RESEARCH ARTICLE | Hypertensive Disorders of Pregnancy: Effects on Mother and Baby

The protective effect of apolipoprotein in models of trophoblast invasion and preeclampsia

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Charlton F, Bobek G, Stait-Gardner T, Price WS, Mirabuto Colafella KM, Xu B, Makris A, Rye K, Hennessy A. The protective effect of apolipoprotein in models of trophoblast invasion and preeclampsia. Am J Physiol Regul Integr Comp Physiol 312: R40–R48, 2017. First published November 2, 2016; doi: 10.1152/ajpregu.00331.2016.—Preeclampsia is a hypertensive disorder of pregnancy. It is associated with abnormal placentalization via poor placental invasion of the uterine vasculature by trophoblast cells, leading to poor placental perfusion, oxidative stress, and inflammation, all of which are implicated in its pathogenesis. A dyslipidemia characterized by low plasma levels of high-density lipoproteins (HDL) and elevated triglycerides has been described in preeclampsia. Apolipoprotein A-I (apoA-I), a constituent of HDL and elevated triglycerides has been described in preeclampsia. Apolipoprotein A-I (apoA-I), a constituent of HDL is an anti-inflammatory agent. This study investigated whether apoA-I protects against hypertension and adverse placental changes in a proinflammatory cytokine (TNF-α)-induced model of preeclampsia. Further, this study investigated whether apoA-I protects against the inhibitory effect of TNF-α in a human in vitro model of trophoblast invasion. Administration of apoA-I to pregnant mice before infusion with TNF-α resulted in a significant reduction in the cytokine-induced increase in systolic blood pressure. MRI measurement of T2 relaxation, a parameter that is tissue specific and sensitive to physiological changes within tissues, showed a reversal of TNF-α-induced placental changes. Preincubation of endothelial cells with apoA-I protected against the TNF-α-induced inhibition of HTR-8/SVneo (trophoblast) cell integration into endothelial (UtMVEC) networks. These data suggest that a healthy lipid profile may affect pregnancy outcomes by priming endothelial cells in preparation for trophoblast invasion.

Preeclampsia, the most significant and common complication of pregnancy, is characterized by hypertension and end-organ dysfunction, predominantly proteinuria. A key factor in this condition’s pathogenesis has been shown to be poor placental invasion of the uterine vasculature by trophoblast cells (23), resulting in poor placental perfusion, placental oxidative stress, cellular damage and inflammation, and the release of antiangiogenic compounds into the maternal circulation (18). This leads to endothelial dysfunction and the maternal hypertensive response.

Dyslipidemia, characterized by increased triglycerides and a decreased level of high-density lipoproteins (HDLs), is associated with increased risk of developing preeclampsia (4, 5, 13, 16, 17). Although a number of studies have shown decreased plasma HDL levels early in pregnancy in cases of preeclampsia, there has been no work looking at the effect of HDL and its components on “appropriate” placental development and, thus, its potentially protective role in the prevention of preeclampsia. As HDLs are known to have anti-inflammatory and anti-oxidant properties (3, 19), the reduction of beneficial HDL and the main HDL apolipoprotein, apoA-I, may be a potential contributor to both the reduced uterine endothelial invasion, as well as to the widespread endothelial cell dysfunction observed in preeclampsia.

Women with preeclampsia display an alteration in the cytokine balance toward an inflammatory response. They have increased levels of TNF-α in both serum and placental tissue (24, 27) and have reduced serum and placental expression of IL-10 (11). A continuous low-dose intravenous infusion of the inflammatory cytokine TNF-α has been demonstrated to induce hypertension in pregnancy in rats (1), baboons (25), and mice (7). Cellular work suggests that this may be a consequence of disruption of normal placental development. Studies using a human trophoblast-endothelium cell interaction model, demonstrated that TNF-α can interfere in vitro with trophoblast migration and integration into endothelial cellular networks (28). This is thought to mimic the early cell-to-cell placental interaction critical to normal placental development.

