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Tumor Necrosis Factor-Alpha Induced VCAM-l Expression is Inhibited by High Density Lipoprotein in Human Astroglioma Cells

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Abstract : Astrocytes, the major glial cells in the central nervous system (CNS), can express vascular cell adhesion molecule-1 (VCAM-1) in response to cytokines, such as TNF-α. In CNS, an increased VCAM-1 expression may contribute to inflammatory processes. We, in the present study, have examined the effect of human plasma High Density Lipoproteins (HDL) and other lipoproteins on VCAM-1 expression in astroglioma cells since astrocytes secrete HDL-like lipoprotein particles which contain apo E and cholesterol, phospholipid. The exposure of astroglioma cells to the major plasma lipoprotein fractions (VLDL, LDL and HDL) had no effect on the VCAM-1 expression. However, TNF-α-induced VCAM-l was inhibited by HDL in a dose-dependent manner, but not by VLDL or LDL. The inhibitory effect of HDL on TNF-α-induced VCAM-l was reversed by the inclusion of Apo A-I antibody, the major apolipoprotein of HDL, demonstrating the specificity of this response. Reconstituted HDL (discoidal complex of apo HDL and DMPC), but not apo HDL or DMPC, was effective in suppressing the VCAM-1 expression. RNase protection assay (RPA) revealed that TNF-α-induced VCAM-l mRNA expression was markedly inhibited by HDL (500 μg cholesterol/mL). These results indicate that HDL-like particles in the CNS may function as an immunosuppressive molecule in pathologic conditions of CNS.

Key words : TNF-α, Astrocytes, Vascular Cell Adhesion Molecule, High Density Lipoproteins

Introduction

Cell adhesion plays a key role in a number of diverse biological processes such as acute and chronic inflammation, autoimmune diseases, and atherosclerosis (Bevilacqua et al. 1994). Specific cellular interactions in these disease processes are mediated by adhesion molecules, a family of proteins that bind specifically to complementary adhesion molecules on other cells.

Astrocytes are the resident glial cells in the CNS that support brain capillary endothelium, and are in intimate contact with brain endothelial cells via perivascular end-feet, and can modulate the endothelial function in response to external stimuli (Hurwitz et al. 1993). Exposure of astrocytes to inflammatory cytokines, such as TNF-α, IL-lβ, and IFN-γ, induce the expression of adhesion molecules such as VCAM-1, ICAM-1 (Shrikant et al. 1994, Rosenman et al. 1995). These adhesion molecules are increased in the CNS particularly during times of inflammation and are thought to contribute to extravasation of leukocytes across the blood-brain barrier (BBB) and into CNS parenchyma (Cannella and Raine 1995). In disease states, ICAM-1 and VCAM-1 expression have been detected on the endothelial cells comprising the BBB, as well as astrocytes and microglia, which contribute to the structural

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integrity of the BBB (Cannella and Raine 1995).

It was demonstrated that astrocytes generate and secrete HDL-like lipoprotein particles, which contain apo E, cholesterol, and phospholipids (Fagan and Holtzman 2000). As well, it has been shown that apo E is abundantly present in the CNS (Koch et al. 2001). Despite the existence of the BBB, lipoprotein particles have been shown to be also present in the CSF. Several studies demonstrated that these particles originate from CNS, although a portion of their protein components may filter through the BBB by a receptor-mediated mechanism called transcytosis (Koch et al. 2001). The lipoprotein particles isolated from human CSF showed size, structure, and composition similar to plasma HDL (Borghini et al. 1995). On the contrary, other reports indicated that CSF lipoproteins are very different in virtually all respects from particles found in the plasma (Puglielli et al. 2003), but it seems most probable that brain lipoproteins participate in lipid transport and homeostasis in CNS as in the vascular compartment (Borghini et al. 1995). In the present study, we have examined the effect of cholesterol-rich native plasma HDL, cholesterol free reconstituted HDL and lipid-free apolipoproteins of HDL on the VCAM-1 expression in astroglioma cells. We demonstrate that TNF-α-induced VCAM-l was inhibited by plasma HDL, but not by VLDL or LDL.

