Inhibition of Arthritis in the Lewis Rat by Apolipoprotein A-I and Reconstituted High-Density Lipoproteins

Ben J. Wu, Kwok L. Ong, Sudichhya Shrestha, Kang Chen, Fatiha Tabet, Philip J. Barter, Kerry-Anne Rye

Objective—This study questions whether high-density lipoproteins (HDLs) and apolipoprotein A-I inhibit joint inflammation in streptococcal cell wall peptidoglycan-polysaccharide (PG-PS)–induced arthritis in female Lewis rats.

Approach and Results—Administration of PG-PS to female Lewis rats caused acute joint inflammation after 4 days, followed by remission by day 8. The animals subsequently developed chronic joint inflammation that persisted until euthanasia at day 21. Treatment with apolipoprotein A-I 24 hours before and 24 hours after PG-PS administration reduced the acute and chronic joint inflammation. Treatment with apolipoprotein A-I at days 7, 9, and 11 after PG-PS administration reduced the chronic joint inflammation. Treatment with apolipoprotein A-I or reconstituted HDLs consisting of apolipoprotein A-I complexed with phosphatidylcholine 24 hours before and at days 1, 7, 9, and 11 after PG-PS administration reduced acute and chronic joint inflammation. Treatment with apolipoprotein A-I also reduced the inflammatory white blood cell count, synovial fluid proinflammatory cytokine levels, synovial tissue macrophage accumulation, as well as toll-like receptor 2, and inflammatory cytokine expression. At the molecular level, preincubation of human monocyte–derived macrophages with apolipoprotein A-I or reconstituted HDLs before PG-PS stimulation inhibited the PG-PS–induced increase in toll-like receptor 2 and myeloid differentiation primary response gene (88) mRNA levels, nuclear factor-κB activation, and proinflammatory cytokine production. The effects of apolipoprotein A-I and reconstituted HDLs were abolished by transfecting the human monocyte–derived macrophages with ATP-binding cassette transporter A1 or G1 siRNA.


Key Words: apolipoprotein A-I, arthritis, rheumatoid, cholesterol, HDL, inflammation

Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes joint destruction and is associated with the migration of inflammatory cells into synovial tissues. Although the causes of RA are unknown, activation of synovial cell toll-like receptors (TLRs) plays a pivotal role in its progression. To date, 10 TLRs have been identified in humans and 12 in mice, including TLR2 that recognizes bacterial components, such as peptidoglycans (PGs). The cytosolic domains of TLRs interact with adaptor proteins, triggering the activation of downstream inflammatory pathways. Myeloid differentiation primary response gene (88) (MyD88), the best characterized TLR adaptor protein, is essential for initiating TLR2 signaling pathways, downstream activation of nuclear factor-κB (NF-κB), and transcription of genes that produce the proinflammatory cytokines, chemokines, and proteases that cause joint inflammation and destruction in arthritis. The high-density lipoproteins (HDLs) in human plasma consist of multiple subpopulations of particles, high levels of which are associated with reduced cardiovascular risk. The most extensively studied function of HDLs and the main HDL apolipoprotein (apolipoprotein A-I) involves their participation in the reverse cholesterol transport pathway, whereby excess cholesterol is exported from peripheral cells and transported to the liver for excretion. These processes are dependent on scavenger receptor scavenger receptor B1 (SR-B1) and the ATP-binding cassette transporters (ABCA1) and ABCG1. HDLs and apolipoprotein A-I are also profoundly anti-inflammatory and inhibit acute and chronic vascular inflammation in vivo.

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HDL cholesterol and apolipoprotein A-I levels are reduced in people with RA relative to healthy controls. HDL cholesterol levels are also significantly decreased in a retrospective
study of blood donors who later developed RA. Conversely, the plasma concentrations of apolipoprotein A-I and HDL cholesterol are increased in patients who respond to treatment with disease-modifying antirheumatic drugs when compared with nonresponders. When considered together, these results suggest that therapies that increase HDL levels may potentially reduce joint inflammation in RA.

We have reported that intravenous infusions of apolipoprotein A-I and reconstituted HDLs (rHDLs) consisting of apolipoprotein A-I complexed with phospholipid inhibit endothelial cell adhesion molecule expression and infiltration of neutrophils into inflamed carotid arteries in New Zealand White rabbits. They also reduce chemokine and chemokine receptor expression in apoE-knockout mice, inhibit inflammatory cytokine production in monocytes by direct contact with stimulated T lymphocytes, and reduce chemokine and chemokine receptor expression in fibroblast-like synoviocytes. Apolipoprotein A-I–containing rHDLs also attenuate adhesion molecule expression in tumor necrosis factor (TNF)-α–stimulated endothelial cells. Moreover, treatment with an apolipoprotein A-I mimetic peptide has been shown to reduce serum inflammatory cytokine levels significantly and to inhibit collagen-induced arthritis in rats. These anti-inflammatory functions of HDLs are particularly relevant in the context of RA in humans, whereas proinflammatory cytokine, chemokine, adhesion molecule, and growth factor production by synovial tissues recruits inflammatory cells into joints, thus exacerbating the disease.

The present study questions whether intravenous infusions of lipid-free apolipoprotein A-I and (A-I)rHDLs inhibit streptococcal cell wall PG-polysaccharide (PG-PS)–induced arthritis in female Lewis rats, an experimental animal model of arthritis that has many features in common with human RA. The results show that apolipoprotein A-I and rHDLs effectively reduce joint inflammation in this animal model by inhibiting TLR2 expression and activation. Additional studies in human monocyte–derived macrophages (HMDMs) have established that apolipoprotein A-I and rHDLs mediate these effects by decreasing NF-κB activation in an ABCA1- and ABCG1-dependent manner.

### Materials and Methods

Materials and Methods are available in the online-only Supplement.

