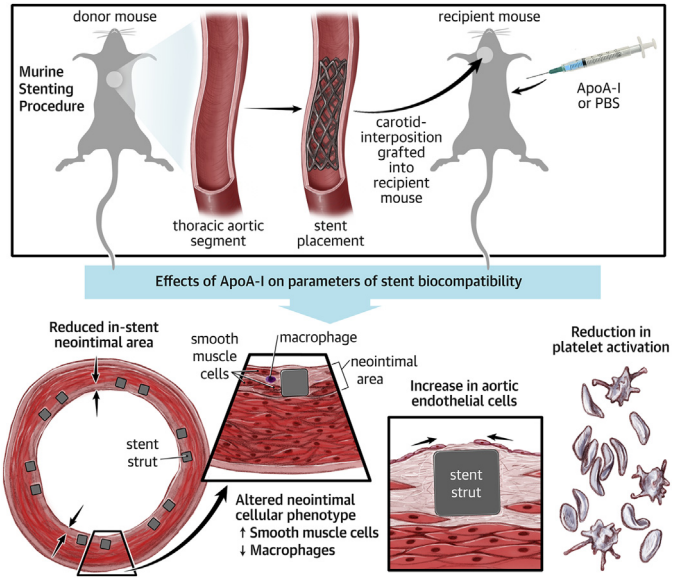
VISUAL ABSTRACT



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HIGHLIGHTS

- **This study shows that apoA-I may be an alternative strategy to improve the biocompatibility of stents.**
- **Systemic infusions of apoA-I reduce in-stent neointimal hyperplasia in a murine model of stenting.**
- **The cellular phenotype of the neointima post-stenting is altered by apoA-I infusions such that the smooth muscle cell phenotype is preserved, and there are fewer macrophages.**
- **There was an increase in endothelial cell content of the arteries post-stenting in the mice infused with apoA-I, indicating an enhancement of endothelialization.**
- **Systemic infusions of apoA-I inhibit platelet activation.**

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SUMMARY

Even the most advanced drug-eluting stents evoke unresolved issues, including chronic inflammation, late thrombosis, and neoatherosclerosis. This highlights the need for novel strategies that improve stent biocompatibility. Our studies show that apolipoprotein A-I (apoA-I) reduces in-stent restenosis and platelet activation, and enhances endothelialization. These findings have therapeutic implications for improving stent biocompatibility. (J Am Coll Cardiol Basic Trans Science 2018;3:200–9)

ABBREVIATIONS AND ACRONYMS

ABCA1 = ATP-binding cassette transporter A1

apoA-I = apolipoprotein A-I

***apoE*$^{-/-}$** = apolipoprotein E deficient

CAD = coronary artery disease

DES = drug-eluting stent(s)

HDL = high-density lipoprotein

PBS = phosphate-buffered saline

PCI = percutaneous coronary intervention

PPAR = peroxisome proliferator-activated receptor

rHDL = reconstituted high-density lipoprotein

SMC = smooth muscle cell

Revascularization due to coronary artery disease (CAD) is commonly achieved by percutaneous coronary intervention (PCI). The minimally invasive approach and immediate clinical benefits make it an attractive revascularization strategy. Refinement in interventional techniques, major improvements in stent design (particularly the advent of drug-eluting stents [DES]), and adjunctive pharmacotherapy with dual-antiplatelet regimens have resulted in marked reduction in the overall rates of stent failure over the last 2 decades. Even with the advancements made in the latest generation of DES, unresolved biological problems persist, including delayed re-endothelialization and neoatherosclerosis, which lead to late expansion of the neointima and late stent thrombosis (1). This points to a need for novel strategies beyond what is currently available to specifically address these pathobiological processes that underpin the residual risk for adverse clinical events (1).

SEE PAGE 210

Denudation of endothelial cells at the site of stent deployment induces a cascade of proinflammatory events allowing the infiltration of monocytes (2). Monocytes release potent cytokines and chemokines promoting medial smooth muscle cell (SMC) migration and proliferation, expanding the neointima. SMC phenotype can change during neointimal development, which is accompanied by an increase in proliferation and a loss in SMC markers including SMC α-actin (3). Atherogenic conditions and cholesterol loading also decrease SMC α-actin expression, with a concurrent increase in classic macrophage markers (4). This suggests that cellular cholesterol content plays an important role in determining SMC phenotype and may cause changes in the balance of SMCs to macrophage-like cells in the neointima (5). This may influence neointimal hyperplasia by creating a proinflammatory environment.

Various iterations in stent design, including DES with biodegradable polymer, polymer-free DES, and bioresorbable vascular scaffolds, have been developed to improve stent biocompatibility (6). Nevertheless, an agent that simultaneously reduces neointimal hyperplasia, promotes healing and re-endothelialization, and has low thrombogenicity would have significant advantages. There is immense therapeutic interest in high-density lipoprotein (HDL) and its main protein constituent, apolipoprotein A-I (apoA-I), due to their potent vasculoprotective properties. Clinical studies show that in patients undergoing PCI, low HDL levels (<0.91 mmol/l) are associated with double the mortality rate of patients with higher HDL levels (1.24 to 3.1 mmol/l), as well as a reduced frequency of repeat interventions due to neointimal thickening (7,8). ApoA-I infusions reduce neointimal formation following carotid artery wire injury (9) and vein grafting (10) in mice and enhance re-endothelialization at sites of endothelial injury (11). Furthermore, a single infusion of reconstituted HDL (rHDL) reduces platelet aggregation in diabetic patients (12). Taken together, these findings suggest a role for HDL in the improvement of key vascular biological responses post-stent deployment.