Materials and Methods

1. Materials

U373-MG human astroglioma cells were obtained from the American Type Culture Collection (Rockville, MD). Human recombinant TNF-α (specific activity: 5.6×10^7 U/mg) was obtained from Genentech Inc. (South San Francisco, CA). Anti-human VCAM-l Abs (clone IG11B1, IgG1 isotype) were obtained from Serotec Inc. (Washington, DC). FITC-conjugated goat anti-mouse IgG was obtained from the Southern Biotechnology Association (Birmingham, AL). pBluescript SK (+/−) containing a fragment of the human VCAM-l cDNA (bp 1307-2811) was obtained from the American Type Culture Collection (Manassas, VA). pAMP-1 vector containing a fragment of human GAPDH cDNA was obtained from Life Technology Inc. (Grand Island, NY). The RNase protection assay (RPA) kit was purchased from Ambion Inc. (Austin, TX). Fresh normolipidemic human plasma was obtained from the Alabama Regional Blood Bank (Birmingham, AL). Very low density lipoproteins (VLDL), LDL and HDL fractions were isolated from plasma by the sequential flotation method (Havel et al. 1955) and purified further by another density gradient ultra-centrifugation step (Chung et al. 1980). Apolipoproteins (apo) of HDL were prepared by delipidating purified HDL with a chloroform:methanol (2 : 1) mixture. Discoidal reconstituted HDL containing apo HDL and phosphatidylcholine (PC) with an apo HDL to PC ratio of 1 : 2 was prepared by mixing apo HDL and Dimyristoylphosphatidylcholine (DMPC) liposomes and subsequently sonicating the mixture. Unilamellar vesicles of DMPC were also prepared by suspending dried DMPC in phosphate buffered saline (PBS) with subsequent sonication of suspended DMPC. DMPC was purchased from Avanti Polar Lipid Inc. (Alabaster, AL). Anti-human apo A-I polyclonal antibody was purchased from Boheringer Mannheim Biochemical Co. (Indianapolis, IN).

2. Cell culture

U373-MG human astroglioma cells were maintained in minimal essential medium (MEM) supplemented with 1 mM Earles BSS media with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and heat-inactivated fetal bovine serum (FBS). For passage, monolayers were rinsed with PBS and then dislodged by 0.25% trypsin. To study the effect of lipoproteins

or TNF-α on the VCAM-l expression, cells in 2～3 replicated wells ($2 \times l0^5$/well, 6 well plates, Nunc) were incubated with serum-free medium alone, TNF-α (50 ng/mL), VLDL (300 μg cholesterol/mL), LDL (800 μg cholesterol/mL), HDL (500 μg cholesterol/mL), or TNF -α plus VLDL, LDL or HDL for 48 h.

3. Analysis of VCAM-1

Production and/or expression of VCAM-1 in cultured astrocytes were measured by the immunofluorescence flow cytometry and by RPA as described previously (Oh et al. 1998).

4. Flow cytometry

U373-MG cells were harvested by mild trypsinization following aspiration of the culture medium and by subsequent washing of cells with PBS. The trypsinized cells were washed once, suspended in PBS containing 0.5% FBS and 0.02% sodium azide, and then incubated with human VCAM-l Ab (1 : 500 dilution) for 30 min. at 4°C. The cells were then washed twice and subsequently stained with FITC-labeled goat anti-mouse IgG (1 : 100 dilution) for 30 min. at 4°C. After washing, cells were fixed in 1% paraformaldehyde, and VCAM-1 expression was measured by fluorescence-activated cell sorting (FACS) (FACStar, Becton Dickinson, Mountain View, CA). Negative controls were incubated with an isotype-matched control anti-mouse IgG. Ten thousand cells were analyzed for each sample. VCAM-1 was expressed as a percentage of positive cells.

