Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression

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Abstract **The ability of different phosphatidylcholine (PC) species to inhibit cytokine-induced expression of vascular cell adhesion molecule 1 (VCAM-1) in human umbilical vein endothelial cells (HUVECs) was investigated. PC species containing palmitoyl- in the *sn*-1 position and palmitoyl- (DPPC), arachidonyl- (PAPC), linoleoyl- (PLPC) or oleoyl- (POPC) in the *sn*-2 position were compared. These PC species were studied as components of reconstituted high density lipoproteins (rHDL) (containing apolipoprotein A-I [apoA-I] as the sole protein) or as small unilamellar vesicles (SUVs). The rHDL containing PLPC and PAPC inhibited VCAM-1 expression in activated HUVECs by 95 and 70%, respectively, at an apoA-I concentration of 16 μM. At this concentration of apoA-I, POPC rHDL inhibited by only 16% and DPPC rHDL did not inhibit at all. These differences could not be explained by differential binding of the rHDL to HUVECs. The same hierarchy of inhibitory activity was observed when these PC species were presented to the cells as SUVs but only when the SUVs also contained an antioxidant. It was concluded that rHDL PC is responsible for their inhibitory activity and that this varies widely with different PC species.**—Baker, P. W., K-A. Rye, J. R. Gable, M. A. Vadas, and P. J. Barter. **Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression.** ***J. Lipid Res.*** **2000.** 41: **1261–1267.**

Supplementary key words vascular cell adhesion molecule 1 • VCAM-1 • human umbilical vein endothelial cells • HUVEC • TNF-α • IL-1 • fatty acid composition • atherosclerosis

Human high density lipoproteins (HDL) inhibit the cytokine-induced expression of adhesion molecules in endothelial cells (1) in a process that may contribute to their antiatherogenicity. This inhibition is concentration dependent within the physiological range of HDL concentrations, although at any given concentration the extent of inhibition varies widely between preparations of HDL isolated from different human subjects (2). The reason for this intersubject variation is not known. The demonstration in a mouse model that HDL inhibits vascular cell adhesion molecule 1 (VCAM-1) expression in endothelial cells in vivo (3) highlights the importance of understanding more about this activity of HDL.

In earlier studies of HDL-mediated inhibition of the tumor necrosis factor α (TNF-α)-induced expression of VCAM-1 in human umbilical vein endothelial cells (HUVECs), we demonstrated that *i*) the inhibitory activity of both HDL (2) and reconstituted HDL (rHDL) (4) is unaffected by replacing all of the HDL apolipoprotein (apo) A-I with apoA-II; *ii*) both spherical and discoidal reconstituted HDL (rHDL) possess inhibitory activity (4); and *iii*) neither the size nor the cholesteryl ester and triglyceride content of rHDL has any effect on their inhibitory activity (4). In contrast to these earlier negative findings, we now report that the inhibitory activity of discoidal rHDL is greatly influenced by its phospholipid composition. Specifically, of three of the most abundant phospholipids in human HDL, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (PAPC), rHDL containing PLPC or PAPC have a much greater inhibitory activity than those containing POPC.

MATERIALS AND METHODS

Preparation of discoidal rHDL

Discoidal rHDL were prepared by the cholate dialysis method precisely as described (5). Their sole lipid component was 1,2-

Abbreviations: HDL, high density lipoproteins; rHDL, reconstituted high density lipoproteins; apo, apolipoprotein; VCAM-1, vascular cell adhesion molecule 1; HUVEC, human umbilical vein endothelial cell; TNF, tumor necrosis factor; IL-1, interleukin 1; PC, phosphatidylcholine; TBS, Tris-buffered saline; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; PAPC, 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PLPC, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; SUV, small unilamellar vesicle; BHT, butylated hydroxytoluene; SKase, sphingosine kinase.

