Cell-derived Apolipoprotein E (ApoE) Particles Inhibit Vascular Cell Adhesion Molecule-1 (VCAM-1) Expression in Human Endothelial Cells*

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Sub-endothelial infiltration of monocytes occurs early in atherogenesis and is facilitated by cell adhesion molecules that are up-regulated on activated endothelium. Apolipoprotein E (apoE) helps protect against atherosclerosis, in part, because apoE particles secreted by macrophages have local beneficial effects at lesion sites. Here, we hypothesize that such protection includes anti-inflammatory actions and investigate whether cell-derived apoE can inhibit tumor necrosis factor-α-mediated up-regulation of vascular cell adhesion molecule-1 (VCAM-1) in human umbilical vein endothelial cells (HUVECs). Two models were used to mimic endothelial exposure to macrophage-derived apoE. In the first, HUVECs were transiently transfected to secrete apoE; VCAM-1 induction inversely correlated with secretion of apoE into the media \( (r = -0.76, p < 0.001) \). In the second, incubation of HUVECs with media from recombinant Chinese hamster ovary (CHO) cells expressing apoE (CHOapoE) also reduced VCAM-1 in a dose-dependent manner \( (r = -0.76, p < 0.001) \). Characterization of CHOapoE cell-derived apoE revealed several similarities to apoE particles secreted by human blood monocyte-derived macrophages. The suppression of endothelial activation by apoE most likely occurs via stimulation of endothelial nitric oxide synthase; apoE increased levels of intracellular nitric oxide and its surrogate marker, cyclic guanosine monophosphate, while the nitric oxide synthase inhibitor, ethylisothiourea, blocked its effect. We propose that apoE secreted locally at lesion sites by macrophages may be anti-inflammatory by stimulating endothelium to release NO and suppress VCAM-1 expression.

Early in atherogenesis circulating monocytes are recruited to the arterial sub-endothelium where they differentiate into macrophages, ingest cholesterol, and develop into “foam cells” (1). Initially, monocytes adhere to activated endothelium on which up-regulated cell adhesion molecules (CAMs) are displayed, a dynamic process sensitive to inflammatory cytokines, shear stress, and oxidative insults (2). Induction of vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin superfamily of CAMs, is increasingly described as the key factor in monocyte infiltration (3, 4).

Apolipoprotein E (apoE) is a 34-kDa polypeptide synthesized mainly by liver and helps protect against atherosclerosis, in part, by mediating hepatic clearance of remnant plasma lipoproteins (5). When apoE is absent or dysfunctional, severe hyperlipidemia and atherosclerosis ensue, while infusion of apoE or hepatic gene overexpression protect (6, 7). ApoE is also abundant in atherosclerotic lesions, secreted by resident cholesterol-loaded macrophages (6). This locally produced apoE is atheroprotective by contributing to reverse cholesterol transport (8), inhibiting smooth muscle cell proliferation (9), preventing oxidation (10), and restricting platelet aggregation (11).

ApoE-deficient mice have elevated VCAM-1 in aortic lesions (3), which enhances monocyte recruitment and adhesion (12), while apoE expression in the artery wall reduces early foam cell lesion formation (13). These findings imply that apoE may influence early inflammatory responses by suppressing endothelial activation and CAM expression. However, we found that plasma-purified apoE did not suppress VCAM-1 induction in cultured human umbilical vein endothelial cells (HUVECs) (14). Nevertheless, if apoE affects CAM expression and atherogenic events in vivo, then most likely it will be released at lesion sites by recruited macrophages. Here, we devise two models to mimic endothelial exposure to locally synthesized, cell-derived apoE and show, in both cases, that apoE limits cytokine-mediated VCAM-1 up-regulation in a dose-dependent manner.

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The abbreviations used are: CAM, cell adhesion molecule; VCAM, vascular cell adhesion molecule; apo, apolipoprotein; HUVECs, human umbilical vein endothelial cells; FBS, fetal bovine serum; CHO, Chinese hamster ovary; MDM, monocyte-derived macrophage; ELISA, enzyme-linked immunosorbent assay; TNF, tumor necrosis factor; HDL-E, apoE-rich high-density lipoproteins; NOS, nitric oxide synthase; ethyl-ITU, 2-ethyl-2-thiopseudourea; W-F, von Willebrand factor; apoER2, apolipoprotein E receptor 2; HSPG, heparan sulfate proteoglycan; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; LDL-R, low-density lipoprotein receptor.