In vivo placental changes in mouse models of preeclampsia have been investigated using MRI (6). A major source of image contrast in MRI studies performed without the use of exogenous contrast agents comes from variation in spin relaxation times of the 1H nuclei, which is largely a function of their...
molecular motion and environment. Relaxation times are, thus, tissue-specific, reflecting differences in tissue composition, water content, oxygen content, and pH. It was reported that high-resolution maps based on \( T_2 \) (spin-spin or transverse) relaxation times in MRI scans of live mouse placenta clearly differentiated between distinct regions of the placenta and that \( T_2 \) values and ratios between regions were sensitive to the physiological changes within the tissue, such as loss of blood flow or TNF-\( \alpha \) infusion (6).

This study investigates whether apoA-I protects against inflammatory cytokine-induced hypertension in pregnancy and examines whether the in vivo placental changes observed by MRI after TNF-\( \alpha \) infusion are ameliorated by administration of apoA-I. This study further examines whether apoA-I provides protection against the deleterious effects of TNF-\( \alpha \) in a human in vitro model of trophoblast invasion and determines whether this protection is provided by modulation of adhesion molecules and markers of invasion that are implicated in trophoblast invasion of the uterine vasculature, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) (26), integrin \( \alpha_\delta \beta_\delta \), integrin \( \alpha_\beta_1 \), epithelial cadherin (E-cadherin), and vascular endothelial cadherin (VE-cadherin) (29).

**MATERIALS AND METHODS**

**Animals.** C57BL/6J Arc mice were obtained from the Animal Resource Centre (Canning Vale, Western Australia, Australia) and were housed in a temperature-controlled room in individually ventilated cages (up to five per cage) maintained in a 12:12-h light-dark cycle with ad libitum access to water and standard rodent chow. All procedures have been approved by the University of Western Sydney Animal Care and Ethics Committee and follow the “Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes” as laid out by the National Health and Medical Research Council of Australia. Radiotemporers were surgically inserted into nonpregnant mice (\( n = 12 \)) for continuous blood pressure measurement, or animals were left without telemeters (\( n = 12 \)) for MRI. Both sets of animals were time-mated, and on \( \text{day 10} \) of gestation, they were randomly assigned to one of four groups: saline infusion (\( n = 3 \)); TNF-\( \alpha \) infusion (500 ng kg\(^{-1}\) day\(^{-1}\)) (\( n = 3 \)); TNF-\( \alpha \) infusion (500 ng kg\(^{-1}\) day\(^{-1}\)) + saline injection (\( n = 3 \)); and TNF-\( \alpha \) infusion (500 ng kg\(^{-1}\) day\(^{-1}\)) + apoA-I injection (40 mg/kg) (\( n = 3 \)).

Preparation of apoA-I from human plasma. Total HDLs were isolated from pooled, autologously donated human plasma (Healthscope, Adelaide, South Australia) by sequential ultracentrifugation and density adjustment (density range: 1.063–1.21 g/ml) with solid potassium bromide (KBr) (10). The HDLs were then delipidated, according to the method of Osborne (21) using chloroform, methanol, and diethyl ether. The resulting apolipoproteins were dried under nitrogen, dissolved in 20 mM Tris (pH 8.2), and lyophilized. The apoA-I was resolved by chromatography on a Q-Sepharose Fast Flow column using an AKTA-FPLC system (GE Healthcare, New York, NY). The apoA-I-containing fractions were pooled, dialyzed against 20 mM NH\(_4\)HCO\(_3\), lyophilized, and stored at \(-20^\circ\)C until use. Prior to use, the purified apoA-I was reconstituted in 10 mM Tris/3 M GdnHCl (pH 8.2) and dialyzed extensively against endotoxin-free PBS. The purity of the reconstituted apoA-I was confirmed by electrophoresis on 20% SDS-polyacrylamide Phast gels with Coomassie Blue R-350 staining, and it was tested for the presence of endotoxin (Endosafe-PTS endotoxin-testing unit). Prior to use in the cell coculture experiments, the lipid-free apoA-I was further dialyzed against EGM-2 MV media.