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dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), PAPC, PLPC, or POPC while their sole protein component was apolipoprotein A-I (apoA-I) (molar ratio of PC to apoA-I of 100:1). Particles were also prepared with apoA-I and mixtures of both PLPC and POPC (molar ratio of PLPC to POPC to apoA-I of 90:10:1, 70:30:1, and 50:50:1). For lipid uptake studies rHDL were prepared with apoA-I and PLPC or POPC labeled with ^{14}C in the *sn*-2 acyl group. Some preparations of rHDL also contained the antioxidant, butylated hydroxytoluene (BHT; molar ratio of PC to BHT to apoA-I of 100:10:1). For binding experiments, apoA-I was labeled with ^{125}I, using Iodobeads as described by the manufacturer (Pierce, Rockville, IL). Iodinated apoA-I was then used to prepare rHDL. Phospholipids were purchased from Sigma (St. Louis, MO). The apoA-I was purified from human HDL as described previously (4). After preparation all rHDL were extensively dialyzed (5 times, 1 L each time) against nitrogen-purged 10 mM Tris-buffered saline (TBS, pH 7.4) containing 150 mM NaCl, 0.03% (w/v) EDTA-Na_2, 0.006% (w/v) NaN_3, and 0.2% (w/v) Chelex (Bio-Rad, Hercules, CA). After dialysis the samples were protected from light, stored under nitrogen, and used within 1 week of preparation. Before addition to HUVECs, the rHDL were dialyzed over 24 h against endotoxin-free phosphate-buffered saline (PBS) (three times, 1 L each time). The size distribution of the rHDL was determined by gradient gel electrophoresis (6). The number of molecules of apoA-I per rHDL particle was determined by cross-linking (7).

Preparation of small unilamellar vesicles

Small unilamellar vesicles (SUVs) containing PAPC, PLPC, or POPC, and BHT (molar ratio of PC to BHT, 10:1), were prepared in PBS precisely as described by Jonas (8). In some experiments the SUVs were prepared without BHT.

HUVEC isolation, culture, and incubation conditions

HUVECs were isolated and cultured as described previously (4). Briefly, confluent preparations of HUVECs (passage 2–4) were washed with 10 mM EDTA-Na_2 (5 mL) in PBS, trypsinised and replated onto 24-well gelatin-coated plates at a density of 3×10^5 cells/mL (500 μl/well). After a 5-h reattachment period a portion of the medium (100 μL) was removed and replaced with PBS alone (100 μL) or with rHDL or SUVs in PBS (100 μL). The cells were then preincubated for 16 h (unless otherwise indicated) before being activated with TNF-α or interleukin 1 (IL-1) (100 U/mL). In some experiments (where indicated) the medium containing the rHDL was removed, the cells were washed with PBS, and fresh medium (not containing rHDL) was replaced before addition of cytokines. Five hours after addition of cytokine the cell surface expression of VCAM-1 was measured by flow cytometry as described (4).

Binding and PC uptake studies

HUVECs were plated onto 6-well gelatin-coated plates at a density of 3×10^5 cells/mL (2.5 mL/well). Binding of rHDL containing iodinated apoA-I to HUVECs, in the absence or presence of unlabeled rHDL, was determined after 2 h at 4°C as described by Vadiveloo and Fidge (9). Binding of labeled rHDL was determined at an apoA-I concentration of 500 nM, consistent with the reported affinity of HDL for endothelial membranes (9–11). Specific binding of labeled rHDL was calculated by subtracting nonspecific binding of labeled rHDL (determined in the presence of a 50-fold excess of unlabeled rHDL) from total binding of labeled rHDL. Uptake of ^{14}C by HUVECs from rHDL containing PLPC or POPC labeled with ^{14}C in the *sn*-2 acyl group was determined after a 16-h incubation of the cells at 37°C with rHDL at an apoA-I concentration of 16 μM. After incubation the cells were washed extensively with PBS, harvested into a minimal volume of PBS, and sonicated. Liquid scintillation counting of an aliquot of the sonicated cells was used to determine the incorporation of ^{14}C into the cells. The amount of ^{14}C bound to the cell surface, a possible confounding factor in determining the cellular uptake of ^{14}C, was determined after a 2-h incubation at 4°C.

Statistical analysis

ANOVA (two-factor with repeated measures) was performed with the data analysis package in Microsoft Excel (version 7.0) by treating all points within a data set as a group. Significance was determined at the 95% confidence interval, that is, $P < 0.05$.