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Cell Culture—HUVECs were isolated, characterized and cultured as described previously (14). Cells were maintained in M199 media with Hank's salts containing 3.6 mM glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin, 20% (v/v) fetal bovine serum (FBS) and 25 µg/ml endothelial growth supplement (Sigma). For experiments, 5% (v/v) FBS was used. Plastic culture ware was precoated with 1% (w/v) gelatin, and cells were used after at least 1–4 passages.

Chinese hamster ovary (CHO) cells secreting human apoE2, apoE3, or apoE4 isoforms (CHO apoE2, CHO apoE3, and CHO apoE4) were produced by stably transfecting CHO cells lacking the dihydrofolate reductase (DHFR) gene with apoE expression plasmids (p7055, apoE2/3/4) encoding the selectable DHFR gene (15). Recombinant CHO apoE cells were maintained in selection media, Isovex's medium plus 2 mM glutamax and 1% (v/v) FBS, while medium for control CHO - cells was supplemented with 0.1 mM hypoxanthine and 16 mM thymidine.

Human monocytes with the apoE22 genotype were isolated by leukapheresis and elutriation then differentiated into macrophages by 12 days of cultivation in RPMI media supplemented with 20% (v/v) pooled human serum. Macrophages were then converted into foam cells by 48 h of incubation with 100 µg of protein/ml of acetylated low-density lipoprotein-loaded monocyte-derived macrophages (MDM) were incubated with serum-free RPMI for characterization of secreted apoE.

Measurement of VCAM-1 by Enzyme-linked Immunosorbent Assay (ELISA)—Analysis of VCAM-1 in quadruplicate wells by ELISA used previously described protocols and reagents (14). Briefly, HUVECs in 96 -well plates were either exposed to cell-derived apoE by transient transfection or conditioning with HDL-E or by incubating non-transfected CHO apoE cell-conditioned media. For the last 6 h in each experiment, tumor necrosis factor-α (TNF-α, human recombinant; Sigma) was added directly into the media to a final concentration of 10 units/ml (0.5 ng/ml) except for basal VCAM-1 controls. Any binding of monoclonal VCAM-1 antibody at 5 µg/ml (clone BBIG-V1; R&D Systems, Abingdon, UK) was detected by StreptABC/HRP Duet kit and O-phenylenediamine chromogenic substrate (both from DAKO Ltd, High Wycombe, UK) was detected by StreptABComplex/HRP Duet kit and O-phenylenediamine chromogenic substrate (both from DAKO Ltd, High Wycombe, UK). Absorbances were measured at 492 nm (A492) by microtiter plate spectrophotometer. Finally, cellular protein per well was determined by solubilizing the monolayers with 0.1 M NaOH, adding Bradford reagent (Bio-Rad Laboratories, Hemel Hempstead, UK), and measuring the absorbance at 620 nm (A620).

VCAM-1 expression was calculated for each well as a ratio of A620 VCAM-1 assay/ A620 protein assay values for TNF-α stimulated cells. Non-cytokine-stimulated wells were subtracted to give “VCAM-1 induction above basal”, and then data was normalized to a percentage of VCAM-1 induction in TNF-α controls.

Measurement of VCAM-1 by Flow Cytometry—Analysis of VCAM-1 by flow cytometry was carried out on confluent HUVECs in 12-well plates as described previously (14). ApoE-enriched high-density lipoproteinContaining HUVECs (from a normal subject homozygous for the e3 allele) were prepared by our standard procedure (17) and added to cells (1.5 mg of protein/ml) for 24 h; TNF-α was added directly into the media to a final concentration of 10 units/ml (0.5 ng/ml) for the last 6 h, except for basal VCAM-1 controls. Primary antibody (monoclonal VCAM-1 antibody at 5 µg/ml) binding was detected using goat anti-mouse fluorescein isothiocyanate-conjugated antibodies (DAKO Ltd) and 5 × 10³ cells were analyzed per well by flow cytometry (Coulter Epics Elite; Coulter, Hialeah, FL).

Transient Transfection of HUVECs—ApoE expression plasmids pCMV-apoE2, pCMV-apoE3, and pCMV-apoE4 were prepared by ligating the corresponding cDNA into pCMV.0, a mammalian expression vector (18). Recombinant CHO apoE cells were maintained in selection media, Isovex's medium plus 2 mM glutamax and 1% (v/v) FBS, while medium for control CHO - cells was supplemented with 0.1 mM hypoxanthine and 16 mM thymidine.