This study directly assessed the effect of systemic apoA-I infusions on stent biocompatibility in a murine model of stent deployment. We found apoA-I reduced in-stent restenosis and platelet activation and increased endothelialization. ApoA-I preserved the SMC content of the neointima, decreased aortic macrophages, and reduced activated circulating monocyte levels. Mechanistically, these differences in neointimal cellular phenotype may be via increased neointimal SMC cholesterol efflux and decreased recruitment of circulating monocytes to the neointima. These findings have implications for therapeutic modulation of neointimal hyperplasia,

thrombosis, and re-endothelialization following stent deployment.

METHODS

PREPARATION OF apoA-I. The HDL fraction (1.063 to 1.21 g/ml) was isolated from pooled samples of autologously donated plasma samples (minimum 5 donors) obtained from the Red Cross (supply agreement 12-08NSW-06) using ultracentrifugation and then dialyzed against phosphate-buffered saline (PBS). For the isolation of apoA-I, HDL was delipidated and subjected to anion-exchange chromatography using a fast protein liquid chromatography system as described previously (13). ApoA-I was reconstituted at a concentration of 10 mg/ml and dialyzed 5 × with 1 l of Tris-buffered saline and 3 × with 1 l of PBS in preparation for injection. Protein concentration was determined by bicinchoninic acid assay. Endotoxin levels were checked to confirm they were below the acceptable range of 1 endotoxin units/ml.

CAROTID INTERPOSITION GRAFT MODEL. The surgical procedure was performed as previously described (14). In chow-fed, 10- to 14-week-old male *apoE*$^{-/-}$ donor mice, for balloon angioplasty, a 1.25 × 0.6-mm balloon angioplasty catheter (Medtronic, Milwaukee, Wisconsin) was inserted retrograde up the thoracic aorta of the donor mouse through an arteriotomy and the balloon inflated to an atmospheric pressure of 12 Pa for 30 s. For stent deployment, the same procedure was followed; however, before insertion of the balloon angioplasty catheter, a stainless steel stent (2.5 × 0.6 mm; Brivant Ltd., Galway, Ireland) was crimped onto the balloon before deployment. The aorta (~10 to 15 mm in length) was harvested by sealing the intercostal branch vessels with electrocautery. The harvested aortas did not exceed a total ischemic time of 15 min and were stored in PBS.

In the recipient mouse, the right common carotid artery was ligated and divided between 7-0 silk ties at its midpoint. Polyethylene cuffs (0.65-mm diameter) were placed over each end and anchored by clamps. The artery was everted over the cuffs and secured with 8-0 silk sutures. The donor aorta was then interposition-grafted by sleeving its ends over the carotid artery cuffs and secured using 8-0 silk sutures. Vessel patency was checked by removing the clamps and restoring blood flow to the vessel.

To investigate the effect of systemically delivered apoA-I on vascular injury and remodeling, chow-fed 10- to 14-week-old male *apoE*$^{-/-}$ recipient mice (n = 9 to 12/group) received either PBS or apoA-I (40 mg/kg) injections intravenously into the tail vein every second day commencing 1 week before either balloon angioplasty or stent implantation, and continued to receive PBS or apoA-I (40 mg/kg) every second day post-surgery for 28 days. Mice received aspirin (10 mg/kg/day) in drinking water for 1 week before surgery and post-surgery for 28 days to reduce the risk of in-stent thrombosis. The mortality rate was <5%. Only male *apoE*$^{-/-}$ mice were used for this study to eliminate the possibility of variation due to hormonal fluctuations.

RESIN EMBEDDING OF STENTED VESSELS. Stented vessels were harvested 28 days after surgery and perfused in situ with 4% phosphate-buffered paraformaldehyde, then excised and embedded into JB-4 (glycol methacrylate) resin (ProSciTech, Thuringowa Central, Australia) according to the manufacturer's instructions for histomorphometry and immunohistochemistry analysis. Transverse sections (5 μm) of the stent were cut using a tungsten-carbide blade on an automatic microtome.

IN-STENT NEOINTIMAL HYPERPLASIA AND THROMBOSIS QUANTIFICATION. For histomorphometric analysis, resin-embedded samples were stained with hematoxylin and eosin. The total neointimal area was quantified on 5 stented sections/vessel that spanned the length of the stented area (2.5 mm) by taking the area inside the internal elastic lamina, minus the lumen. An additional measurement was performed that looked at the stent strut to the edge of the lumen distance (4 to 6 measurements/section). Thrombosis was identified in stented aortas. It was noted that if there was thrombus present, it encompassed a significant proportion of the vessel and/or completely occluded the vessel.