Measurement of blood pressure by radiotelemetry. Animals subject to blood pressure (BP) measurements by radiotelemetry had a BP-transducing catheter (TAIL PA-C10; Data Sciences International, Minneapolis, MN) implanted into their left carotid artery and extended into the aortic arch, with the transmitter body inserted subcutaneously on the right flank. Surgical anesthesia was induced via an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and was monitored by a decrease in respiratory rate and pedal reflex. Analgesia was administered preoperatively by a subcutaneous injection (buprenorphine: 0.1 mg/kg). Animals were then housed individually and allowed to recover (10 days); then, arterial pressures and heart rate were measured for 3 days before mating (baseline) and throughout pregnancy by sampling for 10 s every 10 min. As resting BP measurements were used for the study, data collected during the night, which is the period of greatest activity, were excluded from analysis. All data accrued at an activity level greater than 9 AU were also excluded from analysis. Activity is calculated and measured in terms of distance of the telemeter (implanted in the mouse) from the receiver (positioned under the mouse cage). Data were averaged over the 12:12-h light (resting) period and normalized to gestational day (gd) 9 baseline for each animal and presented as means ± SE.

Tail vein injection of apoA-I and saline. Endotoxin-free apoA-I (150 µl, 40 mg/kg) or saline (150 µl, 0.9%) was administered intravenously via the tail vein on gd 10.5 and gd 12.5 before TNF-\( \alpha \) infusion on gd 13.5.

TNF-\( \alpha \) infusion. A miniosmotic pump (model 1007D; Alzet, Cupertino, CA) that released mouse recombinant TNF-\( \alpha \) (500 ng kg\(^{-1}\) day\(^{-1}\)) (Bio Scientific, Kirrawee, NSW, Australia) was implanted below the right scapulae on gd 13.5. Surgical anesthesia and analgesia were administered as described above. Control animals received a saline implant.

Magnetic resonance imaging. On gd 17.5 of gestation, anesthetized mice were placed in a vertical animal probe and \( ^1 \)H MRI images were obtained using a Bruker Avance 11.74 Tesla wide-bore spectrometer with microimaging probe capable of generating gradients of 0.45 T/m. Anesthesia was induced by inhalation of 4% isoflurane, then 2% for maintenance, before transferring the animals in a supine position to the probe, which was maintained at 28°C. A small collar was used to maintain the head in a vertical position during scanning. A pressure-sensitive pillow was taped to the abdomen to monitor respiration, and the mice were wrapped for insulation. The probe was inserted vertically into the scanner, and the isoflurane concentration was reduced to 1.1–1.7%, so that the respiration rate was ~50–60 breaths per minute. Sequence acquisition was gated on respiration (model no. 1025; Small Animal Monitoring and Gating System, SA Instruments, Stony Brook, NY) to reduce motion artifacts.

A gradient echo fast imaging (GEFI) sequence protocol was used to obtain a series of images across the abdomen to identify the location of the placenta. Thirty contiguous 1-mm slices, with an in-plane resolution of 0.25 mm in either the axial or coronal plane and high-resolution images (in plane resolution of 0.12 mm) were obtained of selected slices. \( T_2 \) measurements using the same geometry were also acquired using a multi-slice multi-echo (MSME) sequence protocol (Bruker MSME-MT-map) with an in-plane resolution of 0.1–0.2 mm. MatLab (The Mathworks, Natick, MA) was used to generate \( 1/T_2 \) maps from the acquired data, and \( T_2 \) values were calculated from three points in each selected region of interest within 2–5 individual placentas. To compare and quantify the change in contrast between the placental regions, the ratio of \( T_2 \) to \( \text{labyrinth to } T_2 \text{ junctional zone was calculated for each placenta.} \)

Cell lines and fluorescent labeling of cells. Primary extravillous trophoblast HTR-8/SVneo cells, which were kindly provided by Prof. C. H. Graham (Queen’s University, Kingston, ON, Canada), were cultured in Roswell Park Memorial Institute medium (RPMI) 1640 with 5% (vol/vol) BCS. Human uterine microvascular endothelial cells UIMVECs (Lonza, Basel, Switzerland) were cultured in EGM-2 MV media until 80% confluent. Cells from passages 6–9 were used in this study. As per previous publications, cells were cultured under normoxic conditions (O\(_2\) tension of 21%) at 37°C with 5% CO\(_2\) (28).

**PROTECTIVE EFFECT OF APOLIPOPROTEIN IN PREGNANCY**

**R417**

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Prior to coculture with UtMVECs, HTR-8/SVneo cells (2 × 10⁶ cells) were labeled with the green fluorophore, PKH67 (Sigma Aldrich, Castle Hill, Australia), according to the manufacturer’s instructions. For the live imaging experiments, UtMVECs were labeled with PKH26 (red, Sigma Aldrich, Castle Hill, Australia) before the preincubation step.