5. RNase protection assay (RPA)

Total cellular RNA was isolated from cell monolayers that have been incubated with VLDL, LDL or HDL in the absence or presence of TNF-α, as previously described (Oh et al. 2001). Briefly, total RNA was extracted using TRIZOL reagent (Life Technologies, Inc., Rockville, MD). A plasmid containing a fragment of the human VCAM-l cDNA (bp 1307-2811) in pBluescript SK (+/−) was linearized with *SpeI*, which digests within the VCAM-l cDNA insert. The *in vitro* transcription of this linearized vector with T7 RNA polymerase results in a 449-bp antisense RNA probe and a protected fragment consisting of 427 nucleotides. A plasmid containing a fragment of human GAPDH cDNA (bp 43-531) in pAMP-1 was linearized with *NcoI* which digests within the GAPDH cDNA insert. *In vitro* transcription of this linearized vector with T7 RNA polymerase results in a 290-bp antisense RNA probe and protected fragment consisting of 230 nucleotides. GAPDH mRNA was utilized as a "house-keeping gene" since its levels are not affected by TNF-α treatment. RPA was conducted with an RPA kit according to the manufacturer's instructions. Briefly, 15 μg/mL of total RNA was hybridized with VCAM-1 (2.5×10^4 cpm) and GAPDH (2.0×10^4 cpm) riboprobes at 42°C overnight in 20 μL of 40 mM PIPES (pH 6.4), 80% deionized formamide, 400 mM NaOAc, and 1 mM EDTA. The hybridized mixture was then treated with RNase A/T1 (1 : 200 dilution in 200 μL of RNase digestion buffer) at room temperature (RT) for 1 h, and RNA was precipitated and analyzed by 5% denaturing (8 M urea) polyacrylamide gel electrophoresis. The gels were exposed to X-ray film, and quantification of protected RNA fragments was performed by scanning with a PhosphorImager (Molecular Dynamics, Sannyvale, CA). Values for VCAM-l mRNA were normalized to GAPDH mRNA levels for each experimental condition.

6. Statistical analysis

Values are presented as mean ± standard deviation. The paired Student's *t-test* was applied to determine whether differences between values were significant. A statistical probability of $p < 0.05$ was considered significant.

Results

1. Response of human astroglioma cells to plasma lipoproteins

To define functional effect of plasma lipoproteins on astroglioma cells, we examined the effect of several lipoproteins on the expression of an adhesion molecule, VCAM-1. This gene was chosen since it was previously shown that astroglioma cells have the capacity to express VCAM-1 in response to cytokine TNF-α (Oh et al. 1998). U373-MG human astroglioma cells were treated with TNF-α (50 ng/mL), VLDL (300 μg/mL), LDL (800 μg/mL), HDL (500 μg/mL) or together for 48 h, and then, VCAM-1 expression was assessed by FACS analyses. As a representative histogram of the FACS results (Fig. 1A) shows, a physiologic level of VLDL, LDL or HDL alone did not influence on VCAM-l expression (lanes 3～5), compared with control (lane 1), and VLDL or LDL showed minimal effects of TNF-α-induced VCAM-l expression (lanes 6 and 7). However, HDL significantly inhibited the TNF-α-induced VCAM-l expression (lane 8, 43% inhibition). Flow cytometric profile of control cells and TNF-α treated cells without or with HDL show that the TNF-α treatment enhances the percentage of VCAM-1 positive cells (Fig. 1B, profile 3), and inclusion of HDL inhibits the number of TNF-α-induced VCAM-1 positive cells (Fig. 1B, profile 2). These results suggest that HDL affects adhesion molecule VCAM-1 expression in astroglioma cells.

2. Inhibition of TNF-α-induced VCAM-1 expression by HDL

Additional study by FACS analysis with different amounts of HDL (0～1,000 μg/mL) was performed. As shown in Fig. 2A, HDL reduced the TNF-α-induced VCAM-l expression in a dose dependent manner; VCAM-1 expression was reduced by 56% at a normal physiologic level of HDL (lane 6, 500 μg HDL choles-