IMMUNOHISTOCHEMISTRY. Resin-embedded sections were subjected to antigen retrieval using a Tris-EDTA buffer (pH 9.0). Vessels were stained for SMC α-actin using a monoclonal anti-mouse antibody conjugated to alkaline phosphatase (clone 1A4, 1:200, Sigma-Aldrich, St. Louis, Missouri) and Vector Red substrate kit as per the manufacturer's instructions. α-Actin^{+} SMCs were expressed as a percentage of the total neointimal area.

IDENTIFICATION OF ENDOTHELIAL CELLS AND MACROPHAGES IN AORTAS AFTER BALLOON ANGIOPLASTY. Carotid interpositioned aortas were harvested 28 days following balloon angioplasty (n = 8/treatment group) from *apoE*$^{-/-}$ mice receiving systemic infusions of PBS or apoA-I (40 mg/kg) on alternate days 1 week before surgery until 28 days post-surgery. A single-cell suspension was created using an enzymatic digestion and manual disruption

of the aorta. An enzymatic digestion buffer was prepared by mixing 125 U/ml collagenase XI (Sigma-Aldrich), 60 U/ml hyaluronidase (Sigma-Aldrich), 60 U/ml DNase I (Sigma-Aldrich), and 450 U/ml collagenase type I (Sigma-Aldrich) in 2.5 ml of PBS. Tissue was cut into small pieces (~1 mm) before being incubated with 2.5 ml of enzymatic digestion buffer for 1 h at 37°C with slight agitation. Tissue was passed through a 70 μm cell strainer to obtain a single-cell suspension.

Red blood cells were briefly lysed, and cells were incubated with a Zombie Aqua viability stain (BioLegend, San Diego, California). Fc receptors were blocked, and the cells were stained with a cocktail of antibodies against CD45 (1:100 rat anti-mouse CD45-APC-Cy7; BD Biosciences, San Jose, California), CD115 (1:100 rat anti-mouse CD115-APC; eBioscience, San Diego, California), CD11b (1:100 rat anti-mouse CD11b-FITC; eBioscience), and CD31 (1:100 rat anti-mouse CD31-Pacific Blue; BioLegend). Macrophages were identified as $CD45^{hi}CD31^{lo}CD115^{hi}CD11b^{hi}$ and endothelial cells as $CD45^{lo}CD31^{hi}$.

REAL-TIME POLYMERASE CHAIN REACTION. Total RNA was extracted from balloon angioplasty-injured aortas in an additional cohort of $apoE^{-/-}$ mice (n = 8/treatment group) using TRI reagent (Sigma-Aldrich). Two hundred nanograms of total RNA was reverse transcribed using iScript cDNA synthesis kit (Bio-Rad, Hercules, California). The expression of *CD68* (forward 5′-GGGGCTCTTGGGAACTACAC-3′ and reverse 5′-GTACCGTCACAACCTCCCTG-3′), ATP-binding cassette transporter A1 (*ABCA1*) (forward 5′- GCGACCATGAAAGTGACACG-3′ and reverse 5′-AGACAGCTGGCAGGACAATC-3′) and peroxisome proliferator-activated receptor (*PPAR*)-γ (forward 5′-CACAATGCCATCAGGTTTGG-3′ and reverse 5′-GCTGGTCGATATCACTGGAGATC-3′) was determined by quantitative real-time polymerase chain reaction using iQ SYBR Green Supermix (Bio-Rad) and 20 pmol each of forward and reverse primers. Relative changes in mRNA levels were normalized using the $^{\Delta\Delta}$CT method to beta-2 microglobulin (forward 5′-TTCTGGTGCTTGTCTCACTGA-3′ and reverse 5′-CAGTATGTTCGGCTTCCCATTC-3′).

CIRCULATING NEUTROPHILS, MONOCYTES, AND MONOCYTE ACTIVATION AFTER BALLOON ANGIOPLASTY. Blood was drawn via tail vein nick 1 and 2 weeks after carotid-interposition grafting of balloon angioplasty-injured vessels into recipient $apoE^{-/-}$ mice. On the fourth week post-surgery, mice were euthanized via cardiac puncture. From each collection of blood, circulating neutrophils, monocytes, and monocyte activation were measured by flow cytometry.

For the identification of monocytes and neutrophils from whole blood, red blood cells were lysed and incubated with a Zombie Aqua viability stain. Fc receptors were blocked and cells were stained with a cocktail of antibodies against CD45 (1:100 rat anti-mouse CD45-APC-Cy7; BD Biosciences), Ly6-C/G (1:100 rat anti-mouse Ly6-C/G-PerCP-Cy5.5; BD Biosciences), CD115 (1:100 rat anti-mouse CD115-APC; eBioscience), and CD11b (1:100 rat anti-mouse CD11b-FITC; eBioscience). Monocytes were identified as $CD45^{hi}CD115^{hi}$ and neutrophils as $CD45^{hi}CD115^{lo}Ly6\text{-}C/G^{hi}$. Monocytes were further subdivided into $Ly6\text{-}C/G^{hi}$ to measure activation/inflammation.