### Results

Apolipoprotein A-I Prevents PG-PS–Induced Arthritis in Female Lewis Rats

Arthritis was induced in female Lewis rats as described in Materials and Methods in the online-only Data Supplement and Figure I in the online-only Data Supplement. Joint inflammation in the animals that received intravenous saline infusions 24 hours before and at days 1, 7, 9, and 11 after PG-PS administration progressed from an initial, acute inflammatory phase during the first 4 days after PG-PS administration to remission by day 8. These animals subsequently developed chronic joint inflammation that persisted until euthanasia at day 21 (Figure 1A–1C, closed circles, S/S). Joint inflammation was not evident in control animals that received intravenous saline.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ABCA1</td>
<td>ATP-binding cassette transporter A1</td>
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<tr>
<td>ABCG1</td>
<td>ATP-binding cassette transporter G1</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<td>HMDMs</td>
<td>human monocyte–derived macrophages</td>
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<td>MyD88</td>
<td>myeloid differentiation primary response gene (88)</td>
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<td>NF-κB</td>
<td>nuclear factor-κB</td>
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<tr>
<td>PG-PS</td>
<td>peptidoglycan-polysaccharide</td>
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<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
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<tr>
<td>rHDL</td>
<td>reconstituted HDL</td>
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<tr>
<td>SR-B1</td>
<td>scavenger receptor B1</td>
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<tr>
<td>TLR</td>
<td>toll-like receptor</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<td>WBC</td>
<td>white blood cell</td>
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Figure 1. Apolipoprotein A-I (ApoA-I) protects against peptidoglycan-polysaccharide (PG-PS)–induced arthritis in rats. Female Lewis rats received a single intraperitoneal (ip) injection of saline or PG-PS (15 mg/kg). The control animals received intravenous (iv) injections of saline 24 hours before and 1, 7, 9, and 11 days after the ip saline (A, S/S, n=7, closed triangles) or PG-PS (A–C, S/S, n=7, closed circles) injection. The remaining animals received apoA-I (8 mg/kg, iv) 24 hours before and 24 hours after the PG-PS injection and iv saline on days 7, 9, and 11 after PG-PS injection (A, A/S, n=6, open circles), iv saline 24 hours before and 24 hours after the PG-PS injection, and then apoA-I (8 mg/kg, iv) on days 7, 9, and 11 after the PG-PS injection (B, S/AI, n=6, open circles), or apoA-I (8 mg/kg, iv) 24 hours before and on days 1, 7, 9, and 11 after the PG-PS injection (C, A/AI, n=3, open circles). Arthritis scores were used to assess joint inflammation as described in the Materials and Methods in the online-only Data Supplement. The pictures in A were taken on day 21 immediately before euthanasia. Data are expressed as mean±SEM. *P<0.05 vs PG-PS treated, saline (S/S) infused rats.
saline infusions 24 hours before and at days 1, 7, 9, and 11 after a single intraperitoneal saline injection (Figure 1A, closed triangles, S/S).

Administration of apolipoprotein A-I 24 hours before and 24 hours after the intraperitoneal PG-PS injection, followed by 3 intravenous saline injections on days 7, 9, and 11 after the PG-PS injection, decreased the acute and chronic phases of arthritis by 63±9% at day 3 and by 61±8% at day 21 (Figure 1A, open circles, AI/S; P < 0.05 for both). Infusion of saline 24 hours before and 24 hours after the PG-PS injection, followed by infusion of apolipoprotein A-I on days 7, 9, and 11 after the PG-PS injection, had no effect on acute joint inflammation but reduced chronic joint inflammation by 43±11% at day 21 (Figure 1B, open circles, S/S; P < 0.05 for both). Apolipoprotein A-I administration 24 hours before the PG-PS injection, and on days 1, 7, 9, and 11 after the PG-PS injection, reduced joint inflammation by 61±5% at day 3 and by 90±5% at day 21 (Figure 1C, open circles, Al/Al; P < 0.05 for both).

**Apolipoprotein A-I Reduces PG-PS–Induced Inflammatory Cell Infiltration and TLR2 Expression in Synovial Tissues**

Joint inflammation in arthritis is mediated by the migration of circulating inflammatory cells into synovial tissues, where they subsequently release proinflammatory cytokines. The present results are consistent with these events. When the animals were treated with intravenous saline on day 1 before and days 1, 7, 9, and 11 after inducing arthritis with an intraperitoneal PG-PS infusion, the total white blood cell (WBC), neutrophil, and monocyte counts increased by 6.1±0.5-, 17±1.4-, and 37±4.9-fold, respectively (Figure 2A–2C, S/S). The number of circulating inflammatory cells increased to 3.7±0.8 pg/mL in the saline-treated animals with arthritis (Figure 2G). Plasma tumor necrosis factor (TNF)–α levels decreased by 40% to 55% (Figure 2H), and IL-1β levels decreased by 42% to 68% (Figure 2I), and IL-1β levels decreased by 40% to 55% (Figure 2I; P < 0.05 for all).

WBCs were not detected in synovial fluid samples from control animals that received a single intraperitoneal injection of saline and were treated with intravenous saline (Figure 3A–3D, S/S). A single intraperitoneal PG-PS infusion, by contrast, induced massive WBC filtration into the synovial fluid (Figure 3A and 3B, closed bars). The major synovial

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**Figure 2.** Apolipoprotein A-I (ApoA-I) reduces circulating inflammatory cells and proinflammatory cytokine levels in rats with peptidoglycan-polysaccharide (PG-PS)–induced arthritis. Female Lewis rats received a single intraperitoneal (ip) injection of saline (open bars) or PG-PS (closed bars) and intravenous injections of saline or apoA-I as described in the legend to Figure 1. Blood was collected at the time of euthanasia on day 21 after the ip PG-PS or saline injection. Total white blood cells (WBCs; A), neutrophils (B), and monocytes (C) were determined as described in the Materials and Methods in the online-only Data Supplement. CD11b/c+ (D), CD44+ (E), and CD11b/c+/CD44+ (F) WBCs were quantified by flow cytometry. Plasma tumor necrosis factor (TNF)–α (G), interleukin (IL)-6 (H), and IL-1β (I) levels were determined by ELISA. Data represent mean±SEM. N.D., Below the level of detection. *P < 0.05 vs PG-PS treated, saline (S/S) infused rats.
fluid WBCs in the PG-PS–treated animals were neutrophils (Figure 3C) and monocytes (Figure 3D). In the animals with arthritis, treatment with apolipoprotein A-I reduced the WBC count by 47% to 78% (Figure 3B), the number of neutrophils by 38% to 72% (Figure 3C), and the number of monocytes by 76% to 84% (Figure 3D; \( P < 0.05 \) for all).

Induction of arthritis with PG-PS also increased synovial fluid TNF-\( \alpha \), IL-6, and IL-1\( \beta \) levels by 56±5.0-, 21±1.6-, and 4.5±0.5-fold, respectively (Figure 3E–3G, closed bars, S/S; \( P < 0.05 \) for all versus control). Treatment with apolipoprotein A-I reduced the TNF-\( \alpha \) (Figure 3E), IL-6 (Figure 3F), and IL-1\( \beta \) (Figure 3G) levels by 65% to 78%, 41% to 61%, and 46% to 65%, respectively, in the animals with arthritis (\( P < 0.05 \) for all).