Preincubation of UtMVECs with apoA-I, or PBS. The UtMVECs (passage 6–9) were cultured to 80% confluency, washed with PBS, and then preincubated for 16 h (final volume 10 ml) with either PBS, or apoA-I (final apoA-I concentration, 1 mg/ml) in EGM-2 MV media. These concentrations have been previously described to have anti-inflammatory properties (8) and are of the magnitude seen in human plasma. Following preincubation, the media were removed, and the cells were washed with PBS and then used for the coculture experiments.

Coculture of human trophoblast (HTR-8/SVneo cells) with human endothelial cells (UtMVECs) on Matrigel. Tissue culture plates (24 wells) were coated with 300 μl/well undiluted Matrigel (BD Biosciences) and were polymerized for 30 min at 37°C. Preincubated UtMVECs (75,000 cells/well) were seeded into each well, and endothelial cell tubular structures were allowed to form (4-h incubation at 37°C). PKH67-labeled HTR-8/SVneo cells (75,000 cells/well) were then added to each well and cocultured with the UtMVECs in the presence and absence of human recombinant TNF-α (final concentration 0.1 ng/ml) (Sigma-Aldrich, Castle Hill, Australia) representing a presence and absence of human recombinant TNF-α (final concentration 0.1 ng/ml) (Sigma-Aldrich, Castle Hill, Australia) before the preincubation step.

Visualization and quantification of cell integration. Integration of HTR-8/SVneo cells into the tubular endothelial networks was visualized by bright-field and fluorescence microscopy (Zeiss 71X, North Ryde, NSW, Australia) and was quantified using ImageJ Software (National Institutes of Health). The total tubular endothelial networks area was measured from bright-field images, and the area of the HTR-8/SVneo migration into the tubular network was measured from the overlaid green fluorescent images. All images were converted to 8-bit grayscale using a defined preset threshold. After conversion into binary black or white images, the network area in each image was calculated as the number of pixels and converted to μm². The ratio of green fluorescence area-to-total endothelial network area was calculated and represents the proportion of the tubular endothelial network with HTR-8/SVneo cell integration. Ratios were converted to percentage, normalized to control experiments (preincubation with PBS and absence of TNF-α), and expressed as means ± SE. Real-time tracking of trophoblast integration into endothelial networks was performed by capturing bright-field and red (UtMVEC) and green (HTR-8/SVneo) fluorescently labeled live cell images over 20 h of coculture with a Zeiss Axiosvert Live Cell Imager (Zeiss Axiovert, North Ryde, NSW, Australia).

RNA extraction and quantitative-PCR analysis. The cocultured cells were retrieved from the tubular structures in Matrigel using BD cell recovery solution (CRS) (500 μl/well; BD Biosciences), according to the manufacturer's instructions. Total RNA was extracted from the cell pellets using a Qiagen RNA extraction kit (Qiagen, Doncaster, Australia), according to the manufacturer's instructions, and the cDNA was reverse transcribed from total RNA (50 ng) using an iSCRIPT cDNA synthesis kit (Bio-Rad), according to the manufacturer’s protocol and carried out in triplicate in a MasterCycler (Eppendorf). Samples were stored at −20°C until use. The cDNA was reverse transcribed from total RNA (50 ng) using an iSCRIPT cDNA synthesis kit (Bio-Rad), according to the manufacturer’s protocol and carried out in triplicate in a MasterCycler (Eppendorf). Samples were stored at −20°C until use. The cDNA was reverse transcribed from total RNA (50 ng) using an iSCRIPT cDNA synthesis kit (Bio-Rad), according to the manufacturer’s protocol and carried out in triplicate in a MasterCycler (Eppendorf). Samples were stored at −20°C until use. The cDNA was reverse transcribed from total RNA (50 ng) using an iSCRIPT cDNA synthesis kit (Bio-Rad), according to the manufacturer’s protocol and carried out in triplicate in a MasterCycler (Eppendorf). Samples were stored at −20°C until use.