PLATELET STUDIES. Eight-week-old male $apoE^{-/-}$ mice (n = 8 to 9) received a single infusion of apoA-I (40 mg/kg) or PBS (control). Two hours following the infusion, blood was collected from the inferior vena cava into a 1-ml syringe containing 100 μl of enoxaparin (400 U/ml). A 1:50 dilution of the whole blood was then stimulated with adenosine diphosphate (ADP) (2 and 20 μmol/l) and collagen (20 μg/ml) for 15 min in a total volume of 25 μl. An antibody against p-selectin (1:10, rabbit anti-mouse CD62P-FITC, BD Pharmingen, San Diego, California) was incubated with the stimulants for the final 10 mins. Changes in p-selectin were then determined using flow cytometry.

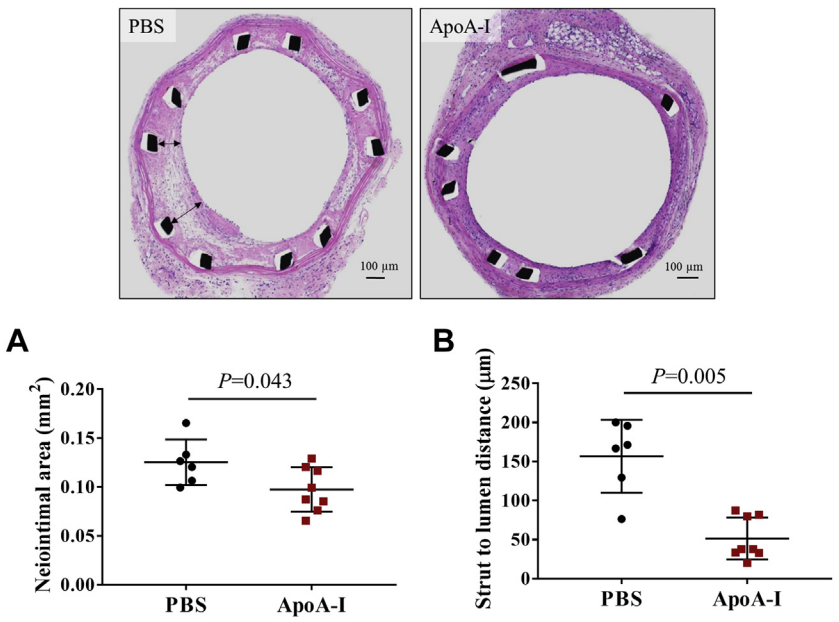
HUMAN apoA-I ANALYSIS. Human apoA-I protein was measured in the plasma of mice that had undergone stenting surgery. The human apoA-I protein was measured using enzyme-linked immunosorbent assay (Cell Biolabs, San Diego, California) according to the manufacturer's guidelines.

STATISTICAL ANALYSIS. Data are expressed as mean ± SD. Differences between treatment groups were calculated using nonparametric Mann-Whitney *U* testing or a 2-way analysis of variance with Bonferroni post hoc test. Where specified, a chi-square test was used. Significance was set at a 2-sided p value of <0.05. GraphPad Prism (version 7, GraphPad Software, La Jolla, California) was used for the statistical analysis.

RESULTS

ApoA-I REDUCES NEOINTIMAL AREA FOLLOWING STENT DEPLOYMENT. Analysis of resin-embedded stented aortic sections revealed that apoA-I infusions reduced total neointimal area ($p = 0.043$) (Figure 1A). Furthermore, the strut-to-lumen distance was smaller in the apoA-I-infused aortas ($p = 0.005$) (Figure 1B), compared with stented arteries from PBS-infused mice. These effects were

FIGURE 1 ApoA-I Reduces Neointimal Area Following Stent Deployment



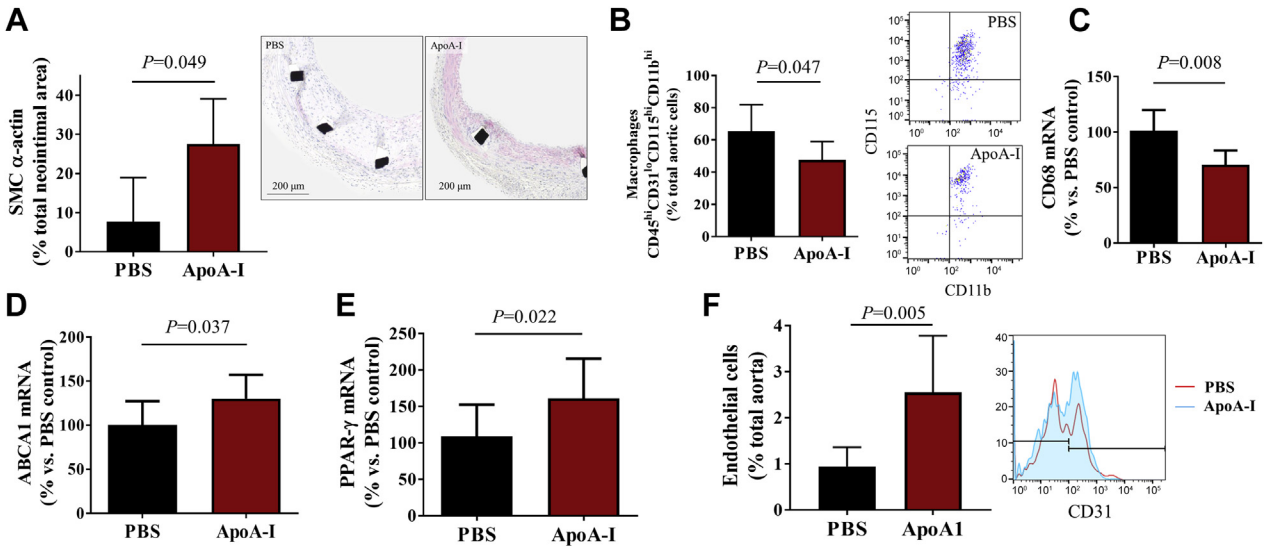
Stents were deployed into the thoracic aorta of donor *apoE*$^{-/-}$ mice. Stented aortas were then carotid interposition grafted into recipient *apoE*$^{-/-}$ mice. Recipient mice received systemic infusions of PBS or apoA-I (40 mg/kg) on alternate days 1 week before surgery and until euthanasia 28 days post-surgery (n = 8 to 10/treatment group). Resin-embedded transverse sections of stented vessels were stained with hematoxylin and eosin, and assessed for changes in **(A)** total neointimal area and **(B)** strut to lumen distance. **Black arrows** indicate an example of the distance measured for the strut-to-lumen measurement. Data are expressed as mean ± SD. apoA-I = apolipoprotein A-I; PBS = phosphate-buffered saline.