Synovial tissue macrophage content (CD68+ cells; Figure 4A) and TNF-\( \alpha \) mRNA levels (Figure 4B) increased by 53±6.3- and 3.5±0.3-fold, respectively, in the saline-treated animals with arthritis (S/S, closed bar), relative to the saline-treated control animals (S/S, open bars; \( P < 0.05 \) for both). Treatment with apolipoprotein A-I reduced the synovial tissue macrophage content by 83% to 90% (Figure 4A) and TNF-\( \alpha \) mRNA levels by 51% to 67% (Figure 4B; \( P < 0.05 \) for all versus saline-treated animals with arthritis).

Synovial tissue TLR2 mRNA levels were increased 3.2±0.3-fold in the saline-treated animals with PG-PS–induced arthritis (Figure 4C, closed bar; \( P < 0.05 \) versus control). Treatment with apolipoprotein A-I reduced TLR2 mRNA levels by 43% to 58% (Figure 4C; \( P < 0.05 \) relative to saline-treated animals with arthritis).

Taken together, these results indicate that treatment of female Lewis rats with PG-PS–induced arthritis with apolipoprotein A-I markedly reduces the number of circulating WBCs in the synovium.
inflammatory cells, that can potentially decrease their migration into synovial tissues and the subsequent release of pro-inflammatory cytokines. These findings also suggest that the ability of apolipoprotein A-I to reduce joint inflammation in rats with PG-PS–induced arthritis may be related to reduced TLR2 expression in macrophages.

**Apolipoprotein A-I Inhibits PG-PS–Induced Inflammation in Macrophages by Attenuating TLR2 Expression and Activation**

The inhibition of PG-PS–induced proinflammatory cytokine production by apolipoprotein A-I and the involvement of TLR2 in this process was investigated in HMDMs. Stimulation of HMDMs with PG-PS increased TNF-α (Figure 5A, closed bar), IL-6 (Figure 5B, closed bar), and IL-1β (Figure 5C, closed bar) secretion into the culture medium by 8.5±0.2-, 12±0.9-, and 2.8±0.4-fold, respectively (P<0.05 for all versus control). Preincubation of the HMDMs with apolipoprotein A-I at a final concentration of 0.25, 0.5, and 1.0 mg/mL inhibited the PG-PS–mediated increase in TNF-α levels by 38±5.6%, 53±6.8%, and 64±8.1%, respectively (Figure 5A), reduced IL-6 levels by 36±10%, 48±12%, and 55±9% (Figure 5B), and decreased IL-1β levels by 34±9.4%, 53±9.1%, and 60±1.0% (Figure 5C; P<0.05 for all).

The ability of apolipoprotein A-I to prevent the PG-PS–mediated increase in TLR2 and MyD88 expression and downstream activation of NF-κB in HMDMs was also investigated. Relative to what was observed for control HMDMs (open bars), incubation with PG-PS increased TLR2 (Figure 5D) and MyD88 (Figure 5E) mRNA levels 3.0±0.3- and 2.9±0.2-fold, respectively (closed bars), whereas nuclear levels of the p65 subunit of NF-κB (Figure 5E, closed bars) increased by 8.2±1.0-fold (P<0.05 for all). Preincubation of HMDMs with apolipoprotein A-I (final concentration 0.25, 0.5, and 1.0 mg/mL) inhibited the PG-PS–mediated increase in TLR2 mRNA levels by 33±3.4%, 48±1.9%, and 59±6.6% (Figure 5D), reduced MyD88 mRNA levels by 32±7.3%, 50±12%, and 62±13% (Figure 5E), and decreased nuclear p65 protein levels by 38±2.3%, 50±8.7%, and 69±8.1% (Figure 5F; P<0.05 for all). When taken together, these results indicate that apolipoprotein A-I inhibits PG-PS–induced inflammation in macrophages by inhibiting TLR2 and MyD88 gene expression and downstream activation of NF-κB.

To confirm that apolipoprotein A-I inhibited the PG-PS–mediated activation of HMDMs in a TLR2-dependent manner, HMDMs were transfected with TLR2 siRNA. TLR2 protein (Figure IIA in the online-only Data Supplement) and mRNA (Figure IIB in the online-only Data Supplement) levels in the transfected cells were decreased by 81±10% and by 71±11%, respectively (P<0.05 for both), relative to HMDMs transfected with scrambled siRNA.

When the scrambled siRNA-transfected cells were incubated with PG-PS, HMDM TLR2 mRNA levels increased by 2.9±0.5-fold (Figure IIC in the online-only Data Supplement, open bars; P<0.05). Preincubation of the scrambled siRNA-transfected cells with apolipoprotein A-I before stimulation with PG-PS decreased HMDM TLR2 mRNA levels by 58±3.8% (Figure IIC in the online-only Data Supplement, open bars; P<0.05 relative to PG-PS–treated cells preincubated with PBS). Incubation with PG-PS increased TLR2 mRNA levels in the TLR2 siRNA-transfected cells by 2.6±0.4-fold (P<0.05; Figure IIC in the online-only Data Supplement, closed bars). TLR2 mRNA levels were similarly increased when the cells were preincubated with apolipoprotein A-I before stimulation with PG-PS.

Incubation of scrambled siRNA-transfected HMDMs with PG-PS increased the concentration of TNF-α in the culture medium from 0.09±0.02 to 0.88±0.07 ng/mL (Figure IID in the online-only Data Supplement, open bars). Preincubation of the scrambled siRNA-transfected HMDMs with apolipoprotein A-I before stimulation with PG-PS decreased the TNF-α concentration in the medium by 61±11% (Figure IID in the online-only Data Supplement; P<0.05 versus PG-PS–treated cells preincubated with PBS).