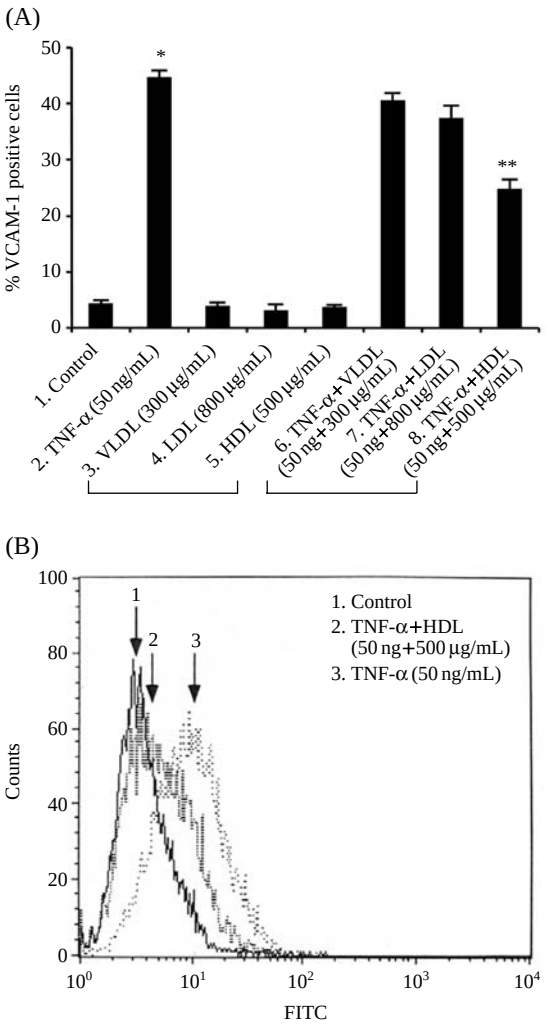

Fig. 1. Effect of plasma lipoproteins and/or TNF-α on VCAM-l expression on astroglioma cell lines. Panel A, U373-MG cells were incubated with either medium alone, TNF-α (50 ng/mL), VLDL (300 μg cholesterol), LDL (800 μg cholesterol) or HDL (500 μg cholesterol) or TNF-α with lipoproteins (VLDL, LDL or HDL) for 48 h. Cells were harvested and analyzed for VCAM-1 expression by FACS. Values expressed as the means±SD. $^{**}p<0.01$ versus TNF-α-treated control group. Panel B, U373-MG cells were incubated with either medium alone or TNF-α (50 ng/mL) with or without HDL (500 μg/mL) for 48 h. The cells were then trypsinized, and VCAM-1 expression was assessed by FACS analysis. Flow cytometric profile 1 shows control cells, and profiles 2 and 3 show cells incubated with TNF-α with or without HDL, respectively. A representative of three experiments.

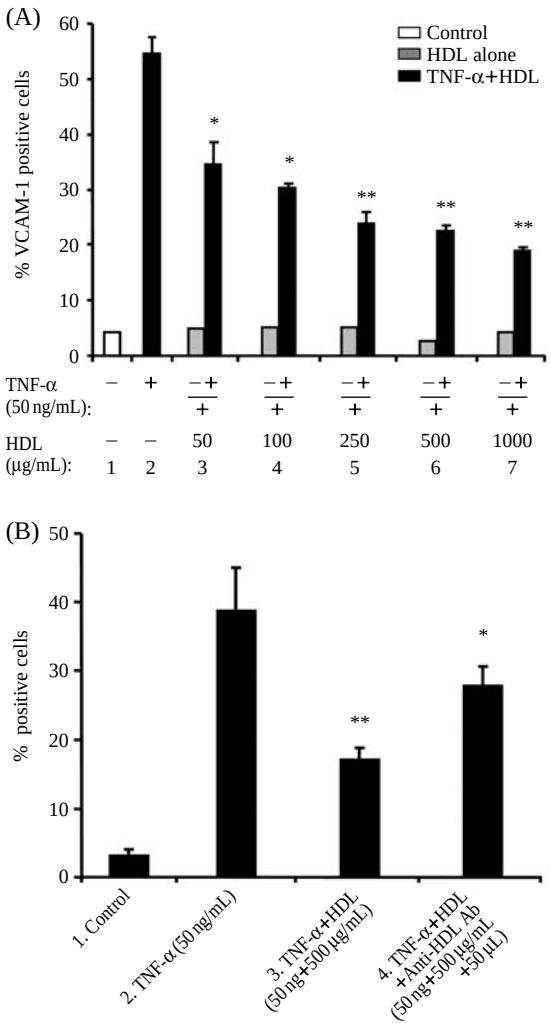

Fig. 2. The inhibitory effect of HDL on TNF-α-induced VCAM-1 expression. Panel A, U373-MG cells were incubated with either medium alone, TNF-α (50 ng/mL), or increasing concentrations of HDL (50～1,000 μg HDL cholesterol/mL) with or without TNF-α (50 ng/mL) for 48 h. Cells were harvested and analyzed by FACS for VCAM-1 expression. Values expressed as the means±SD of three separate experiments. *$p<0.05$ or **$p<0.01$ versus TNF-α-treated control goup. Panel B, U373-MG cells were incubated with either medium alone or TNF-α (50 ng/mL), TNF-α plus HDL (500 μg cholesterol/mL) or TNF-α plus HDL plus anti apo A-I Ab (50 μL of 1 : 100 dilution) for 48 h. Cells were harvested and analyzed for VCAM-1 expression. Values expressed as the means±SD of three separate experiments. *$p<0.05$ or **$p<0.01$ versus TNF-α-treated group.