Human monocytes with the apoE22 genotype were isolated by leukapheresis and elutriation then differentiated into macrophages by 12 days of cultivation in RPMI media supplemented with 20% (v/v) pooled human serum. Macrophages were then converted into foam cells by 48 h of incubation with 100 µg of protein/ml of acetylated low-density lipoprotein-loaded monocyte-derived macrophages (MDM) were incubated with serum-free RPMI for characterization of secreted apoE.

Characterization of ApoE Particles—To characterize apoE particles secreted from CHO apoE cells and to compare them with those secreted by MDM, cells were incubated for 24 h with serum-free media. Media was then concentrated 10-fold in Vivaspin concentrators (10,000 molecular weight cut off; Vivasinc Ltd, Lincoln, UK), and apoE particles were analyzed for electrophoretic mobility in agarose hydrogels (Sebia, Issy-les-Moulineaux, France) by apoE immunoblotting (8) or for molecular weight cut off using FPLC gel filtration on a Superose 6 column (Amersham Pharmacia Biotech). Fractions (0.5 ml) were blotted onto nitrocellulose and analyzed for apoE by immunoblotting and densitometry.

Characterization of ApoE Receptor 2 (ApoER2) in HUVECs—Polyadenylated RNA was extracted from HUVECs, and the full-length open reading frame of apoER2 was amplified by reverse transcription-polymerase chain reaction to detect splice variants as described previously (20). To detect protein, HUVECs were labeled for 4 h with [35S]methionine, and solubilized membranes were immunoprecipitated with our anti-aptoprotein sera, “anti-apoER2nus” (20). Precipitated proteins were reduced, separated by 8% SDS-polyacrylamide gel electrophoresis, and analyzed by fluorography. Similar analyses were carried out using CHO cells expressing apoER2 (CHO apoER2) as positive control.

Characterization of Tumor Necrosis Factor-α—This was performed for independent experiments using GraphPad InStat version 3.01 for Windows 95, choosing a Student’s t-test analysis, test of variance test, or Pearson correlation, as appropriate. Results are shown as mean ± S.E. and p < 0.05 was considered significant.

RESULTS

Inability of HDL-E to Down-regulate VCAM-1—Plasma-purified apoE does not inhibit endothelial CAM expression (14). However, as the purification process may attenuate apoE biological activity (21), we studied effects of HDL-E, the minor apoE-containing subclass of bulk plasma HDL, on VCAM-1 up-regulation. The fluorescein isothiocyanate fluorescence profiles showed basal VCAM-1 expression was negligible, with most cell fluorescence values under 10 units (Fig. 1A), similar to isotype-treated controls or of cells incubated with HDL-E alone (not shown). After TNF-α treatment, the fluorescence increased to a marked level at night, indicating up-regulation of cell-surface VCAM-1 (Fig. 1B). However, pretreatment with HDL-E did not suppress this induction of VCAM-1 expression; the fluorescence profile or mean fluorescence intensity was not altered (Fig. 1C).
of protein/ml) before addition of TNF-α/H9251 bound ELISA (data not shown). Strikingly, cells secreting apoE and HDL-E was confirmed in independent assays using different batches of HUVECs (data not shown). Samples were analyzed in duplicate, and findings were difficult to transfect and we estimated our transfection efficiency to be typically ~1% by treating parallel wells with a plasmid encoding green fluorescent protein (pCMV.GPF; data not shown). Further support against an intracellular action of apoE was obtained by transferring media from transfected HUVECs containing 0.12 ± 0.03 μg of apoE/ml to untransfected cells; induction of VCAM-1 was inhibited by 12.9 ± 2.2% (p < 0.05, Student’s t test, data not shown). Additionally, when HUVECs were co-cultured with CHOapoE2/3/4 cells expressing apoE2, VCAM-1 induction was down-regulated by 16.1 ± 2.4% (p < 0.05, Student’s t test) when the concentration in the media was 0.14 ± 0.01 μg/ml, as compared with incubations with control CHOΔapoE cells (data not shown).