independent of changes in lipids (total, low-density lipoprotein, and HDL); however, human apoA-I was detected in the plasma of apoA-I-infused mice but not PBS-infused mice (Supplemental Table 1).

ApoA-I CHANGES THE CELLULAR COMPOSITION OF THE NEOINTIMA AFTER PCI. Immunohistochemical analysis of the neointima of stented vessels revealed that there was a significantly higher number of SMC α-actin^{+} cells in the neointima of mice receiving apoA-I compared with the PBS control group (p = 0.049) (Figure 2A). We also assessed the aortic content and mRNA expression levels of macrophages. In mice receiving apoA-I infusions, CD45^{hi}C-D31^{lo}CD115^{hi}CD11b^{hi} macrophage content and CD68 mRNA levels were both significantly lower (p = 0.047 and p = 0.008, respectively) (Figures 2B and 2C), compared with the PBS controls. To determine whether the increase in SMC α-actin expression was via enhanced apoA-I-mediated cholesterol efflux from the neointima, 2 genes involved in cholesterol efflux (*ABCA1*) and cholesterol transfer (*PPAR-γ*) were measured in aortas post-PCI. Following systemic infusions of apoA-I, the aortic mRNA levels of ABCA1 (p = 0.037) (Figure 2D) and PPAR-γ (p = 0.022) (Figure 2E) were significantly elevated. Finally, we found a significant increase in the number of endothelial cells in balloon-injured vessels of mice receiving apoA-I infusions (p = 0.005) (Figure 2F).

ApoA-I REDUCES CIRCULATING ACTIVATED MONOCYTES AFTER PCI. To determine whether the changes in neointimal cellular phenotype by apoA-I were also modulated by contributions from infiltrating circulating leukocytes, we measured neutrophils and monocytes and monocyte activation in the blood 1, 2, and 4 weeks after balloon angioplasty by flow cytometry. The apoA-I-infused mice had significantly lower circulating neutrophil (CD45^{hi}CD115^{lo}Ly6-C/G^{hi}) levels 4 weeks after vessel injury (p = 0.050) (Figure 3A). There were significantly fewer circulating monocytes (CD45^{hi}CD115^{hi}) 1 week post-surgery in the apoA-I-infused mice (p = 0.014) (Figure 3B). Circulating monocytes were also measured for markers of activation (Ly6-C/G^{hi}), and interestingly, 4 weeks

FIGURE 2 ApoA-I Changes the Cellular Composition of the Neointima After PCI



The cellular composition of the neointima of balloon angioplasty injured or stented vessels was investigated in *apoE*$^{-/-}$ mice receiving systemic infusions of PBS or apoA-I (40 mg/kg) on alternate days one week before surgery until euthanasia 28 days post-surgery (n = 8/treatment group). **(A)** SMC α-actin expression (as a percentage of neointima area), determined using immunohistochemistry on resin-embedded stented sections. **(B)** CD45^{hi}CD31^{lo}CD115^{hi}CD11b^{hi} macrophages were measured by flow cytometry. **(C)** CD68 gene expression, measured by quantitative real-time PCR, normalized to beta-2 microglobulin. Cholesterol efflux-related gene expression of **(D)** ABCA1 and **(E)** PPAR-γ were measured by quantitative real-time PCR, normalized to beta-2 microglobulin. **(F)** CD45^{lo}CD31^{hi} endothelial cells were measured by flow cytometry. Data are expressed as mean ± SD. ABCA1 = ATP-binding cassette transporter A1; *apoE*$^{-/-}$ = apolipoprotein E deficient; PCI = percutaneous coronary intervention; PCR = polymerase chain reaction; PPAR = peroxisome proliferator-activated receptor; SMC = smooth muscle cell; other abbreviations as in Figure 1.

post-surgery, circulating monocytes in mice receiving apoA-I infusion were significantly less activated compared with the PBS-infused mice (p = 0.048) (Figure 3C).