Table 1. Genes, primer sequences, and annealing temperature for quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer Sequence</th>
<th>Reverse Primer Sequence</th>
<th>Ta, °C</th>
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<tr>
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<td>TCTAACTCTAATGATTTCTCTCC</td>
<td>TCTCATCTGTCACATAAACCC</td>
<td>54</td>
</tr>
<tr>
<td>Integrin-β₁</td>
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<td>AGCTCTGAGAGGGCTGCTCTAC</td>
<td>54</td>
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<tr>
<td>Integrin-β₄</td>
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<td>AACTGATCCACATCTCTTC</td>
<td>54</td>
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<tr>
<td>E-cadherin</td>
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<td>55</td>
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<tr>
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<td>60</td>
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<tr>
<td>β-actin</td>
<td>ATGGGGCTGAGGCTTGTGTTT</td>
<td>AGTGGGCTGAGGCTTGTGTTT</td>
<td>60</td>
</tr>
</tbody>
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Ta, annealing temperature.

Fig. 1. Systemic apoA-I protects against inflammatory cytokine-induced hypertension in pregnancy. Change in systolic blood pressure of saline-infused control (n = 5, red), TNF-α infused (n = 5, blue), TNF-α infused + apoA-I infused (n = 3, turquoise) and TNF-α infused + saline infused (n = 3, gray) mice from gestation day (gd) 9 to 17. Intravenous injections of apoA-I or saline were administered on gd 10 and 12 before TNF-α infusion from gd 13.5. Data are expressed as means ± SE. Differences between groups were assessed by one-way ANOVA with Dunnett’s multiple-comparison test as the post hoc test. *P < 0.05 TNF-α vs. TNF-α + apoA-I at gd 14. **P < 0.05 TNF-α vs. TNF-α + apoA-I at gd 17. *P < 0.05 saline control vs. TNF-α at gd 14. #P < 0.05 saline control vs. TNF-α at gd 17.
was used to evaluate the differences in $T_2$ values and the $T_2$ labyrinth/$T_2$ junctional zone ratios. Data are expressed as means ± SE with the level of significance being $P < 0.05$. A logarithmic transformation was carried out on ratios before statistical analysis (6).

Statistical significance of cell integration studies were calculated using the nonparametric Mann Whitney $U$-test. Although the overall significance was set at 0.05, the sharpened Bonferroni method was used to adjust the individual $P$-level when multiple comparisons were performed. Statistical analysis of BP studies and quantitative PCR

Fig. 3. Systemic apoA-I restores the pattern of $T_2$ contrast in murine placenta. Comparison of the $T_2$ labyrinth/$T_2$ junctional zone ratios between saline-infused control; TNF-α-infused, TNF-α infused + saline-injected, and TNF-α-infused + apoA-I-injected animals at day 17.5 of gestation. $T_2$ values are calculated from three points in each region, from two to five placentas per animal, and three animals per group. Data are expressed as means ± SE and analyzed by generalized estimating equation modeling, clustering placenta within animals, and animals within treatment groups. *$P < 0.001$, **$P < 0.05$, ***$P < 0.05$.

Fig. 4. Pretreatment with apoA-I protects against TNF-α-induced disruption of integration of HTR-8/SVneo trophoblast cells into UMVEC endothelial networks. UMVECs were preincubated for 16 h with PBS or lipid-free apoA-I (final apoA-I concentration 1 mg/ml) before seeding on Matrigel-coated plates and allowed to form tubular networks. After 4 h, HTR-8/SVneo cells were added to the wells and cocultured with the UMVECs in the presence or absence of TNF-α (final concentration 0.1 ng/ml). After 20 h of coculture, fluorescent and bright-field images were captured and analyzed for the proportion of tubular endothelial network with HTR-8/SVneo cell integration. Data are normalized to control cocultures and expressed as means ± SE ($n = 6$). Statistical significance was calculated using the nonparametric Mann Whitney $U$-test *$P < 0.001$ **$P < 0.001$. 

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**Fig. 2.** Representative MRI images showing patterns of contrast in pregnancies. The pattern of contrast in the placenta (arrows) is altered in TNF-α-infused animals. $T_2$ map of a gestation day 17.5 mouse showing contrast in the placenta of a normal pregnant mouse (A), a saline-injected, TNF-α-treated mouse (B), a TNF-α-treated (C), and a TNF-α-treated mouse injected with apoA-I (D). [Panels A and C were reproduced from Bobek et al. 2013; this article was published in an open-access journal, which has distributed this article under the Creative Commons Attribution License; see http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0059971.].