RESULTS

Discoidal rHDL containing different PC species

Discoidal rHDL were prepared by complexing apoA-I to a number of different PC species. The rHDL containing DPPC, PLPC, or POPC were homogeneous in size, with average Stokes' diameters of 10.0, 9.5, or 9.9 nm, respectively. In each of these preparations there were two molecules of apoA-I per particle. The rHDL containing PAPC were heterogeneous, including several populations of particles with Stokes' diameters ranging from 8.5 to 16.4 nm. While most of the PAPC rHDL contained two molecules of apoA-I per particle, about 20% contained three or four molecules of apoA-I per particle. The final molar ratio of PC to apoA-I was about 100:1 in all rHDL preparations.

Inhibition of VCAM-1 expression by rHDL containing different PC species

HUVECs were preincubated for 16 h with rHDL containing PAPC, PLPC, or POPC before being activated with TNF-α. Preincubation with PLPC or PAPC rHDL led to a marked reduction in the subsequent expression of VCAM-1 (**Fig. 1**). In contrast, preincubation with POPC rHDL had little effect on TNF-α-induced VCAM-1 expression. At an apoA-I concentration of 16 μM the inhibition achieved by PLPC, PAPC, and POPC rHDL was 95, 70, and 16%, respectively (Fig. 1).

To determine whether the extent of inhibition was influenced by the duration of the preincubation, HUVECs were preincubated with rHDL containing PLPC or POPC for 1 h or 16 h before activation with TNF-α. After 1 h of pre-incubation at an apoA-I concentration of 16 μM, PLPC rHDL inhibited VCAM-1 expression by 40%; when the preincubation was extended to 16 h, the inhibition was 95%. At the same concentration of apoA-I, POPC rHDL inhibited VCAM-1 expression by approximately 16% when preincubated with the cells for 16 h but did not inhibit at all when preincubated for only 1 h (results not shown).

In all the experiments described so far, the rHDL were present in the medium at the time of addition of TNF-α; furthermore, the rHDL remained in the medium for the next 5 h until the incubation was terminated and VCAM-1 expression was determined. In previous studies with HDL, we have found that the inhibition of VCAM-1 expression

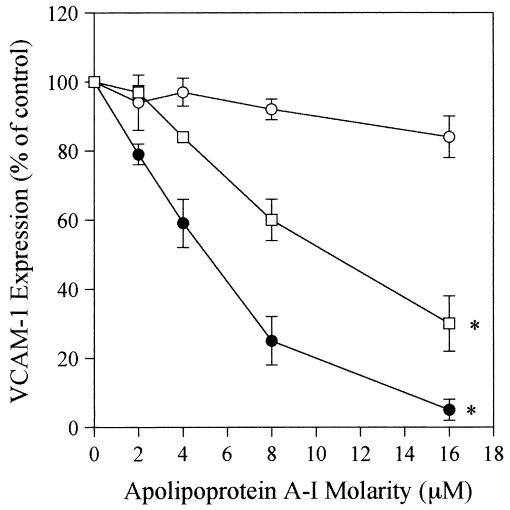

Fig. 1. Inhibition of TNF-α-induced VCAM-1 expression in HUVECs after preincubation with rHDL containing different PC species. Discoidal rHDL were prepared with POPC (○), PAPC (□), or PLPC (●) as the sole lipid and apoA-I as the sole protein at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. HUVECs were preincubated with the rHDL for 16 h before being activated with TNF-α. The rHDL remained in the medium during activation with TNF-α. VCAM-1 expression was determined 5 h after addition of TNF-α. Results are expressed as means ± SEM (n = 4). An asterisk (*) indicates a significantly greater inhibitory activity when compared with that achieved with POPC rHDL ($P < 0.05$, ANOVA).