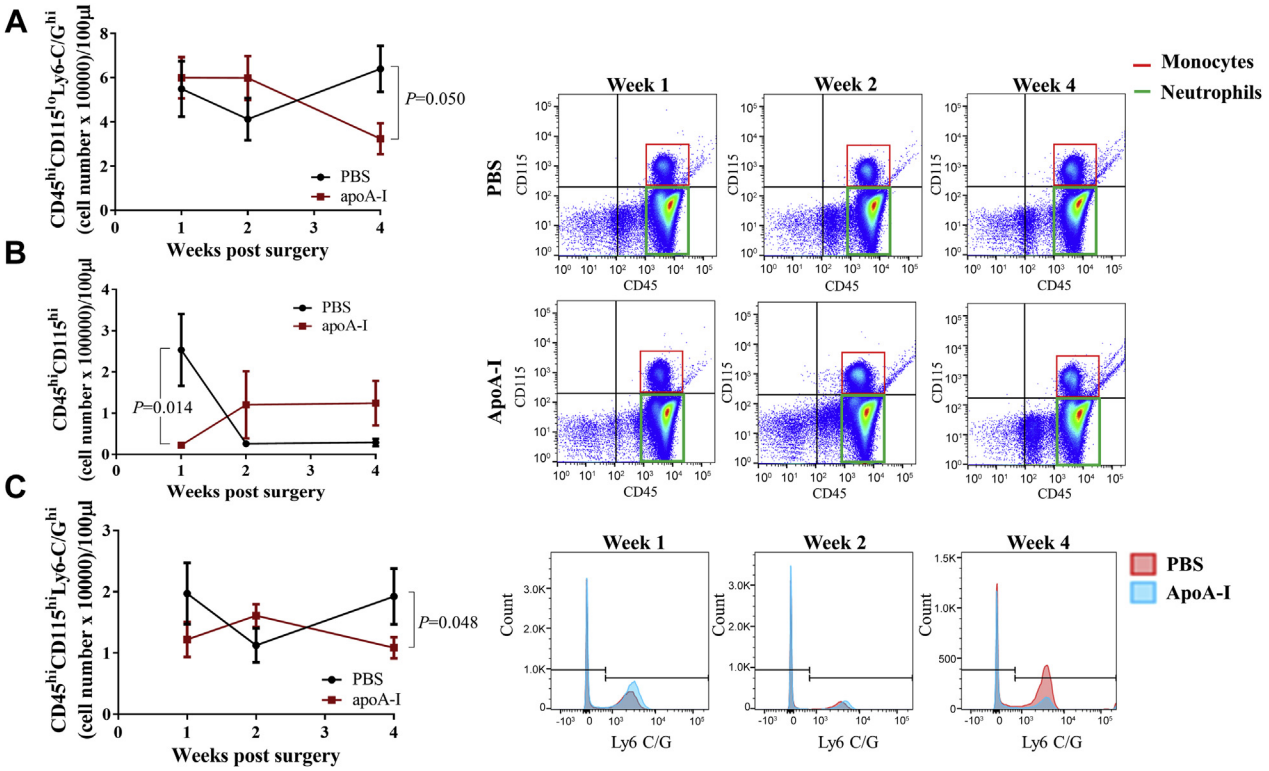
ApoA-I REDUCES THE INCIDENCE OF THROMBOSIS AND PLATELET ACTIVATION FOLLOWING STENT DEPLOYMENT. A major cause of stent failure is stent thrombosis. We found, in a separate cohort of mice, that apoA-I infusions strikingly suppressed p-selectin activation 2 h post-infusion in response to stimulation with ADP at 2 μm (p = 0.034) and 20 μm (p = 0.040) and collagen (p = 0.045) (Figure 4). It was also observed that mice in the apoA-I infusion group experienced a lower incidence of thrombosis post-stenting than the PBS control group, although this did not reach significance (Supplemental Table 2).

DISCUSSION

Reintervention after initially successful PCI is necessary if neointimal hyperplasia or neoatherosclerosis occurs. A holistic approach that favorably influences multiple facets of the vascular injury in response to stenting is likely to improve stent biocompatibility. In this study, we found that apoA-I, the main protein constituent of HDL, reduces in-stent restenosis, enhances aortic endothelialization, and inhibits platelet activation. ApoA-I, therefore, exerts a number of key vascular biological effects that are associated with improved stent biocompatibility. Our studies reveal a novel role for apoA-I in enhancing stent biocompatibility.