TNF-α was not detected in the medium when HMDMs were transfected with TLR2 siRNA (Figure IID in the online-only Data Supplement, closed bars). Preincubation of the TLR2 siRNA-transfected HMDMs with PBS or apolipoprotein A-I

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**Figure 5.** Apolipoprotein A-I (ApoA-I) inhibits peptidoglycan-polysaccharide (PG-PS)–induced inflammation in macrophages by reducing toll-like receptor (TLR) 2 and myeloid differentiation primary response gene 88 (MyD88) mRNA levels and nuclear factor-κB (NF-κB) activation. Human monocyte-derived macrophages were preincubated with PBS (open bars) or apoA-I (final concentration 0.25, 0.5, and 1.0 mg/mL) for 16 hours. The apoA-I was removed and the cells were washed with fresh medium before stimulation for 6 hours with PG-PS (final concentration 20 μg/mL, closed bars). Tumor necrosis factor (TNF)-α (A), interleukin (IL)-6 (B), and IL-1β (C) levels in the culture medium were determined by ELISA. TLR2 (D) and MyD88 mRNA levels (E) were quantified by quantitative polymerase chain reaction. Nuclear p65 levels were determined by SDS-PAGE and Western blotting using β-actin as a loading control (F). Data represent mean±SEM of 3 to 6 independent experiments. *P<0.05 vs PG-PS–treated cells incubated with PBS.
before stimulation with PG-PS increased the concentration of TNF-α in the medium to 0.16±0.02 and 0.11±0.01 ng/mL, respectively (Figure IID in the online-only Data Supplement).

**Apolipoprotein A-I Decreases PG-PS–Induced TLR2 and NF-κB Activation in Macrophages in an ABCA1-Dependent Manner**

To investigate the mechanism by which apolipoprotein A-I prevents the PG-PS–induced increase in TLR2 and MyD88 mRNA levels and nuclear translocation of NF-κB, HMDMs were transfected with ABCA1 siRNA, ABCG1 siRNA, SR-B1 siRNA, and scrambled siRNA (siControl) for 24, 48, and 72 hours. ABCA1 protein levels were reduced by 29±18%, 76±9.9% (P<0.05), and 75±13%, respectively, after 24, 48, and 72 hours of transfection with ABCA1 siRNA (Figure IIIA in the online-only Data Supplement). Transfection of HMDMs for 24, 48, and 72 hours with ABCG1 siRNA decreased ABCG1 protein levels by 48±9.0%, 69±9.3%, and 77±7.3%, respectively, (Figure IIIB in the online-only Data Supplement; P<0.05 for all). SR-B1 protein levels were decreased by 70±11% after 48 hours and by 89±5.0% after 72 hours of transfection with SR-B1 siRNA (Figure IIIC in the online-only Data Supplement; P<0.05 for both). ABCA1, ABCG1, and SR-B1 mRNA levels were decreased by 71±8.4%, 62±9.1%, and 64±7.8% after 48 hours of transfection with their respective siRNAs (Figure IIID–IIIF in the online-only Data Supplement; P<0.05 for all).

Incubation of the scrambled siRNA-transfected HMDMs with PG-PS increased TLR2 mRNA levels 3.3±0.4-fold (Figure 6A, open bars; P<0.05 relative to control). A similar increase in TLR2 mRNA levels was also observed in HMDMs that were transfected with ABCA1 siRNA, ABCG1 siRNA, and SR-B1 siRNA and incubated with PBS before stimulation with PG-PS (Figure 6A, closed bars). This indicates that the PG-PS–induced expression of TLR2 in HMDMs is independent of ABCA1, ABCG1, and SR-B1. Preincubation of the scrambled siRNA-transfected cells with apolipoprotein A-I inhibited the PG-PS–mediated increase in TLR2 mRNA levels by 53±6.9% (Figure 6A, open bars; P<0.05). TLR2 mRNA levels were similarly inhibited in HMDMs transfected with ABCG1 siRNA and SR-B1 siRNA then preincubated with apolipoprotein A-I before stimulation with PG-PS. Preincubation with apolipoprotein A-I, by contrast, did not inhibit the PG-PS–mediated increase in TLR2 mRNA levels in HMDMs transfected with ABCA1 siRNA (Figure 6A, closed bars). This indicates that the ability of apolipoprotein A-I to inhibit the PG-PS–mediated increase in TLR2 mRNA levels in HMDMs is dependent on ABCA1 but not on ABCG1 or SR-B1.

To determine whether apolipoprotein A-I also inhibits the PG-PS–mediated increase in HMDM MyD88 mRNA levels, nuclear p65 levels, and TNF-α secretion in an ABCA1-dependent manner, HMDMs were transfected with scrambled siRNA and ABCA1 siRNA. Incubation with PG-PS increased MyD88 levels (Figure 6B), nuclear p65 levels (Figure 6C), and TNF-α secretion (Figure 6D) in HMDMs transfected with scrambled siRNA (open bars) and ABCA1 siRNA (closed bars). Preincubation of the scrambled siRNA-transfected HMDMs with apolipoprotein A-I inhibited the PG-PS–mediated increase in MyD88 mRNA levels (Figure 6B, open bars), nuclear p65 expression (Figure 6C, closed bars), and TNF-α secretion (Figure 6D, closed bars).
online-only Data Supplement) levels by 3.1±0.6-, 3.2±0.3-, and 22±3.0-fold, respectively (closed bars; \( P<0.05 \) for all), whereas the concentration of TNF-α (Figure IVD in the online-only Data Supplement), IL-6 (Figure IVE in the online-only Data Supplement), and IL-1β (Figure IVF in the online-only Data Supplement) in the culture medium increased by 6.2±0.7-, 46±5.8-, and 4.5±1.1-fold (closed bars; \( P<0.05 \) for all). Preincubation of the cells with CSL-111 inhibited the PG-PS–mediated increase in TLR2 mRNA levels by 58±12% (Figure IVA in the online-only Data Supplement), MyD88 mRNA levels by 60±4.5% (Figure IVB in the online-only Data Supplement), and nuclear p65 expression by 73±13% (Figure IVC in the online-only Data Supplement). TNF-α (Figure IVD in the online-only Data Supplement), IL-6 (Figure IVE in the online-only Data Supplement), and IL-1β (Figure IVF in the online-only Data Supplement) levels in the culture medium were reduced by 58±7.6%, 51±6.0%, and 73±15%, respectively (\( P<0.05 \) for all). Comparison with Figure 5 indicates that CSL-111 inhibits TLR2 expression and downstream NF-κB activation as effectively as apolipoprotein A-I in HMDMs.