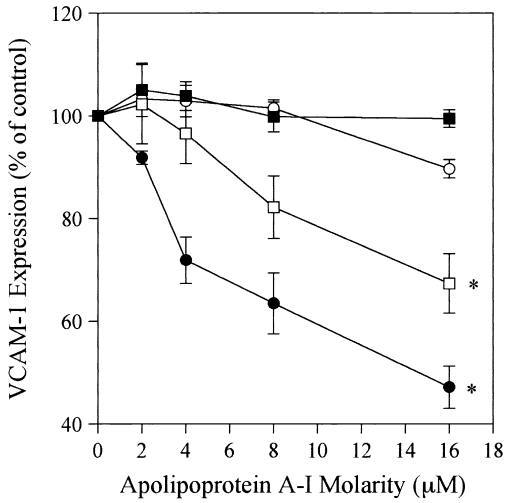

Fig. 2. Effect of removing the rHDL from the medium before activating HUVECs with TNF-α. Discoidal rHDL were prepared with DPPC (■), PAPC (□), PLPC (●), or POPC (○) as the sole lipid and apoA-I as the sole protein at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. HUVECs were preincubated with the rHDL for 16 h. The medium containing the rHDL was then removed, the cells were washed with PBS, and fresh medium (not containing rHDL) was added before activation of the cells with TNF-α. VCAM-1 expression was determined 5 h after activation with TNF-α. Results are expressed as means ± SEM (n = 3). An asterisk (*) indicates a significantly greater inhibitory activity when compared with that achieved by POPC rHDL ($P < 0.05$, ANOVA).

in HUVECs persisted even if the HDL were removed from the medium before activation of the cells with TNF-α (1, 2, 12). We therefore conducted studies to determine whether this also applies to discoidal rHDL and, if so, whether the hierarchy of inhibitory activities observed above with rHDL containing different PC species persists.

Effect of removing rHDL before activating the cells with TNF-α

These studies were conducted not only with rHDL containing PLPC, PAPC, or POPC as in Fig. 1 but also with rHDL containing DPPC. HUVECs were preincubated for 16 h with these rHDL preparations before the medium containing the rHDL was removed, the cells were washed with PBS, and fresh medium (not containing rHDL) was added. The cells were then activated with TNF-α and the expression of VCAM-1 determined 5 h later. Despite being removed before the cells were activated, rHDL containing PLPC or PAPC still inhibited VCAM-1 expression in a concentration-dependent manner (**Fig. 2**); at an apoA-I concentration of 16 μM, the inhibition achieved by PLPC and PAPC rHDL was 53 and 33%, respectively. The rHDL containing POPC had minimal inhibitory activity and those containing DPPC did not inhibit at all (Fig. 2).

Binding of rHDL containing PLPC or POPC to HUVECs

Studies were conducted to determine whether the differing inhibitory activities of rHDL containing PLPC or POPC were related to differences in their binding to HUVECs. Binding of rHDL to HUVECs was assessed with preparations in which the apoA-I was labeled with ^{125}I. The specific activity of the rHDL apoA-I was approximately 150 cpm/ng. Specific binding of POPC rHDL (shaded column) and PLPC rHDL (open column) to cells was comparable (**Fig. 3A**), with approximately 0.03% of the added rHDL being bound to the cell surface. Competitive binding studies of labeled POPC rHDL in the presence of an equimolar amount of unlabeled PLPC rHDL (Fig. 3B, shaded column) and vice versa (Fig. 3B, open column) demonstrated that each rHDL preparation had a similar affinity for the cell surface. After incubation all the unbound apoA-I was recovered in the medium.

Cellular uptake of PLPC or POPC from rHDL

Having demonstrated that rHDL containing PLPC or POPC bind with similar affinity to the surface of HUVECs, studies were conducted to determine the extent to which these cells take up ^{14}C from rHDL containing PLPC or POPC labeled with ^{14}C in the *sn*-2 acyl group. The specific activity of the rHDL PC was approximately 800 cpm/μg. After a 16-h incubation at 37°C the cells were found to have taken up 1.4% of the ^{14}C contained in the rHDL PC (**Fig. 4A**). There was no difference in the uptake of ^{14}C from rHDL containing POPC or PLPC (Fig. 4A). Approximately 98% of the phospholipid added to the cells was recovered in the medium after incubation. When the incubation was conducted at 4°C, few counts were associated with the cells, between 0.02 and 0.03% of the added counts (Fig. 4B).