Neointimal hyperplasia is characterized by the uncontrolled proliferation and migration of medial SMCs, leading to a decrease in the luminal area and eventual stent failure. Although previous studies have shown that apoA-I reduces neointimal formation following carotid artery wire injury (9) and vein grafting (10) in mice, these studies do not fully replicate the physiological process and conditions of vascular interventions in humans. This is the first study to our knowledge conducted in a murine model of stenting that allows for the important mechanical aspects of clinical PCIs to be reproduced and addressed. In this study, we find that apoA-I reduces neointimal hyperplasia. This is supported by in vitro studies that have found apoA-I inhibits SMC

FIGURE 3 ApoA-I Reduces Circulating Activated Monocytes After PCI



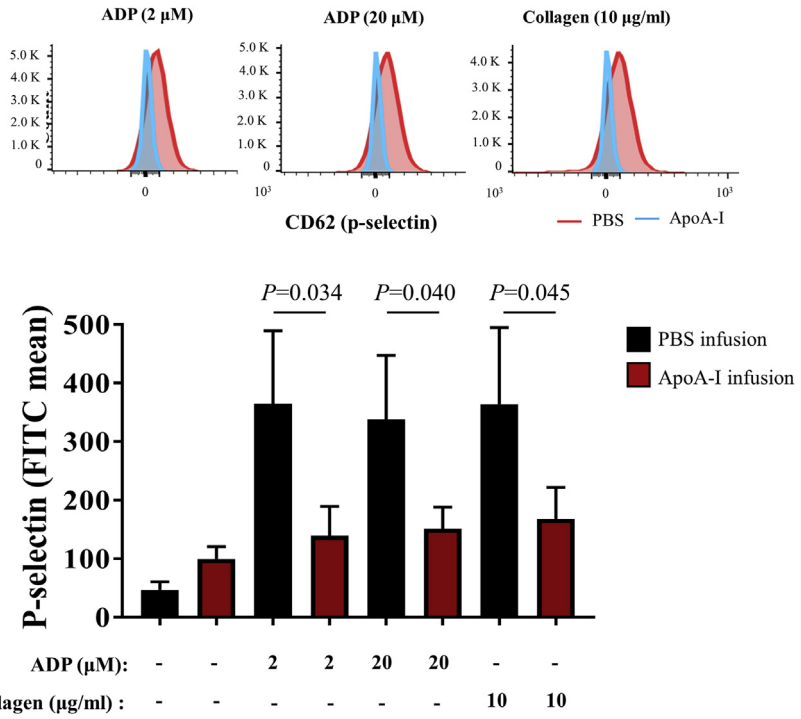
Donor thoracic aortas from *apoE*$^{-/-}$ were subjected to balloon angioplasty and then carotid interposition grafted into recipient *apoE*$^{-/-}$ mice. Recipient mice received systemic infusions of PBS or apoA-I (40 mg/kg) on alternate days 1 week before surgery and until euthanasia 28 days post-surgery (n = 8/treatment group). One, 2, and 4 weeks post-surgery, circulating **(A)** CD45^{hi}CD115^{lo}Ly6-C/G^{hi} neutrophils, **(B)** CD45^{hi}CD115^{hi} monocytes, and **(C)** Ly6-C/G^{hi} activated monocytes were measured by flow cytometry. Data are expressed as mean ± SD. Abbreviations as in Figures 1 and 2.

proliferation and inflammation (13), key contributors to neointimal hyperplasia.

The cellular composition of the neointima greatly affects the chance of stent failure after PCI. In vitro and in vivo studies have shown that SMCs can differentiate into macrophage-like cells in hyperlipidemic conditions, which is accompanied by a decrease in the expression of SMC α-actin (4). This suggests that cellular cholesterol content may play an important role in determining SMC phenotype (4), and a retention in α-actin^{+} SMCs would lead to neointima with a less inflammatory environment. Furthermore, increases in macrophage content are likely to lead to an increase in the release of potent cytokines that promote local inflammation, accelerate SMC proliferation, and thus increase the risk of neointimal hyperplasia (15). Interestingly, we found that the neointimas of apoA-I-infused mice had higher SMC α-actin expression concomitant with a lower macrophage content. Mechanistically, we found that apoA-I promoted the expression of *ABCA1* and *PPAR-γ*, 2 key genes involved in cholesterol efflux. Taken together, this suggests that apoA-I may increase cholesterol efflux from neointimal SMCs, preserving SMC phenotype and preventing a switch into a more macrophage-like state, which is supported by the decrease in aortic macrophages. Consistent with these findings, in vitro studies have found that incubation with apoA-I reverses the induction of macrophage phenotype following cholesterol loading in vascular SMCs (16). Overall, these phenotypic modifications by apoA-I are likely to play an important anti-inflammatory role in the neointima and provides an explanation for our observed reduction in in-stent restenosis by apoA-I.

Inadequate re-endothelialization is associated with neointimal expansion and an increased risk of thrombosis after PCI. We found that apoA-I increased aortic

FIGURE 4 **ApoA-I Reduces the Incidence of Thrombosis and Platelet Activation Following Stent Deployment**



Mice were infused with PBS or apoA-I (40 mg/kg), and 2-h post-infusion, platelet p-selectin was measured in whole blood after ADP (2 or 20 μmol/l) or collagen (10 μg/ml) stimulation using flow cytometry. Data are expressed as mean ± SD. ADP = adenosine diphosphate; other abbreviations as in Figure 1.

endothelial cell numbers following balloon angioplasty, indicative of enhanced re-endothelialization. This finding is consistent with previous studies that show HDL/apoA-I promote endothelial cell proliferation (17) and enhance re-endothelialization after vein grafting (10). An increase in neointimal endothelial cells is important for stent biocompatibility as the rate of re-endothelialization inversely associates with neointimal thickening and thrombosis (18).