The ability of CSL-111 to inhibit PG-PS–mediated increase in TLR2 mRNA levels in HMDMs was also found to be ABCA1 and ABCG1 but not SR-B1 dependent (Figure V in the online-only Data Supplement). Incubation of scrambled siRNA-transfected HMDMs with PG-PS increased TLR2 mRNA levels by 3.5±0.2-fold (Figure V in the online-only Data Supplement, open bars; \( P<0.05 \)). TLR2 mRNA levels were similarly increased in HMDMs transfected with ABCA1 siRNA, ABCG1 siRNA, and SR-B1 siRNA then preincubated with PBS before stimulation with PG-PS (Figure V in the online-only Data Supplement, closed bars). Preincubation of the scrambled siRNA-transfected cells with CSL-111 inhibited the PG-PS–mediated increase in TLR2 mRNA levels by 46±4.1% (Figure V in the online-only Data Supplement, open bars; \( P<0.05 \)). TLR2 mRNA levels were similarly inhibited when the HMDMs were transfected with SR-B1 siRNA and preincubated with CSL-111 before stimulation with PG-PS. Preincubation with CSL-111, by contrast, did not inhibit the PG-PS–mediated increase in TLR2 mRNA levels in HMDMs transfected with ABCA1 siRNA or ABCG1 siRNA (Figure V in the online-only Data Supplement, closed bars).

The ability of CSL-111 to inhibit PG-PS–induced inflammation in vivo was also determined. Arthritis was induced in female Lewis rats with a single intraperitoneal infusion of PG-PS. The animals also received intravenous injections of saline (Figure VI in the online-only Data Supplement, closed circles) or CSL-111 (Figure VI in the online-only Data Supplement, open circles) 24 hours before and at days 1, 7, 9, and 11 after the PG-PS injection. Treatment with CSL-111 reduced the acute and chronic phases of joint inflammation by 44±4% at day 3 and by 43±9% at day 21 (Figure VI in the online-only Data Supplement, open circles; \( P<0.05 \) for both).

**Discussion**

The ability of apolipoprotein A-I and HDL to inhibit vascular inflammation is well established in vitro and in vivo. This report demonstrates for the first time that apolipoprotein A-I and to a lesser extent the rHDL preparation, CSL-111, also inhibit joint inflammation in female Lewis rats with PG-PS–induced arthritis.

As CSL-111 is likely to be cleared from the circulation less rapidly than lipid-free apolipoprotein A-I, we anticipated that it would be a more effective inhibitor of joint inflammation when compared with apolipoprotein A-I. Comparison of the results in Figure 1 and Figure VI in the online-only Data Supplement indicate that this is not the case. This unexpected result may be a consequence of association with lipid markedly altering the secondary structure of the C-terminal domain of apolipoprotein A-I in CSL-111 and decreasing surface exposure of specific apolipoprotein A-I residues or peptide sequences with anti-inflammatory or chemotactic properties.

Given that recruitment of circulating leukocytes is a key event in the pathogenesis of joint inflammation in RA, it is possible that the apolipoprotein A-I–mediated reduction in circulating inflammatory cell levels may have been directly responsible for the decreased number of inflammatory cells and the reduction in proinflammatory cytokine levels in the joints of the PG-PS–treated rats. As HDLs from mice transgenic for human apolipoprotein A-I have recently been shown to suppress myeloid cell proliferation, it is also possible that the reduced number of circulating inflammatory cells in the PG-PS–treated animals may have been caused by an apolipoprotein A-I–mediated decrease in leukocyte production. It is also conceivable that apolipoprotein A-I may have crossed the synovial membrane and inhibited macrophage proinflammatory cytokine production in the joints of the PG-PS–treated animals directly.

The finding that apolipoprotein A-I inhibits PG-PS–induced inflammation in HMDMs by decreasing TLR2 and MyD88 mRNA levels and inhibiting NF-κB activation in an ABCA1-dependent manner convincingly demonstrates that the previously reported anti-inflammatory properties of apolipoprotein A-I and HDLs extend beyond their potential benefit in cardiovascular disease and that they may both have therapeutic applications in other inflammatory pathologies, particularly in humans with RA exacerbations. rHDL infusions and other HDL-raising agents that are currently under investigation in large-scale cardiovascular clinical outcome trials may, therefore, offer an alternative approach for people who do not respond to or cannot tolerate conventional RA therapies, such as TNF inhibitors and the disease-modifying antirheumatic drug, methotrexate. This approach would have the added benefit of reducing cardiovascular risk that is known to be elevated in people with RA. The observations that apolipoprotein A-I inhibits PG-PS–induced inflammatory responses by reducing TLR2 and MyD88 mRNA levels and inhibiting NF-κB activation in an ABCA1-dependent manner convincingly demonstrates that the previously reported anti-inflammatory properties of apolipoprotein A-I and HDLs extend beyond their potential benefit in cardiovascular disease and that they may both have therapeutic applications in other inflammatory pathologies, particularly in humans with RA exacerbations. rHDL infusions and other HDL-raising agents that are currently under investigation in large-scale cardiovascular clinical outcome trials may, therefore, offer an alternative approach for people who do not respond to or cannot tolerate conventional RA therapies, such as TNF inhibitors and the disease-modifying antirheumatic drug, methotrexate. This approach would have the added benefit of reducing cardiovascular risk that is known to be elevated in people with RA.
of apolipoprotein A-I are also apparent in an entirely different pathology. Apolipoprotein A-I and the apolipoprotein A-I mimetic peptide, 4F, have also been reported to decrease TLR4 expression and protect against lipopolysaccharide-induced inflammation in HMDMs. Similarly, the increased HDL levels in mice transgenic for human apolipoprotein A-I are associated with decreased TLR4 expression in the lung and protect against lipopolysaccharide-induced lung inflammation.

The current results showing that CSL-111 and apolipoprotein A-I inhibit macrophage and joint inflammation by decreasing TLR expression and signaling, as well as the expression of inflammatory genes in an ABCA1- and ABCG1-dependent manner, are consistent with what was reported for the first time in 2008 by Yvan-Charvet et al., who found that ABCA1-/ABCG1-deficient macrophages with high cholesterol levels had increased TLR4 and MyD88/TRIF expression. HDLs also inhibit NF-κB–dependent responses to TLR ligands in an ABCA1-dependent manner, possibly by decreasing the cholesterol content of lipid rafts and reducing MyD88-dependent TLR trafficking to these domains. This observation is consistent with the finding that the anti-inflammatory effects of HDLs and apolipoprotein A-I are enhanced in cholesterol-depleted macrophages and monocytes. When these findings are considered in light of the current results, which establish that apolipoprotein A-I inhibits PG-PS–induced inflammation in HMDMs in an ABCA1-dependent manner, it follows that the observed reduction in the inflammatory response in the HMDMs in the present study may have been related to a reduction in the cholesterol content of the cells. Although it could be argued that a reduction in cell cholesterol levels cannot explain why CSL-111 inhibited inflammation in HMDMs in an ABCA1-dependent manner, it is noteworthy that this result is in agreement with a recent report in which the ability of the closely related formulation, CSL-112, to efflux cholesterol from macrophages was shown to be dependent on ABCA1. Although the possibility of carrying out bone marrow transplantation studies to test this possibility in vivo is appealing, such an approach is unlikely to provide a definitive answer. The confounding issue in experiments of this type is that the infused apolipoprotein A-I and CSL-111 would incorporate rapidly into the endogenous HDL fraction, where they would be remodeled by plasma factors. This would result in the generation of several subpopulations of HDL particles with the capacity to accept cellular cholesterol by multiple pathways.