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were performed using GraphPad Prism version 5.0d for Mac OSX (GraphPad Software, San Diego CA, www.graphpad.com). Differences between treatment groups were assessed by one-way ANOVA with Dunnett’s multiple-comparisons post hoc test, with $P < 0.05$ being considered significant.

RESULTS

Systemic apoA-I protects against inflammatory cytokine-induced hypertension in pregnancy. Experimental animals infused with TNF-α showed a significant increase in systolic BP (16.2 mmHg ± 1.71 mmHg) compared with saline-infused control animals (−5.71 mmHg ± 1.49 mmHg) ($P < 0.05$) at gestational day 17 (gd 17) (Fig. 1). The increase in BP was already evident on gd 14, the day following the insertion of the TNF-α osmotic pump [12.39 mmHg ± 0.99 mmHg (TNF-α)] compared with 3.93 mmHg ± 1.22 mmHg (Control)] $P < 0.05$.

Administration of two intravenous injections of apoA-I at gd 10 and gd 12, before the TNF-α infusion at gd 13, resulted in a significant reduction in the cytokine-induced increase in systolic BP at gd 17 (7.59 mmHg ± 0.85 mmHg, $P < 0.05$), which emerged as early as gd 14 (3.85 mmHg ± 0.83 mmHg, $P < 0.05$). Administration of two intravenous injections of saline before TNF-α infusion showed an inconsistent pattern, not significantly different from TNF-α-infused animals at gd 15 and 16 but significantly different at gd 14 (3.26 ± 0.85 mmHg, $P < 0.05$) and gd 17 (8.28 ± 1.25 mmHg) ($P < 0.05$).

Systemic apoA-I restores pattern of $T_2$ contrast in murine placenta. Three distinct morphological regions of contrast based on $T_2$ relaxation times were clearly discernable in the MRI images of the mouse placenta at gd 17.5. These regions correlate to the labyrinth, junctional zone, and decidua (Fig. 2). As previously reported, $T_2$ contrast between placental regions is sensitive to perfusion and the ratio of $T_2$ labyrinth to $T_2$ junctional zone can be used to quantify these changes (6).

In saline-infused control mice the ratio of $T_2$ labyrinth to $T_2$ junctional zone was 2.35 ± 0.06 ($n = 3$), whereas TNF-α infusion significantly reduced the contrast between regions with the ratio of $T_2$ labyrinth to $T_2$ junctional zone decreasing to 1.74 ± 0.19 ($n = 3$, $P < 0.001$) (Fig. 3), indicating blood flow-sensitive changes, as previously reported (6). The treatment with apoA-I on gd 10 and 12 before TNF-α infusion restored the $T_2$ labyrinth-to-$T_2$ junctional zone ratios to those that were not significantly different to saline-infused control
mice, 2.164 ± 0.053 (n = 3, P = 0.182), indicating a protective effect of apoA-I against the deleterious effects of TNF-α infusion on placental blood flow. TNF-α infused + saline-injected mice had T2 labyrinth-to-T2 junctional zone ratios that were not significantly different to TNF-α-infused values, 1.81 ± 0.05 (n = 3, P = 0.307) but were significantly smaller than TNF-α-infused + apoA-I-injected values (P < 0.05) (Fig. 3).

Preincubation with apoA-I protects against the deleterious effects of TNF-α on human trophoblast-endothelium cell interaction. The effect of apoA-I on trophoblast incorporation into endothelial tubules was examined. In the presence of TNF-α, integration of HTR-8/SVneo primary extravillous trophoblast cells into human uterine microvascular endothelial cells (UtMVEC) networks was significantly decreased (82% ± 3.7%) compared with control cells (100%, P < 0.001) (Fig. 4). However, when the HTR-8/SVneo cells were cocultured with UtMVECs that had been preincubated with apoA-I, the inhibitory effect of TNF on trophoblast incorporation into endothelial networks was abolished (n = 6, P < 0.001) (Fig. 4). The bright-field and fluorescent images of the integration of the HTR-8/SVneo cells into the endothelial networks after 20 h are presented in Fig. 5. In the absence of TNF-α apoA-I had no effect on the integration of trophoblasts into the endothelial cell network (data not shown).