Cell-derived ApoE Has an Inherent Ability to Down-regulate VCAM-1—To verify that apoE secreted by CHOapoE2/3/4 cell-conditioned media was added directly to HUVECs. Although cell-derived apoE is undetectable in pCMV.0 media, it was readily detected in pCMV.apoE2/3 transfectants with the accumulated levels of apoE2 and apoE3 being similar (0.10 ± 0.02 versus 0.12 ± 0.02 μg/ml, respectively, n = 4). Levels of apoE4 were much lower, close to the detection limit of 10–20 ng/ml, possibly due to enhanced re-uptake by the cells or of increased association with cell-surface heparan sulfate proteoglycan (HSPG) (16). Variable apoE handling and retention by HSPG, with isoform-dependence, has been reported for various cultured cells including neurons, fibroblasts, CHO, and HepG2 cells (22). However, while HSPG-mediated accumulation of apoE4 occurs less readily than apoE3 in these cell types, in macrophages apoE4 has increased surface binding to HSPG (16). Whether there is differential apoE isoform retention by endothelial cells is unknown but merits further investigation.

HUVECs transiently transfected with pCMV.0 responded to TNF-α by a 10-fold induction of VCAM-1 as measured by cell-bound ELISA (data not shown). Strikingly, cells secreting apoE2, apoE3, or apoE4 had significantly reduced (all p < 0.01, Student’s t test) VCAM-1 compared with pCMV.0 transfection (67.8 ± 8.3%, 61.0 ± 12.0%, and 41.4 ± 7.4% inhibition, respectively; Fig. 2A), although the differences between the isoforms was not significant (p > 0.05, analysis of variance test). Further experiments revealed that VCAM-1 induction was inversely correlated with concentration of secreted apoE2 (r = −0.76, Pearson correlation, p < 0.001; n = 20, Fig. 2B) or apoE3 (r = −0.81, Pearson correlation, p < 0.001; n = 8, data not shown).

One explanation for VCAM-1 suppression would be an intracellular action of apoE trafficking in successfully transfected cells, as this influences several processes (23). However, this possibility is unlikely to account for down-regulation of up to 70% of VCAM-1; primary endothelial cells are notoriously difficult to transfect and we estimated our transfection efficiency to be typically ~1% by treating parallel wells with a plasmid encoding green fluorescent protein (pCMV.GFP; data not shown). Further support against an intracellular action of apoE was obtained by transferring media from transfected HUVECs containing 0.12 ± 0.03 μg of apoE/ml to untransfected cells; induction of VCAM-1 was inhibited by 12.9 ± 2.2% (p < 0.05, Student’s t test, data not shown). Additionally, when HUVECs were co-cultured with CHOapoE2/3/4 cells expressing apoE2, VCAM-1 induction was down-regulated by 16.1 ± 2.4% (p < 0.05, Student’s t test) when the concentration in the media was 0.14 ± 0.01 μg/ml, as compared with incubations with control CHOΔapoE cells (data not shown).

Cell-derived ApoE Has an Inherent Ability to Down-regulate VCAM-1—To verify that apoE secreted by CHOapoE2/3/4 cells inhibits VCAM-1 expression, CHOapoE2/3/4 cell-conditioned media was added directly to HUVECs. Although VCAM-1 suppression was less than by pCMV.apoE2/3/4 transfections, induction of VCAM-1 inversely correlated with apoE2 (r = −0.70, p < 0.001; n = 10, Fig. 3A) or apoE3 and apoE4 concentration (r = −0.64, p < 0.001, n = 4 and r = −0.53, p < 0.001, n = 4, respectively; data not shown) in the conditioned media. By contrast, CHOΔapoE cell-derived apoE did not affect expression of other endothelial markers; at 5.5 ± 0.2 μg of apoE/ml of expression of both constitutive vWF and TNF-α-induced E-selectin were unchanged, even though VCAM-1 was suppressed by 23.4 ± 2.2% (p < 0.001; n = 3, Fig. 3B) in the same experiments. Thus,
CHOapoE2 cell-conditioned media (5.5 ± 0.2 μg of apoE2/ml) suppressed TNF-α-stimulated VCAM-1 induction by 23.4 ± 2.2% (*p < 0.001, Student’s t test, n = 3), expression of TNF-α-induced E-selectin and constitutive vWF in parallel wells were unaffected as assessed by cell-bound ELISAs (grey bars). Control percentage antigen expression was 100 ± 1.2% (black bar), and data is from three independent experiments performed using two batches of HUVECs.

Unlike plasma-purified apoE, which was inactive (14), CHOapoE cell-derived apoE was a selective inhibitor of VCAM-1 expression, without affecting the constitutive levels of vWF or the degree of E-selectin up-regulation in response to TNF-α.