In conclusion, this study provides mechanistic insights into the potential therapeutic benefit of apolipoprotein A-I and HDL infusions in arthritis. The results also suggest that HDL- and apolipoprotein A-I–raising agents that are currently being investigated in large-scale cardiovascular clinical outcome trials may be useful in other inflammatory disorders that can be refractory to conventional treatments.

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Disclosures
None.

References


**Significance**

The ability of high-density lipoproteins (HDLs) and the main HDL apolipoprotein A-I to inhibit vascular inflammation is well established. The present study shows that the anti-inflammatory properties of HDLs and apolipoprotein A-I extend beyond the vasculature to the inhibition of joint inflammation and joint destruction in an animal model of arthritis. These results were associated with reduced accumulation of inflammatory cells, as well as decreased expression of proinflammatory cytokines, toll-like receptor 2, and the master regulator of inflammation, nuclear factor-κB, in the joints. In vitro studies established that these effects of apolipoprotein A-I and HDLs were dependent on the ATP-binding cassette transporters A1 and G1 that export cholesterol from cells. When taken together, these results suggest that the HDL- and apolipoprotein A-I–raising agents currently being investigated in large-scale clinical cardiovascular outcome trials may also be beneficial for people with rheumatoid arthritis, especially those who are refractory to, or unable to tolerate, conventional therapies.
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MATERIALS AND METHODS

Inhibition of Arthritis in the Lewis Rat by Apolipoprotein A-I and Reconstituted High Density Lipoproteins

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Materials and Methods

Animal studies

Arthritis was induced in five groups of female Lewis rats (Groups 2, 3, 4 and 5, n=6-7/group, approximate weight 150 g, Animal Resources Centre, Perth, Australia) with a single intraarticular (ip) injection of peptidoglycan-polysaccharide (PG-PS, 15 mg/kg body weight, BD Pharmingen, Australia, Catalogue Number: 210866) (Supplemental Fig. 1). Control animals (n=7/group) received an equivalent volume of ip saline (Group 1).

The animals in Groups 1 (n=7/group) and 2 (n=7/group) received an intravenous (iv) saline infusion 1 day prior to and on days 1, 7, 9 and 11 after the ip saline or PG-PS administration (Fig. 1). The animals were treated with lipid-free apolipoprotein (apo) A-I as follows: (Group 3) two iv infusions of apoA-I (8 mg/kg) 24 h before and 24 h after the ip PG-PS injection, followed by three iv saline infusions on days 7, 9 and 11 after the PG-PS injection (n=6/group), (Group 4) two iv infusions of saline 24 h before and 24 h after the ip PG-PS injection, followed by three iv apoA-I (8 mg/kg) infusions on days 7, 9 and 11 post-PG-PS injection (n=6/group), and (Group 5) two iv infusions of apoA-I (8 mg/kg) 24 h before and 24 h after the ip PG-PS injection and three iv apoA-I infusions (8 mg/kg) on days 7, 9 and 11 post-PG-PS injection (n=3/group) (Supplemental Fig. 1). The iv infusions of saline and apoA-I were administered via a tail vein.

Joint inflammation was assessed by two blinded observers and quantified as a combined forepaw and hindpaw score using a standard joint inflammation score1: 0–no inflammation, 1–slight redness and swelling of the foot, 2–swelling of the foot such that the tendons are no longer visible, 3–swelling extending to the ankle joint and 4–gross inflammation and deformity of the ankle joint.

The ability of HDL to inhibit joint inflammation was investigated in an additional two groups of female Lewis rats (n=6/group) that received a single ip injection of PG-PS (15 mg/kg) and iv infusions of saline or a preparation of reconstituted HDL consisting of apoA-I (8 mg/kg) complexed with soybean phosphatidylcholine 24 h prior to and at days 1, 7, 9, 11 after the PG-PS injection. The reconstituted HDL preparation (CSL-111) was donated by CSL, Ltd, Parkville, Australia.

All the animals were sacrificed on day 21 after ip PG-PS or saline administration. Blood was obtained at sacrifice and plasma isolated by centrifugation. Synovial lavage fluids were collected after an intra-articular injection of saline (200 µL) into the ankle. The right hind ankles were removed and fixed in 4% (v/v) formaldehyde for immunohistochemical assessment. Synovial tissues were isolated from the left hind ankles. All of the procedures were approved by the Sydney Local Health Network Animal Welfare Committee (Approval Number 2008/011).

Immunohistochemistry

Tibiotalarsal joints were fixed for 4 days in formaldehyde, then decalcified in 0.3 M/L EDTA-Na₂ (pH 7.0) for 4 weeks2. The joints were dehydrated with 70% (v/v) alcohol and embedded in paraffin. Longitudinal sections (5 µm) were incubated overnight at 4 °C with a mouse anti-rat CD68 monoclonal antibody (1:100) (AbD Serotec, Raleigh, NC, Catalogue Number: MCA341R), treated with Horse Radish Peroxidase (HRP)-3,3' Diaminobenzidine (DAB) (Envision Mouse Kit, DAKO, Glostrup, Denmark, Catalogue Number: K4007), and counter stained with haematoxylin. The sections were imaged using an upright light microscope (Zeiss, Jena, Germany) at 50x magnification. DAB staining was quantified with ImageJ software (http://rsb.info.nih.gov/ij/). The threshold for positive staining was defined by an independent observer that was blinded to the treatment. Data are expressed as the intensity (pixels) of CD68 positive staining.

White blood cell count and quantification of cytokine levels

White blood cell (WBC), neutrophil and monocyte counts, were determined as described3. Briefly, total WBCs were manually counted using a hemocytometer. Neutrophils and
monocyte numbers were determined on blood smears treated with Diff Quik Stain (Lab. Aids, Australia).