The incorporation of the trophoblast cells into the endothelial network were followed by live cell imaging (Fig. 6). In the absence of TNF-α, the trophoblast cells exhibit directional movement toward the endothelial cells and were fully incorporated into the endothelial network by 7 h (Fig. 6, A–C). In the presence of TNF-α the trophoblast lost their directional movement with little incorporation into the endothelial network occurring during the 7 h (Fig. 6, D–F). Pretreatment of endothelial cells with apoA-I restored the directional movement of the trophoblasts and as shown in Fig. 5C, they become fully integrated into the endothelial network.

Preincubation with apoA-I alters the gene expression of adhesion molecules and invasion markers in cocultured cells. The gene expression of cell adhesion markers and other molecules involved in trophoblast invasion (integrins α1β1 and α6β4, E-cadherin, VE-cadherin, ICAM-1, and VCAM-1) were examined in the cocultured cells in the absence and presence of TNF-α and apoA-I after both 6 h and 20 h of coculture (Fig. 7). The presence of TNF-α alone in the cocultures resulted in a significant increase in gene expression of ICAM-1 and VCAM-1 after 20 h of coculture. Only VCAM-1 gene expression was increased at 6 h. There was no significant change in integrins α1β1 and α6β4, E-cadherin, VE-cadherin, at either time point. A reduction in the TNF-α-induced gene expression of VCAM-1 (P < 0.05) was observed in cocultured cells after 20 h in the endothelial cells that had been pretreated with apoA-I before incubation with TNF-α (P < 0.05). This reduction was not yet evident after 6 h of coculture. Despite any upregulation of integrin α1β1 and E-cadherin in the presence of TNF-α alone, preincubation with apoA-I resulted in a significant decrease in their expression (P < 0.05) after 20 h coculture. At the earlier time point, an initial increase in integrin α6β4 gene expression was observed. No significant changes were observed in the gene expression of integrin α1β1, or VE-cadherin.

DISCUSSION

This study explored the potential of apoA-I, a major lipoprotein constituent of HDL, for improving or reversing the hypertension and placental changes observed in an animal model of inflammatory cytokine-induced hypertension in pregnancy. It further examined whether apoA-I has a protective effect in an in vitro model of human trophoblast invasion and whether this is related to alterations in adhesion molecules and markers of invasion.

This study found that intravenous administration of lipoprotein apoA-I before the inflammatory cytokine insult partly reversed the TNF-α-induced increase in blood pressure in pregnant mice. Administration of saline before TNF-α infusion appears to also have a partial effect in decreasing BP, but unlike apoA-I, this was not sustained over the whole period of the TNF-α infusion. These saline changes are likely due to blood volume effects. MRI measurement of T2 relaxation, a parameter that is tissue-specific and sensitive to physiological changes within tissues, showed a reversal of TNF-α-induced placental changes. The results from our complementary in vitro
Fig. 7. Pretreatment with apoA-I alters the gene expression of adhesion molecules and invasion markers in cocultured cells. Relative gene expression of integrin αβ1 (A and B), integrin α6β4 (C and D), E-cadherin (E and F), VE-cadherin (G and H), ICAM-1 (I and J), and VCAM-1 (K and L) in UtMVEC/HTR-8/SVneo cocultured cells after preincubation of endothelial cells with apoA-I or PBS (control) and subsequent coculture with trophoblast cells in the presence or absence of TNF-α for 6 h (A, C, G, K) or 20 h (B, D, F, H, J, L). Data are normalized to control cocultures, expressed as means ± SE, and are analyzed by one-way ANOVA with Dunnett’s multiple comparisons as the post hoc test. **P < 0.01; n = 6.

studies indicated that apoA-I provides protection against the deleterious effects of TNF-α on human trophoblast-endothelium cell interaction.

The protective effect conferred by apoA-I on the cytokine-induced hypertension in pregnancy is likely to be due to tissue uptake rather than continuous presence in the bloodstream. The animals received the final apoA-I injections 24 h before TNF-α infusion. Kinetic studies by others have shown that after intravenous tail vein injection of apoA-I, less than 10% of the injected dose remains in the bloodstream after 24 h with the majority (~70%) cleared after only 5 h (14). The MRI data, showing that apoA-I has physiological effects on the placenta that can be detected by MRI using $T_2$ relaxation measurements, suggests that at least some of the tissue uptake is by the placenta. The results presented here demonstrate that the ratio of $T_2$ labyrinth to $T_2$ junctional zone, is significantly decreased by TNF-α infusion, indicating blood flow-sensitive changes, as previously reported (6). However, this change is ameliorated by the injection of apoA-I, indicating a protective effect of apoA-I against the deleterious effects of TNF-α infusion on placental blood flow.