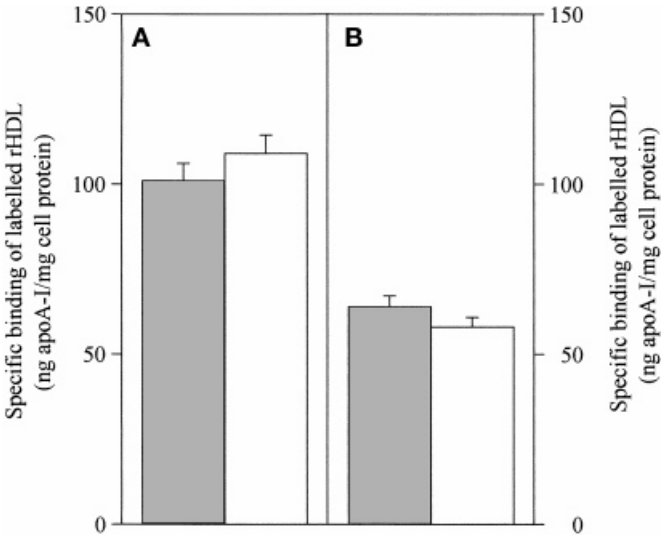

Fig. 3. Binding of rHDL containing POPC or PLPC to HUVECs. Discoidal rHDL were prepared with POPC or PLPC as the sole lipid and unlabeled apoA-I or ^{125}I-labeled apoA-I as the sole protein at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. HUVECs were incubated for 2 h at 4°C with labeled rHDL and specific binding was determined by correcting for nonspecific binding as described in Materials and Methods. (A) Specific binding of POPC rHDL (shaded column) and PLPC rHDL (open column). (B) Specific binding of labeled POPC rHDL in the presence of an equimolar amount of unlabeled PLPC rHDL (shaded column) and vice versa (open column); rHDL were added at an apoA-I concentration of 500 nM. Results are expressed as means ± SEM (n = 3).

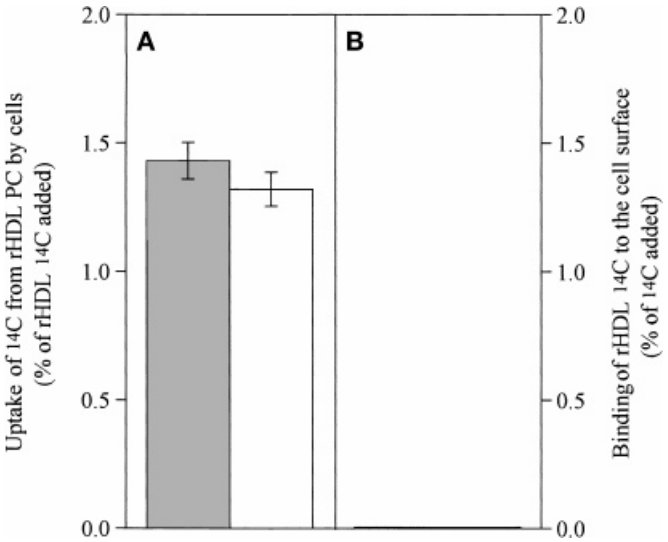

Fig. 4. Uptake of rHDL PC by HUVECs. Discoidal rHDL were prepared with POPC or PLPC labeled with ^{14}C in the *sn*-2 acyl group as the sole lipid and unlabeled apoA-I as the sole protein at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. HUVECs were incubated with labeled rHDL for 16 h at 37°C (A) or for 2 h at 4°C (B). The uptake of ^{14}C by the cells was then determined as described in Materials and Methods. Results are expressed as means ± range (duplicate determination).

Effects of discoidal rHDL containing mixtures of PLPC and POPC

Having observed large differences in the ability of discoidal rHDL containing PLPC or POPC to inhibit endothelial cell VCAM-1 expression, studies were conducted to investigate the possibility of interactions between these PC species. In these studies discoidal rHDL were prepared with apoA-I and mixtures of PLPC and POPC. The molar ratios of PLPC to POPC in these mixtures were 100:0, 90:10, 70:30, 50:50, and 0:100. All had a molar ratio of PC to apoA-I of about 100:1 and comprised single populations of particles with Stokes' diameters that increased progressively from 9.6 nm when the particles contained 100% PLPC to 10.0 nm when the particles contained 100% POPC.