terol/mL) compared with TNF-α alone (lane 2, 50 ng/mL). HDL (50～1,000 μg/mL) alone had no effects on VCAM-1 expression, similar with control (lane 1). To test the specificity of these inhibitory effects, antibody against apo A-I, the major apolipoprotein species of HDL, was used. For this test, anti-apo A-I antibody was pre-incubated with HDL for 30 min at RT and these complexes were added to the cells, and then TNF-α was treated for additional 48 h. As shown in Fig. 2B, TNF-α-induced VCAM-1 expression was inhibited by HDL (～58%). This inhibitory effect by HDL was significantly abolished (～31%) by the addition of anti-apo A-I Ab, suggesting that inhibition by HDL is specific. Preincubation of HDL with a control IgG had no effect in this response (data not shown).

3. Inhibition of TNF-α-induced VCAM-1 expression by Reconstituted HDL

Recent studies have shown that reconstituted HDL particles prepared from a major apolipoprotein species of HDL (apo A-I) and phosphatidylcholine (PC), but not apo A-I or PC alone, are effective in inhibiting TNF-α-induced VCAM-1 expression in endothelial cells (Cockerill et al. 1995, Calabresi et al. 1997). Therefore, we tested the effect of HDL and cholesterol-free reconstituted HDL prepared from delipidated HDL (apo HDL) and DMPC on VCAM-1 expression in U373-MG cells. As shown in Fig. 3, reconstituted HDL had an inhibitory effect on TNF-α-induced VCAM-1 expression (lane 4, 38%), but the effect was lower than that of native HDL (lane 3, 45%). Apo HDL or DMPC liposomes alone had a minimal effect on TNF-α-induced VCAM-1 expression (lanes 5 and 6).

4. HDL inhibits TNF-α induced VCAM-1 mRNA expression

We further examined whether HDL could modulate VCAM-1 mRNA expression. As illustrated in Fig. 4A/B, U373-MG cells are constitutively negative for VCAM-1 mRNA (lane 1), while TNF-α induces VCAM

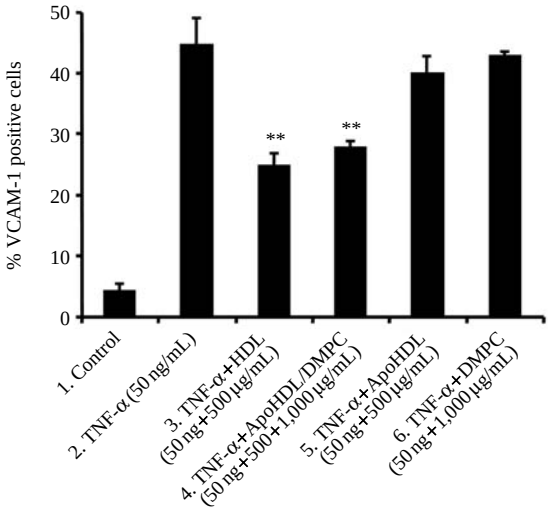

Fig. 3. Effect of various HDL forms on the TNF-α-induced VCAM-I expression. U373-MG cells were incubated with either medium alone, TNF-α (50 ng/mL), TNF-α. plus native HDL (500 μg protein/mL), TNF-α plus reconstituted HDL (500 μg apo HDL and 1,000 μg DMPC/mL), TNF-α plus apo HDL (500 μg protein/ mL) or TNF-α. plus unilamellar DMPC liposomes (1,000 μg/ mL) for 48 h. Cells were harvested and analyzed for VCAM-1 expression. Values expressed as the means ± SD of three separate experiments. $^{**}p < 0.01$ versus TNF-α-treated control group.