Characterization of ApoE Particles—Characterization of CHOapoE cell-derived apoE revealed several similarities to particles secreted by MDM. Both had a similar heterogeneity by agarose gel electrophoresis (Fig. 4A). Particles were spherical and of a similar diameter by negative staining electron microscopy (mean diameter 14 nm; range 8–20 nm) (Fig. 4B). Additional analyses showed that, for both cells, most apoE particles had a mean molecular mass of 400 kDa (range 150–1200 kDa; Fig. 4C) with preα-mobility. A minor 90-kDa (50–150 kDa) particle with preβ-mobility was also found. Additionally, MDM media contained a very large particle (>1200 kDa) not seen in CHOapoE cell media.

Evidence That Cell-derived ApoE Down-regulates VCAM-1 via the ApoER2-NO Pathway—ApoE inhibits platelet aggregation by stimulating NOS III (endothelial NOS) (11), the NO released elevating anti-aggregatory cGMP. The initial step was considered to be binding of apoE by apoER2, a member of the low-density lipoprotein receptor (LDL-R) family localized to caveolae signaling microdomains within the plasma membrane (24). Upon binding, a signal transduction cascade to activate NOS was postulated (20, 25). As NO is an inhibitor of VCAM-1 expression (26–28), we examined whether this apoE-NOS pathway might down-regulate VCAM-1 in endothelial cells. Suppression of VCAM-1 induction in HUVECs by CHOapoE2 cell-conditioned media was completely blocked by the NOS inhibitor, ethyl-ITU (Fig. 5A), while intracellular cGMP levels were increased by 46.5 ± 15% (p < 0.05, Student’s t test, n = 3), indicating NO release (Fig. 5B). However, it is unlikely that cGMP itself mediates the action of apoE because the inhibition of VCAM-1 by NO is not a result of increases in cyclic nucleotides (27, 28). When intracellular NO production was measured directly, using the cell-permeable fluorescent indicator DAF-2 DA (18), NO levels increased 62% from basal levels of 100.0 ± 5.6% in HUVECs exposed to control-conditioned media) to 161.9 ± 24.2% (p < 0.001, Student’s t test, n = 5) during a 2-h incubation with CHOapoE2 cell-conditioned media (Fig. 5C). Moreover, this apoE-induced rise in NO production was due to activation of NOS since the effect was essentially blocked using the NOS inhibitor (p < 0.001, Student’s t test, n = 5).

We then confirmed, that apoER2 mRNA was present in HUVECs (29) by long-range polymerase chain reaction (Fig. 6A). As in platelets and the megakaryocytic cell line, HEL (20), the predominant transcript (~70% by densitometry) lacked binding repeats 4–6 (apoER2Δ4–6) but contained the 177-base pair cytoplasmic insert, although minor transscripts of full-length apoER2+ cytoplasmic insert (Ins) were also detected. To verify protein expression we immunoprecipitated solubilized HUVEC membranes with anti-apoER2Abs (20). As the variant lacking the cytoplasmic insert is not recognized by this anti-antipede antibody, only two immunoreactive bands were detected, a major one at 130 kDa and a minor one at 180 kDa.

**Fig. 4.** CHOapoE2 cell-derived and MDM apoE particles are similar. Secreted apoE particles in CHOapoE2 cell-conditioned media were heterogeneous by agarose gel electrophoresis and apoE immunoblotting (A), by negative staining electron microscopy (B; electron micrographs taken at ×125,000 magnification, white scale bar represents 100 nm), and by gel filtration (C; the bars indicate fractionation of molecular-size standards: A, dextran blue, 2000 kDa; B, thyroglobulin, 669 kDa; C, catalase 232 kDa; D, albumin, 67 kDa; profiles for apoE content in media from the different cell types are overlaid (solid line for MDM, dashed line for CHOapoE2), but have many similarities to those in MDM media.
corresponding, respectively, to apoER2Δ4–6 and full-length apoER2 (Fig. 6B).

DISCUSSION

This study provides the first evidence that cell-secreted apoE may be anti-inflammatory by suppressing VCAM-1 induction, most probably because endothelial cells are stimulated to release NO. By contrast, an apoE-enriched lipoprotein, HDL-E, did not inhibit VCAM-1 expression, endorsing the view that plasma apoE does not modulate endothelial CAMs (14). This was important; previously we used plasma-purified apoE3 complexed to phospholipid (apoE3:DMPC), and, although active in other systems (11, 30), this did not inhibit VCAM-1 expression (14) presumably because the purification process (21) or lipid environment (5) diminished biological potency. Indeed, in a direct comparison of anti-platelet activity we found that cell-derived apoE3 was 4-fold more active than apoE3:DMPC (data not shown). As activated endothelium is exposed to locally secreted apoE from cholesterol-loaded macrophages within atherosclerotic plaques (6) our findings suggest a new atheroprotective role for apoE: the restriction of endothelial activation by down-regulating VCAM-1 induction. This is consistent with macrophage apoE acting early in lesion development (13).