The rHDL containing 100% PLPC inhibited VCAM-1 expression in a concentration-dependent manner, with the inhibition reaching 77% at an apoA-I concentration of 16 μM, while the rHDL containing 100% POPC achieved minimal inhibition at this molarity of apoA-I (**Fig. 5**). Replacement of 10% of the rHDL PLPC with POPC reduced the inhibition from 77 to 64% at an apoA-I concentration of 16 μM. Replacement of 30% of the rHDL PLPC with POPC further reduced the inhibition to 51%, while replacement of half the rHDL PLPC with POPC reduced the inhibition to 31%.

Effects of SUVs containing different PC species

Studies were conducted to determine whether the effects of discoidal rHDL could be replicated by SUVs composed of PAPC, PLPC, or POPC. PAPC and PLPC SUVs prepared without BHT were cytotoxic to HUVECs as judged by trypan blue exclusion, making it impossible to assess their effects on adhesion molecule expression. In contrast, PAPC and PLPC SUVs containing BHT were not cytotoxic (95% viability after 16 h of preincubation) and inhibited the TNF-α-induced expression of VCAM-1 in a concentration-dependent manner, comparable to that of PLPC and PAPC rHDL (**Fig. 6**). POPC SUVs, with or without BHT, were not cytotoxic to HUVECs; in neither case did they inhibit VCAM-1 expression (Fig. 6). As with PLPC and PAPC rHDL, the inhibition mediated by PAPC and PLPC SUVs was apparent whether the SUVs were present during activation of the cells with cytokine (Fig. 6) or had been removed prior to activation (**Fig. 7**).

Effects of rHDL and SUVs containing PLPC or POPC on IL-1-induced VCAM-1 expression

In all the experiments shown above, the HUVECs were activated with TNF-α. Further experiments were conducted to determine whether the differing inhibitory activities of rHDL and SUVs containing PLPC or POPC were also apparent when IL-1 rather than TNF-α was used to activate the cells. In these experiments rHDL (Fig. 7A) or SUVs (containing BHT) (Fig. 7B) were preincubated with HUVECs for 16 h. The rHDL and SUVs were then removed, fresh medium (not containing rHDL or SUVs) was added, and the cells were activated with IL-1. PLPC, whether present as a component of rHDL or SUVs, inhibited the IL-1-mediated expression of VCAM-1 in HUVECs in a concentration-dependent manner comparable to that observed when the cells were activated by TNF-α. Also

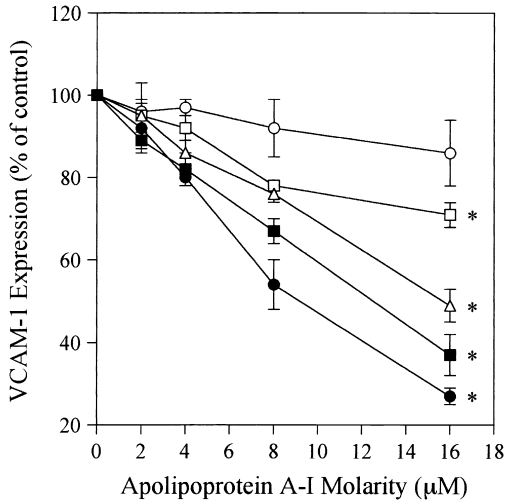

Fig. 5. Inhibition of TNF-α-induced VCAM-1 expression in HUVECs by preincubating the cells with rHDL containing mixtures of POPC and PLPC. Discoidal rHDL were prepared with apoA-I and mixtures of PLPC and POPC at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. HUVECs were preincubated with the different preparations of rHDL for 16 h before being activated with TNF-α. The rHDL remained in the medium during activation of the cells with TNF-α. VCAM-1 expression was determined 5 h after addition of TNF-α. The molar ratios of PLPC to POPC to apoA-I in the different rHDL preparations were 100:0:1 (●), 90:10:1 (■), 70:30:1 (△), 50:50:1 (□), and 0:100:1 (○). Results are expressed as means ± SEM (n = 3). The inhibition mediated by all rHDL preparations was significantly greater than that observed with rHDL containing 100% POPC ($P < 0.05$, ANOVA).