Leukocyte infiltration contributes to the promotion of SMC proliferation and neointimal formation after vessel injury (19). The current study found circulating neutrophil numbers were lower in apoA-I–infused mice 4 weeks after vessel injury. This supports the observed decrease in neointimal area with apoA-I (20). The activation state of circulating monocytes is critical for monocyte infiltration and the progression of neointimal hyperplasia (21). Monocytes can become activated after PCI, and their activation correlates with recurrent disease (22). Four weeks post-surgery, the mice receiving apoA-I showed a significant reduction in circulating monocyte activation. This is consistent with previous studies that found lower circulating monocyte activation after infusion of rHDL into endarterectomy patients (23). A reduction in circulating activated monocytes may have also contributed to the reduction in aortic macrophages by apoA-I post-PCI.

Advances in interventional procedures and stent design together with prolonged dual-antiplatelet therapy have resulted in marked reductions in the rates of stent thrombosis; nevertheless, due to the drastic consequences of stent thrombosis, adjunctive therapies to reduce the incidence remain significantly important (1). Platelet activation was significantly inhibited in apoA-I–infused mice, suggesting apoA-I may be a promising therapy that further reduces stent thrombosis rates or for those patients with dual-antiplatelet therapy intolerance. We also found that, although not significant, the rate of thrombosis was lower post-stenting in mice infused with apoA-I. This is interesting because the stented mice had aspirin

supplemented into their drinking water, which would have substantially reduce platelet activation in both groups, making a benefit from apoA-I difficult to achieve. Our findings are consistent with an epidemiological study in which low HDL levels were found to be a predictor of major cardiac events including death due to in-stent thrombosis in patients receiving DES (24). Furthermore, these observations are in accordance with studies in humans that show infusions of rHDL reduced p-selectin in diabetic patients (12).

Our alternate-day infusions of apoA-I caused transient peaks in circulating apoA-I and HDL-cholesterol concentrations. We have shown that for apoA-I, the peak occurs 2 h after infusion (69.4 μmol/l) (25). After 24 h, apoA-I levels then drop to 11% of the maximum concentration and are then completely cleared by 48 h. Peak HDL-cholesterol concentration occurs later at 8 h (1.6 mmol/l), which then declines more gradually than apoA-I, reaching baseline at 72 h post-infusion (25). Our alternate-day infusion protocol should, therefore, cause elevations in plasma apoA-I and HDL cholesterol throughout the 28-day experimental period.

Although a high HDL-cholesterol level inversely associates with cardiovascular disease benefit in a vast number of epidemiological in vitro and in vivo studies, large clinical trials aimed at raising HDL-cholesterol levels in patients with advanced atherosclerosis have been largely neutral (26–28). One reason proposed for the failure of these trials is that the functionality of HDL is compromised in CAD and with aging (29–31). Because HDL-raising therapies have, until now, targeted elderly patients with advanced atheroma, it is perhaps not surprising that raising endogenous HDL has limited benefit. Our study, however, is aimed at the alternate pathology of stent biocompatibility in which HDL infusions may show more efficacy. Support for this has been shown in human intervention studies with rHDL that have found significant reductions in platelet activation (12) and inflammation (23), and an enhancement in endothelial progenitor cell mobilization (32), all key elements of improved stent biocompatibility.

CONCLUSIONS

We have shown that apoA-I significantly reduces in-stent restenosis, promotes endothelialization, and reduces platelet activation, key biological processes associated with enhanced stent biocompatibility. Furthermore, apoA-I preserved the in-stent neointimal α-actin SMC phenotype and decreased aortic macrophage content post-PCI. Our data suggest that this is via increases in SMC cholesterol efflux (via ABCA1 and PPAR-γ) and the inhibition of monocyte activation and neointimal infiltration. These findings show that apoA-I is a promising candidate for the improvement of stent biocompatibility.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Overall, through the improved understanding of the effects of apoA-I on the vascular biological processes associated with stent biocompatibility, and with an improved strategy for translation, these studies may have implications for the prevention and treatment of stent failure and for the future of stent design. These studies established that apoA-I is a promising candidate to improve all key aspects of stent biocompatibility. Ultimately, this will have an impact on cardiovascular outcomes in the increasing number of patients who require PCIs, reducing both health and financial burden.

TRANSLATIONAL OUTLOOK: Therapies that elevate HDL levels endogenously via pharmacological agents have not been successful at reducing cardiovascular disease due to off-target effects, such as increased blood pressure. Acute infusions of reconstituted HDL (rHDL, apoA-I + phospholipid) have, however, shown more promise and suppress many cardiovascular risk factors. Our findings that apoA-I improves the key vascular biological processes associated with stent biocompatibility suggest that localized delivery of HDL on a stent may be the next translation step. This will use the beneficial properties of apoA-I in a way that will not evoke the unwanted side effects caused by pharmacological HDL raising but rather maximize its effects by targeting apoA-I locally to the site of stent deployment. Our findings have implications for improving the inherent biocompatibility of stents and may realize a long-recognized potential of HDL-related therapies that will have an impact on cardiovascular disease.

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APPENDIX For supplemental tables, please see the online version of this paper.