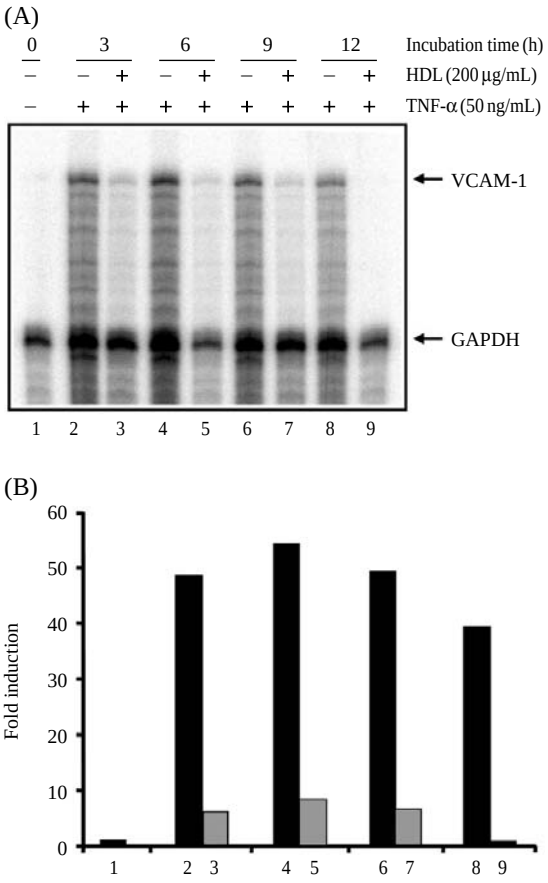

Fig. 4. HDL inhibition of TNF-α-induced VCAM-1 mRNA expression. Panel A, U373-MG cells were incubated with medium alone for 3 hr (lane 1) or with medium containing TNF-α ± HDL for 3 h (lanes 3 and 2), 6 h (lanes 5 and 4), 9 h (lanes 7 and 6) or 12 h (lanes 9 and 8). After incubation, RNA was extracted and analyzed for VCAM-1 and GAPDH mRNA by RPA. Panel B, Values for VCAM-1 mRNA expression were normalized to GAPDH mRNA levels for each experimental condition. Quantification of the experiment shown in A is depicted. Constitutive expression of VCAM-1 mRNA was set at 1, and each treatment was compared with control levels to arrived at the fold induction value. A Representative of two separate experiments.

-l mRNA expression (3 h ~ 12 h; lanes 2, 4, 6, 8), and inclusion of HDL inhibits TNF-α-induced VCAM-1 mRNA expression by more than 80% with the lapse of time (3 h ~ 12 h; lanes 3, 5, 7, 9). GAPDH was utilized as a house-keeping gene. These findings at the mRNA level are comparable to what was observed at the VCAM-l protein level (Figs. 1 and 2).

Discussion

In the present study, we investigated whether plasma HDL regulates VCAM-1 expression in human astroglioma cells. Our findings show that HDL alone does not induce VCAM-1 expression; rather it can inhibit TNF-α-induced VCAM-1 expression. Moreover, our results demonstrate a selective inhibitory effect of HDL on TNF-α-induced VCAM-1 expression with specific inhibition observed by anti-HDL antibodies.

HDL exerts their anti-inflammatory properties me-

diated multiple mechanisms, including G-proteins, MAPKs (Nofer and Assmann 2005). Furthermore, HDL prevents endothelial cell dysfunction and activation, and reduces the deleterious effect of ox-LDL by regulating membrane cholesterol level (Nofer and Assmann 2005, Negre-Salvayre et al. 2006). Sato et al. also reported that HDL-like lipoproteins derived from CSF had ability to alter the migration of neural cells (Sato et al. 2007), that is similar function to that of plasma HDL shown in endothelial cells (Negre-Salvayre et al. 2006), suggesting their functional similarity. Therefore, native plasma HDL inhibition of VCAM-1 expression (in this study) raises a possibility that HDL-like particles in the CSF may play a role of modulating VCAM-l expression in glial cells. The apo E and/or apo A-1 containing HDL-like particles in the CNS have been suggested be formed via a recombinant process involving the uptake of cellular phospholipids by apo E synthesized by astrocytes and other glial cells, and by apo A-I transported from periphery into the brain parenchyma or synthesized by brain endothelial cells via ABCA1 transporter (Ito et al. 2002). We also observed in this study that plasma HDL was effective in suppressing VCAM-1 expression in cultured astrocytes, suggesting that apo A-I containing HDL-like particles, formed by recombinant process in the CNS, may play a role of regulating VCAM-1 expression.