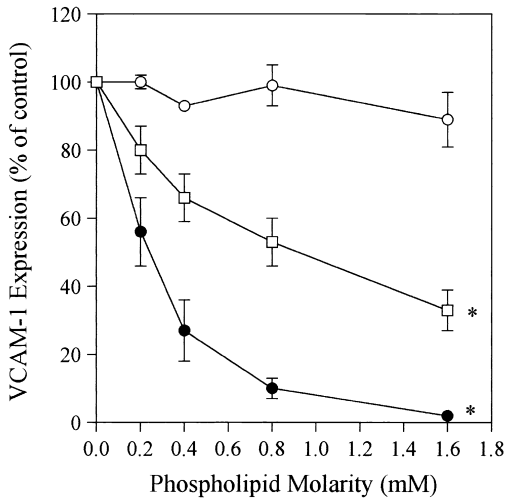

Fig. 6. Inhibition of TNF-α-induced VCAM-1 expression in HUVECs after preincubation with SUVs containing different PC species. SUVs were prepared with PAPC (□), PLPC (●), or POPC (○) as the sole lipid and BHT as an antioxidant at a molar ratio of PC to BHT of 10:1, as described in Materials and Methods. HUVECs were preincubated with the SUVs for 16 h before being activated with TNF-α. The SUVs remained in the medium during activation with TNF-α. VCAM-1 expression was determined 5 h after addition of TNF-α. Results are expressed as means ± SEM (n = 3). An asterisk (*) refers to a significant difference in inhibitory activity compared to POPC rHDL ($P < 0.05$, ANOVA).

consistent with the results with TNF-α, POPC had little to no effect on the IL-1-induced expression of VCAM-1, whether present during preincubation as a component of rHDL or SUVs (Fig. 7).

DISCUSSION

These studies demonstrate for the first time that three of the more abundant PC species in human HDL, PAPC, PLPC, and POPC (13, 14), differ markedly in their abilities to inhibit the TNF-α-induced expression of VCAM-1 in HUVECs. These differences were observed whether the PC species were presented to the cells as components of discoidal rHDL or as SUVs and they could not be explained by differences in rHDL binding to the cells or the uptake of rHDL PC by the cells. PAPC and PLPC were cytotoxic to HUVECs unless they were components of rHDL in which they were complexed to apoA-I or were components of SUVs that also contain the antioxidant BHT. This latter observation is consistent with reports that apoA-I has antioxidant properties (15). It may also explain why low density lipoproteins (LDL), whose lipids are more readily oxidisable than the lipids in HDL (16), do not inhibit adhesion molecule expression (17, 18).

We have reported that both HDL and rHDL inhibit endothelial sphingosine kinase (SKase) (12). SKase is an enzyme that catalyses a key step in the pathway by which TNF-α stimulates endothelial cell VCAM-1 and E-selectin expression (19). The precise mechanism by which HDL interact with the cell to achieve this inhibition is still not known. There is good evidence, however, that it does not involve interference by HDL with the binding of TNF-α to the cell surface (12). This conclusion is supported by the observation in the present study that the inhibitory activity of both rHDL and SUVs remained apparent even when they were removed before the cells were activated with TNF-α or IL-1.

The explanation for the differing inhibitory activities of rHDL and SUVs containing different PC species is not known. Regardless of the mechanism, however, the observation has obvious pathophysiological implications. About 80% of the phospholipid in human HDL is PC, with PLPC accounting for about 40% of the PC (13, 14). The importance of the fatty acid composition of plasma phospholipids [which reflects that of HDL phospholipids (13, 14)] has been highlighted in population studies in which the linoleic acid composition of phospholipids is predictive of the development of coronary heart disease (20–23). It will, therefore, be of considerable interest to determine whether differing inhibitory activities of HDL isolated from different human subjects (2) can be explained by variations in the composition of HDL phospholipids. It will also be worth investigating whether differences in phospholipid composition can explain the superior inhibitory activity of HDL_3 compared with that of HDL_2 (2).