Total synovial fluid WBCs, neutrophils and monocytes were manually counted at a high magnification (40x objective) using synovial fluid smears prepared with a Cytospin 4 cytocentrifuge (Thermo Scientific Cytospin 4, Kalamazoo, MI) and treated with Diff Quik Stain. Plasma and synovial fluid TNF-α, IL-1β and IL-6 levels were determined by ELISA according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, Catalogue Number: DY510, DY501 and DY506, respectively).

Flow cytometry
For WBC immunofluorescence staining, whole blood was treated with lysing buffer (Gibco by Life Technologies, Australia) to remove red blood cells. The samples were then incubated on ice for 30 min with FITC mouse anti-rat CD44 and/or PE mouse anti-rat CD11b/c antibodies (BD Pharmingen, Australia, Catalogue Number: 550974 and 554862, respectively), washed with PBS, fixed in 1% (v/v) paraformaldehyde and analyzed using a FACS Vantage flow cytometer (BD Biosciences, Franklin Lakes, NJ). Data was analyzed with Cell Quest software. Typically, 10,000 cells were analyzed based on forward versus side scatter gating.

Preparation of lipid-free apoA-I
High density lipoproteins (HDL) were isolated from pooled samples of normal human plasma (Healthscope Pathology, South Australia) by sequential ultracentrifugation (1.063<d<1.21 g/ml)¹. The HDL were delipidated and apoA-I was isolated by chromatography on a Q Sepharose Fast Flow column attached to an Äkta–FPLC system (GE Healthcare, Chalfont St Giles, Bucks, UK)². The purified apoA-I was lyophilized, reconstituted in 3 M/L guanidine hydrochloride/10 mM/L Tris/0.01% (w/v) paraformaldehyde and dialyzed against endotoxin-free PBS (pH 7.4) before use.

Cell Culture and transfections
Human monocyte-derived macrophages (HMDMs) were prepared using white cell buffy coats obtained from healthy donors (Australian Red Cross Blood Services, Sydney, Australia) in a Beckman Avanti J-20 XPI centrifuge as previously described³, then differentiated at 37 °C in RPMI (Roswell Park Memorial Institute) 1640 medium containing heat-inactivated human serum supplemented with L-glutamine (20 mM/L), penicillin (100 i.u./mL) and streptomycin (100 µg/mL) in a 5% CO₂ incubator over 9 days.

The HMDMs (1x10⁶ cells/well) were incubated for 16 h at 37 °C with PBS, lipid-free apoA-I (final concentration 0.25, 0.5 and 1.0 mg/mL) or CSL-111 (final apoA-I concentration 1.0 mg/mL) in serum-free RPMI 1640 medium. The medium containing apoA-I and CSL-111 was removed and the cells were washed with medium that did not contain apoA-I. Fresh medium that did not contain apoA-I was added to the cells, which were then stimulated for 6 h with PG-PS (final concentration 20 µg/mL). TLR2 and MyD88 mRNA levels were quantified by qPCR. The p65 subunit of nuclear factor (NF)-κB was quantified by western blotting. TNF-α, IL-1β and IL-6 levels in the culture media were determined by ELISA according to the manufacturer’s recommendations (R&D Systems, Minneapolis, MN).

TLR2 was silenced by transfecting HMDMs at 37 °C for 24 h with specific TLR2 siRNAs (Santa-Cruz Biotechnology, Catalogue Number: sc-40256) or scrambled siRNA (control) (Santa-Cruz Biotechnology, Catalogue Number: sc-37007). ABCA1, ABCG1 and SR-B1 were silenced by transfecting HMDMs at 37 °C for 48 h with specific ABCA1, ABCG1 and SR-B1 siRNAs (200 pmol, SMARTpool, mixture of four different target-specific sequences,) or scrambled siRNA (control) (Thermo Scientific, Lafayette, CO, Catalogue Number: L-008615-00-005, L-004128-00-0005, L-01592-00-0005 and D-001810-10-15, respectively) using the Opti-MEM/Lipofectamine system (Invitrogen, Carlsbad, CA, Catalogue Number: 772661).
Western Blotting
Nuclear proteins from HMDMs were isolated using the NE-PER extraction kit (Pierce, Rockford, IL, Catalogue Number: 78833), and lysed in 20 mM/L Tris buffer (pH 7.5) containing 0.5 mM/L EDTA-Na2, 0.5 mM/L EGTA-Na2 and protease inhibitors. The nuclear lysates were subjected to SDS-PAGE, transferred to nitrocellulose membranes (Invitrogen, Carlsbad, CA, Catalogue Number: NP0321) and incubated overnight with an NF-κB p65 rabbit anti-human polyclonal antibody (1:500) (Santa-Cruz Biotechnology, Catalogue Number: sc-372), or TLR2 mouse anti-human (1:200) (Santa-Cruz Biotechnology, Catalogue Number: sc-166900), ABCA1 mouse anti-human (1:500) (Abcam, Catalogue Number: ab18180), ABCG1 rabbit anti-human (1:500) (Abcam, Catalogue Number: ab52617), or SR-BI rabbit anti-human (1:200) (Epitomics, Catalogue Number: 1971-1) monoclonal antibodies using a mouse anti-human monoclonal antibody against β-actin (1:3000) (Sigma-Aldrich, Catalogue Number: A1978) as a loading control. Anti-rabbit and anti-mouse IgG-HRP (Santa-Cruz Biotechnology, Catalogue Number: sc-2004 and sc-2005, respectively) were used as secondary antibodies. Immunoreactive proteins were detected by ECL and analysed with Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA).