HDL induces FGF-2 expression in astrocytes, which is associated with ERK activation and PLC activation and Ca^{2+} mobilization (Malchinkhuu et al. 2003) and inhibits TNF-α-induced VCAM-1 expression in endothelial cells (Park et al. 2003). We demonstrated the ability of natural HDL, their lipids, apolipoprotein components, and reconstituted HDL to inhibit TNF-α-induced VCAM-1 expression in astrocytes. VCAM-1 expression on these cells depends on the activation of transcription factors, in particular NF-κB (Park et al. 2003); HDL inhibits the activation of NF-κB induced by TNF-α, resulting in inhibition of VCAM-1 expression in endothelial cells (Xia et al. 1999, Park et al. 2003). Xia et al. reported that HDL inhibited TNF-α stimulated sphingosine kinase activity in endothelial cells, resulting in a decrease in both sphingosine 1-phosphate production and TNF-α induced VCAM-1 expression, suggesting the importance of sphingosine kinase pathway (Xia et al. 1999). As well, it is known recently that HDL induces TGF-β expression in endothelial cells (Norata et al. 2005) and TGF-β inhibits TNF-α induced VCAM-1 expression in astrocytes (Winkler and Beveniste 1998), raising a possibility that HDL inhibition of TNF-α induced VCAM-1 expression shown in this study may be occurred by TGF-β induced by HDL. This possibility is being currently investigated.

Although the mechanism whereby HDL inhibits TNF-α induced VCAM-1 expression in the CNS is not fully understood, in any circumstances, if the integrity of BBB is destroyed by traumatic injury, hemorrhage or neuroinflammatory diseases, plasma HDL and proinflammatory cytokines including TNF-α could infiltrate into the CNS, and HDL may function as an immunosuppressive role in inhibiting cytokine-induced adhesion molecules expression, together with HDL-like particles derived from CNS.

References

Bevilacqua MP, Nelson RM, Mannori G, Cecconi O : Endothelial-leukocyte adhesion molecules in human disease. Annu Rev Med 45: 361-378, 1994.

Borghini I, Barja F, Pometta D, James RW : Characterization of subpopulations of lipoprotein particles isolated from human cerebrospinal fluid. Biochim Biophys Acta 1255: 192-200, 1995.

Calabresi L, Franceschini G, Sirtori CR, De Palma A, Sareselle M, Ferrante P, Taramelli D : Inhibition of VCAM-1 expression in endothelial cells by reconstituted high density lipoproteins. Biochem Biophys Res Commun 238: 61-65, 1997.

Cannella B, Raine CS : The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 37: 424-435, 1995.

Chung BH, Wilkinson T, Geer JC, Segrest JP : Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. J Lipid Res 21: 284-291, 1980.

Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ : High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 15: 1987-1994, 1995.

Fagan AM, Holtzman DM : Astrocyte lipoproteins, effects of apoE on neuronal function, and role of apoE in amyloid-beta deposition in vivo. Microsc Res Tech 50: 297-304. 2000.

Havel RJ, Eder HA, Bragdon JH : The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34: 1345-1353, 1955.

Hurwitz AA, Berman JW, Rashbaum WK, Lyman WD : Human fetal astrocytes induce the expression of blood-brain barrier specific proteins by autologous endothelial cells. Brain Res 625: 238-243, 1993.

Ito J, Nagayasu Y, Kato K, Sato R, Yokoyama S : Apolipoprotein A-I induces translocation of cholesterol, phospholipid, and caveolin-1 to cytosol in rat astrocytes. J Biol Chem 277: 7929-7935, 2002.

Koch S, Donarski N, Goetze K, Kreckel M, Stuerenburg HJ, Buhmann C, Beisiegel U : Characterization of four lipoprotein classes in human cerebrospinal fluid. J Lipid Res 42: 1143-1151, 2001.

Malchinkhuu E, Sato K, Muraki T, Ishikawa K, Kuwabara A, Okajima F : Assessment of the role of sphingosine 1-phosphate and its receptors in high-density lipoprotein-induced stimulation of astroglial cell function. Biochem J 370: 817-827, 2003.

Negre-Salvayre A, Dousset N, Ferretti G, Bacchetti T, Curatola G, Salvayre R : Antioxidant and cytoprotective properties of high-density lipoproteins in vascular cells. Free Radic Biol Med 41: 1031-1040, 2006.

Nofer JR, Assmann G : Atheroprotective effects of high-density lipoprotein-associated lysosphingolipids. Trends Cardiovasc Med 15: 265-271, 2005.

Norata GD, Callegari E, Marchesi M, Chiesa G, Eriksson P, Catapano AL : High-density lipoproteins induce transforming growth factor-beta2 expression in endothelial cells. Circulation 111: 2805-2811, 2005.