Mimicking endothelial exposure to macrophage apoE in vitro is complex; a simple coculture would expose endothelial cells not only to apoE but also to diverse macrophage secretory factors, including inflammatory cytokines (31). To circumvent this problem, we first transiently transfected HUVECs to expose them to locally synthesized native apoE, albeit self-secreted; all three common apoE isoforms, apoE2, apoE3, and apoE4, strongly inhibited VCAM-1 induction even though media levels of apoE were <200 ng/ml. Moreover, VCAM-1 down-regulation was not solely due to intracellular actions or autocrine functions of apoE synthesized in successfully transfected cells; cross-incubation studies, adding media from transfected HUVECs to fresh non-transfected cells and the low efficiency of transfection (<1%) excluded these possibilities. Rather, and in contrast to HDL-E or plasma-purified apoE, the potency of apoE reflected its inherent cell-derived nature. Thus, in other experiments, CHOapoE cell-conditioned media also down-regulated VCAM-1 induction, correlating with apoE content. Significantly, this cell-derived apoE was secreted as particles that closely resembled macrophage apoE.
Intriguingly, suppression of VCAM-1 was most marked in transfected HUVECs when the media content of apoE was 50–75 times lower than in CHOapoE cell-conditioned media. One explanation is that in transfected wells entrapment of secreted apoE by endothelial HSPG (32) may result in high local concentrations at the cell surface and enhanced biological activity. Indeed, HSPG is abundant on the cell-surface of cultured endothelial cells, and its production is rapidly stimulated by apoE (33). Although our transfection studies are not directly relevant to the situation in vivo, as endothelium does not synthesize apoE, they do have implications for gene therapy; transfecting endothelial cells to express apoE may limit endothelial activation. Indeed, this approach prevents lesion development in apoE-deficient mice (34).

ApoE activates NOS III in platelets (11) and NOS II in macrophages (35). Because NO is a potent intracellular messenger and inhibitor of atherogenesis, in part by suppressing cytokine-induced VCAM-1 expression and reducing monocyte adherence to endothelium (26–28), we investigated whether apoE might stimulate endothelial NO production. Supporting this mechanism, the NO inhibitor, ethyl-ITU, blocked the inhibitory effect of apoE on VCAM-1 expression, while CHOapoE cell-conditioned media not only increased cGMP, a surrogate marker for NO release (36), but also increased intracellular NO levels in a fluorescent assay for direct NO detection. Importantly, E-selectin, a cytokine-induced endothelial CAM not regulated by NO in HUVECs (26), was unaffected by apoE; rather apoE selectively inhibits VCAM-1.

Indirect evidence also implicates apoE-mediated release of NO in down-regulating VCAM-1. In platelets, binding of apoE by its receptor, apoER2, appears to initiate a signal transduction cascade to up-regulate NOS (20, 25). As we found apoER2 mRNA and protein in HUVECs, a similar apoE-apoER2-NOS pathway may function in endothelium to limit VCAM-1 induction. Indeed, this constitutes the first report of apoER2 protein being identified in vascular endothelial cells in non-neuronal tissues. A role for apoER2 is also suggested by the similar efficacy of apoE2 and apoE3; apoE2 binds poorly to the LDL-R and LDL-R-related protein, thereby discounting them in inhibiting VCAM-1, whereas apoER2 and its closest mammalian homolog, the very-low-density receptor (VLDL-R), bind both isoforms efficiently (5, 20, 37). Also relevant is the proposal that both apoER2 and VLDL-R initiate tyrosine kinase signaling to modulate neuronal positioning in brain development (38), a process dependent on neuronal cell adhesion molecules (39, 40). Since VLDL-R is also present in HUVECs (41), we cannot exclude coordinate apoE signaling by the two receptors in HUVECs to activate NOS and limit VCAM-1 induction.

In summary, we have shown that cell-derived apoE inhibits cytokine-induced VCAM-1 expression on endothelial cells and propose that this occurs through activation of NOS to release NO. However, further work will be needed to confirm an apoE-NO link in endothelium and to delineate the steps involved, not least because VCAM-1 can also be suppressed by NO-independent mechanisms (42).

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