The results from our in vitro work indicated that apoA-I provides protection against the detrimental effects of TNF-α on human trophoblast-endothelium cell interaction. While HTR8/SVneo cells form tubules when cultured on their own on Matrigel [data not shown and (12)], our observations show that when cocultured with UtMVEC, rather than with their own independent tubules, they exhibit directional movement toward endothelial cells and integrate into the existing endothelial networks. These results confirm that the proinflammatory cytokine TNF-α disrupts the movement of trophoblast cells toward endothelial cells and diminishes the extent of trophoblast integration into vascular networks of uterine endothelial cells (28). Preincubation of uterine endothelial cells with apoA-I, a component of HDL, reverses the inhibitory effect of TNF-α on trophoblast-like cell integration into endothelial cellular networks. The trophoblast cells retain their directional movement toward the endothelial cells despite having no direct contact with apoA-I. This suggests that a chemotactic signal is released by the endothelial cell or a cell-signaling pathway is activated to coordinate integration of the trophoblast cells, and that apoA-I protects against disruption of this by TNF-α.

These experiments explored the potential mechanism of protection by apoA-I focusing on key molecules involved with invasion and phenotype switching. In preeclampsia, the invading cytotrophoblasts fail to switch expression of adhesion receptors to those that are expressed by vascular cells, integrin α1β1, and VE-cadherin, instead retaining integrin α6β4 and E-cadherin. Defective remodeling may be due to a failure of the cells to mimic a vascular adhesion phenotype (29). Cocultures of UtMVECs with HTR-8/SVneo trophoblast cells showed that despite an insult with TNF-α, when endothelial cells are pretreated with apoA-I, the switch to the more invasive phenotype was preserved. The analysis of gene expression of cell adhesion molecules and markers establishes that apoA-I shifts the $α_1β_1$-to-$α_6β_4$ ratio to one that favors an invasive trophoblast phenotype. This is apparent from the reduction in $α_6β_4$ despite an absence of change in integrin $α_1β_1$. Similarly, the reduction in E-cadherin mRNA levels after pretreatment with apoA-I indicates a preservation of the switch to the more invasive phenotype despite no apparent change in VE-cadherin mRNA levels. While the aim of this study is the examination of gene expression upon interaction of the endothelial and trophoblast cells, this is at the same time a limitation because if one of the cell types has constitutive high levels of expression such as VE-cadherin on endothelial cells, then any modulation in expression on the other cell type may be hidden.

It is interesting to note that at the 6-h time point, integrin $α_6β_4$ shows increased gene expression. This is possibly due to the
introduction of the trophoblast cells into the endothelial network only being partially complete at this stage.

In preeclampsia, circulating levels of VCAM-1 are significantly higher than in normal pregnancy, indicating an overall proinflammatory systemic response (2, 26). VCAM-1 is rapidly induced by the inflammatory cytokines IL-1 and TNF-α (20), and HDLs are known to inhibit TNF-α-induced VCAM-1 expression in endothelial cells (9). Reductions in TNF-α-induced gene expression of VCAM-1 in the cocultured cells after preincubation of endothelial cells with apoA-I indicates that apoA-I may also promote changes in gene expression toward a less inflammatory and, thus, a more favorable environment for placental development.

In conclusion, the results presented here demonstrate the protective effect conferred by apoA-I on the inflammatory cytokine-induced hypertension in a pregnant animal and suggest that this is likely to be due to tissue uptake and protection against inflammatory cytokine-induced alterations in the placenta. These results confirm the sensitivity of VCAM-1 toward a less inflammatory and, thus, a more favorable environment for placental development.

Perspectives and Significance

The beneficial effect of HDL in protecting women from placental diseases, such as preeclampsia, is likely to be conferred by apolipoprotein elements and by reducing inflammation at a tissue level. This has been associated in this study with an improvement in maternal physiological response to placental inflammation by control of hypertension. Identification of mechanisms of healthy placental growth will help us develop strategies to treat and prevent common maternal diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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