Oh JW, Drabik K, Kutsch O, Choi C, Tousson A, Benveniste EN : CXC chemokine receptor 4 expression and function in human astroglioma cells. J Immunol 166: 2695-2704, 2001.

Oh JW, Van Wagoner NJ, Rose-John S, Benveniste EN : Role of IL-6 and the soluble IL-6 receptor in inhibition of VCAM-1 gene expression. J Immunol 161: 4992-4999, 1998.

Park SH, Park JH, Kang JS, Kang YH : Involvement of transcription factors in plasma HDL protection against TNF-alpha-induced vascular cell adhesion molecule-1 expression. Int J Biochem Cell Biol 35: 168-182, 2003.

Puglielli L, Tanzi RE, Kovacs DM : Alzheimer's disease: the cholesterol connection. Nat Neurosci 6: 345-351, 2003.

Rosenman SJ, Shrikant P, Dubb L, Benveniste EN, Ransohoff RM : Cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1) by astrocytes and astrocytoma cell lines. J Immunol 154: 1888-1899, 1995.

Sato K, Malchinkhuu E, Horiuchi Y, Mogi C, Tomura H, Tosaka M, Yoshimoto Y, Kuwabara A, Okajima F : HDL-like lipoproteins in cerebrospinal fluid affect neural cell activity through lipoprotein-associated sphingosine 1-phosphate. Biochem Biophys Res Commun 359: 649-654, 2007.

Shrikant P, Chung IY, Ballestas ME, Benveniste EN : Regulation of intercellular adhesion molecule-1 gene expression by tumor necrosis factor-alpha, interleukin-1 beta, and interferon-gamma in astrocytes. J Neuroimmunol 51: 209-220, 1994.

Winkler MK, Beveniste EN : Transforming growth factor-beta inhibition of cytokine-induced vascular cell adhesion molecule-1 expression in human astrocytes. Glia 22: 171-179, 1998.

Xia P, Vadas MA, Rye KA, Barter PJ, Gamble JR : High density lipoproteins (HDL) interrupt the sphingosine kinase signaling pathway. A possible mechanism for protection against atherosclerosis by HDL. J Biol Chem 274: 33143-33147, 1999.

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사람별아교모세포종세포에서 High Density Lipoprotein이 Tumor Necrosis Factor-Alpha에 의해 증가된 VCAM-1 발현을 억제한다

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간추림 : 중추신경계에서 교세포의 대다수를 차지하는 Astrocytes는, 사이토카인 TNF-α 등의 자극으로 여러 혈관세포유착인자들 중, vascular cell adhesion molecule-1 (VCAM-1)의 발현을 증가시킨다. 이렇게 증가된 VCAM-1의 발현은 중추신경계의 염증과정에서 중요한 역할을 수행한다.

Astrocyte cells이 apo E 등을 포함하는 HDL-like lipoprotein particles을 분비함이 최근에 밝혀졌기 때문에, 우리는 astroglioma 세포를 가지고 VCAM-1 발현변화에 초점을 두고, 인체 혈장에서 분리된 HDL, VLDL, 그리고 LDL 등 다른 lipoproteins들의 VCAM-1 발현 조절 가능성을 조사하였다. Astroglioma세포에 VLDL, LDL 그리고 HDL 등의 단독처리는 VCAM-1 발현변화에 아무런 영향이 없었다. 그러나 Astroglioma세포에서 HDL이 흥미롭게도 농도의존성으로 TNF-α에 의해 증가된 VCAM-1 발현을 억제시켰다. 그러나 비슷한 구조를 갖는 VLDL, LDL은 이러한 억제성을 보여주질 못했다. 특이성 실험에서, TNF-α에 의해 증가된 VCAM-1 발현에 대한 HDL억제성은 HDL의 주요 apolipoprotein 구성원인 Apo A-1 항체의 전처리로 인해 다시 증가됨을 확인하였다. 이는 곧 HDL의 Apo A-1이 이러한 억제기능에 중요한 역할이 있음을 의미한다. 게다가 Reconstituted HDL (apo HDL과 DMPC의 dis-coidal complexes) 또한 VCAM-1 발현증가를 억제함을 알 수 있었다. RNase protection assay (RPA) 실험에서, TNF -α에 의해 증가된 VCAM-1 mRNA 발현증가 역시 HDL에 의해 억제되었다.

이러한 결과들은 질환상태의 중추신경계에서 HDL-like particles이 면역억제기능을 수행할 수 있음을 제시한다.

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