qPCR
Synovial tissues were incubated in RNAlater solution (Ambion, Austin, TX, Catalogue Number: 7020) at 4 °C for 24 h, then stored at -80 °C until use. Total RNA was isolated from the frozen tissues using TRIzol (Invitrogen, Carlsbad, CA, Catalogue Number: 15596018), and from HCAECs using TRI reagent (Sigma-Aldrich, Catalogue Number: 93289). RNA concentrations were normalized to 100 ng/µL using the SYBR Green II assay (Molecular Probes, Invitrogen, Carlsbad, CA, Catalogue Number: S7568) and reverse transcribed using iSCRIPT/iQ SYBR Green Supermix in a BioRad iQ5 thermocycler. Relative changes in mRNA levels were determined by the ΔΔCT method, using β-actin as a control. The PCR primers were: rat β-actin: Sense 5′-GCCCTGGCTCTAGCACC-3′, Antisense 5′-CCACCAATCCACACAGAATT-3′; rat TNF-α: Sense 5′-TACTGAATTTCCGGGTATTTGTC-3′, Antisense: 5′-CAGCCTTGTTCCAAGAGAAA-3′; rat TLR2: Sense 5′-CTCCTGTGAACTCCTGTCCTT-3′, Antisense: 5′-AGCTGTCTGGCAGTTGCAAC-3′; human β-actin: Sense 5′-GATCAGCTGACCGTATGAG-3′, Antisense 5′-GTCGTACTCCTGCTTGGT-3′, human TLR2: Sense 5′-GGCCAGCAATTACCTGTGTG-3′, Antisense 5′-AGGGGAACATCTGGAACCT-3′; human MyD88: Sense 5′-GAGCGGGATGTGCTTATC-3′, Antisense 5′-GGATCCGAGAAATCTGACAAATCAAC-3′; human ABCA1: Sense 5′-GAGGCTCCGGAGTGTGTTG-3′, Antisense 5′-GTATTTGAGCCCTCATTTGGA-3′; human ABCG1: Sense 5′-GCTACTGACCTGACAGCATC-3′; human SR-B1: Sense 5′-GTTCCCTGTGCATCTGCACC-3′, Antisense 5′-CTCCTATCTTGAGCCCTT-3′.

Statistics
Data are expressed as the mean±SEM. One-way ANOVA and the Newman-Keul’s post-hoc test were used to evaluate differences between groups. Statistical analyses were carried out using GraphPad Prism software Version 4.03 (GraphPad Software, Inc. San Diego, CA). A value of p<0.05 was considered significant. All the data fulfilled the criteria for normal distribution as determined using the Kolmogorov-Smirnov test (SPSS Software v21).
References


SUPPLEMENTAL MATERIAL

Inhibition of Arthritis in the Lewis Rat by Apolipoprotein A-I and Reconstituted High Density Lipoproteins

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**Supplemental Fig. I.** Schematic representation of the study design for the prevention of experimental arthritis in female Lewis rats by apoA-I. ip, intraperitoneal injection; iv, intravenous infusion.
Supplemental Fig. II. ApoA-I inhibits the PG-PS-mediated activation of HMDMs in a TLR2-dependent manner. HMDMs were transfected with scrambled siRNA (siControl [siCtrl], open bars) or TLR2 siRNA (siTLR2) (closed bars). Transfection efficiency was monitored by western blotting with anti-TLR2 antibodies using β-actin as a loading control (Panel A), and by TLR2 mRNA levels (Panel B). Transfected HMDMs were then incubated with PBS or apoA-I (final concentration 1 mg/mL) for 16 h, prior to stimulation for a further 6 h in the absence or presence of PG-PS (final concentration 20 µg/mL). TLR2 mRNA levels were quantified by qPCR (Panel C). TNF-α levels in the culture medium were determined by ELISA (Panel D). Data represent mean±SEM (n=3). N.D = below the level of detection. *p<0.05 vs relative HMDMs transfected with scrambled siRNA. #p<0.05 vs PG-PS-treated cells incubated with PBS.
Supplemental Fig. III. Transfection of HMDMs with ABCA1 siRNA, ABCG1 siRNA and SR-B1 siRNA: Effects on protein and mRNA levels. HMDMs were transfected with scrambled siRNA (siCtrl, open bars), ABCA1 siRNA (siABCA1), ABCG1 siRNA (siABCG1), or SR-B1 siRNA (siSR-B1) (closed bars). Transfection efficiency was monitored by western blotting with anti-ABCA1 (Panel A), ABCG1 (Panel B) and SR-B1 (Panel C) antibodies at 24, 48 and 72 h using β-actin as a loading control. ABCA1, ABCG1 and SR-B1 mRNA levels were quantified at 48 h (Panels D, E and F, respectively). Data represent the mean±SEM (n=3 for all conditions except for HMDMs transfected with ABCA1 siRNA, where n=2). *p<0.05 vs scrambled siRNA.
Supplemental Fig IV. CSL-111 inhibits PG-PS-induced TLR2 expression and NF-κB activation in HMDMs. HMDMs were pre-incubated for 16 h with PBS or CSL-111 (final apoA-I concentration 1.0 mg/mL), then incubated for a further 6 h in the absence (open bars) or presence of PG-PS (final concentration 20 µg/mL, closed bars). TLR2 (Panel A) and MyD88 (Panel B) mRNA levels were quantified by qPCR. Nuclear fractions of cell lysates were subjected to SDS-PAGE and Western blotting with an anti-NF-κB p65 subunit (p65) antibody using β-actin as a loading control. Data are expressed as the intensity of the p65 band relative to β-actin (Panel C). TNF-α (Panel D), IL-6 (Panel E) and IL-1β (Panel F) levels in the culture medium were determined by ELISA. Data represent mean±SEM of 3-6 independent experiments. *p<0.05 vs PG-PS-treated cells incubated with PBS.
Supplemental Fig. V. CSL-111 inhibits the PG-PS-mediated activation of HMDMs in an ABCA1- and ABCG1-dependent manner. TLR2 mRNA levels were determined in HMDMs transfected with scrambled siRNA (siControl, open bars), ABCA1 siRNA (siABCA1), ABCG1 siRNA (siABCG1), or SR-B1 siRNA (siSR-B1) (closed bars), then incubated with PBS or apoA-I (final concentration 1 mg/mL) for 16 h prior to stimulation for a further 6 h in the absence or presence of PG-PS (final concentration 20 µg/mL). Data represent mean±SEM of 3-6 independent experiments. *p<0.05 vs PG-PS-treated cells treated incubated with PBS.
Supplemental Fig VI. CSL-111 protects against PG-PS-induced arthritis in female Lewis rats. Female Lewis rats received a single ip injection of PG-PS (15 mg/kg) and iv injections of saline or CSL-111 (8 mg/kg apoA-I) 24 h prior to and at days 1, 7, 9, 11 after the PG-PS injection. Pictures and quantification of joint inflammation 21 days after the ip PG-PS or saline injection and iv infusion of saline (n=6, closed circles) or CSL-111 (n=6, open circles) are shown. Arthritis scores were determined as described in Materials and Methods. Data are expressed as mean±SEM. *p<0.05 vs PG-PS treated, saline infused rats.