When the different PC species were presented to the cells as components of SUVs, the hierarchy of inhibitory activities was the same as that observed when they were

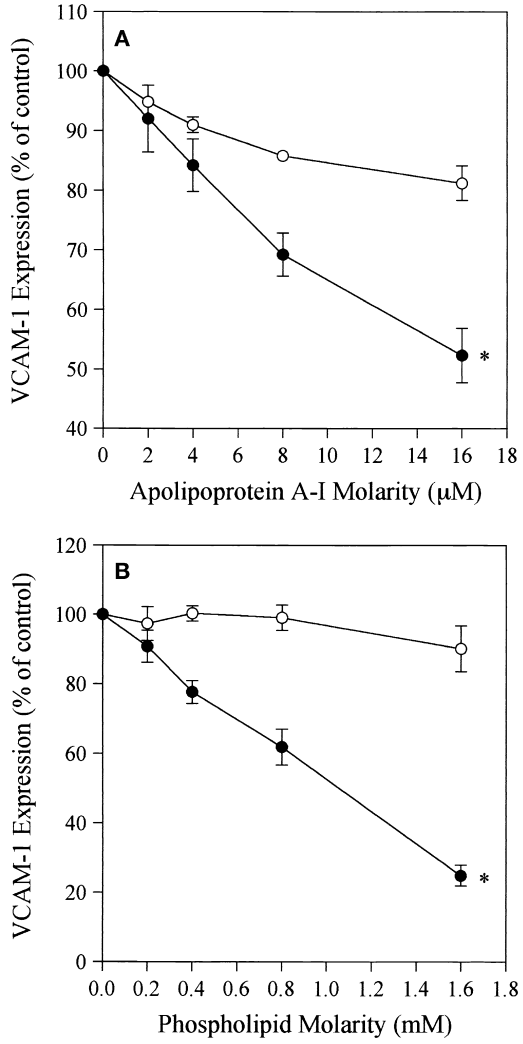

Fig. 7. Inhibition of IL-1-induced VCAM-1 expression in HUVECs by preincubating the cells with rHDL (A) or SUVs (B) containing different PC species. Discoidal rHDL were prepared with PLPC (●) or POPC (○) and apoA-I at a molar ratio of PC to apoA-I of 100:1, as described in Materials and Methods. SUVs were prepared with PLPC (●) or POPC (○) as the sole lipid and BHT as an antioxidant at a molar ratio of PC to BHT of 10:1, as described in Materials and Methods. HUVECs were preincubated with the different preparations of rHDL or SUVs for 16 h before being activated with IL-1. The medium containing the rHDL or SUVs was removed before activation with TNF-α. VCAM-1 expression was determined 5 h after activation with IL-1. Results are expressed as means ± SEM (n = 4). An asterisk (*) refers to a significant difference in inhibitory activity compared with POPC rHDL or POPC SUVs ($P < 0.05$, ANOVA).

components of rHDL. This raises the possibility that PC may be the active component of HDL responsible for its inhibitory activity. The fact that these PC species as components of SUVs are effective only in the presence of an antioxidant suggests that the inhibitory activity of the PC in HDL may depend on it being protected by the antioxidant properties of these lipoproteins (15, 16, 24).

The cytokine-induced expression of endothelial cell VCAM-1 is also inhibited by nonesterified fatty acids (NEFA) (25, 26). Consistent with the inhibition by phospholipids in the present study, the inhibition by nonesterified linoleic acid is superior to that of nonesterified oleic acid (26). There are, however, clear differences between the inhibition mediated by NEFA and that mediated by the PC associated with HDL. For example, there is no evidence of a lag phase with the inhibition mediated by HDL (1) or rHDL, whereas the inhibition mediated by NEFA occurs only after a lag phase of about 6 h (25). In addition, maximal inhibition of VCAM-1 expression by NEFA requires preincubation for 48–72 h (25, 26) compared with 16 h or less in the case of HDL or rHDL (1, 2, 4). Interestingly, the time course for the inhibitory effects of NEFA parallels that of their incorporation into cellular lipids (25). We postulate that the importance of HDL in this process relates to their ability to transport PC in an environment in which it is protected against oxidation prior to being delivered to endothelial cells.

The pathophysiological relevance of these findings to the ability of HDL to protect against atherosclerosis remains to be determined. Although the results of in vivo studies are conflicting (3, 27–29), several studies have shown that HDL do inhibit endothelial cell adhesion molecule expression in vivo (3, 27, 28). To the extent that this antiinflammatory property of HDL is antiatherogenic, the present studies suggest that the phospholipid composition may be of greater importance than previously recognized.

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