Abstracts

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Abstracts for the oral and poster presentations are provided in this special on-line supplement.
to oxidized lipids are the mechanisms by which GPx inhibits atherosclerosis. Unexpectedly, heterozygous mutation to GPx4 did not increase atherosclerotic lesions and oxidized lipids in ApoE−/− mice.

**Mac-I Mediates CD40L-induced Atherogenesis**

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Strong evidence supports a role for CD40L as marker and mediator of inflammatory diseases such as atherosclerosis. Despite extensive characterization of CD40, the classical receptor for CD40L, in immune defense, its role in inflammatory diseases remains uncertain. This study aims to investigate whether CD40L can be used to monitor inflammatory states in vivo. We first generated a truncated apoA-V, comprising amino acids 1–292, was generated. Far UV circular dichroism spectra of full-length apoA-V and apoA-IV[1−292] were similar with ~50% alpha helical content. In guanidine HCl denaturation experiments, both full-length and truncated apoA-V yielded biphasic profiles consistent with the presence of two structural domains. The denaturation profile of the lower stability component, but not the higher stability component, was affected by the truncation. In fluorescent dye binding experiments, apoA-V[1−292] contained fewer swollen exposed hydrophobic sites than full-length apoA-V. Truncated apoA-V displayed an attenuated ability to solubilize DMPC phospholipid vesicles compared to full-length apoA-V but it bound to a bovine/intermediates with faster kinetics. Taken together, the data suggest the concept that the 51 amino acid segment C-terminal to the tetra-proline sequence, is not required for apoA-V to adopt a folded protein structure yet functions to modulate apoA-V lipid binding activity and thereby, may be relevant to the mechanism whereby it influences plasma TG levels.

**Disruption of Glycosylation at Asparagine-116 of Endothelial Lipase Enhances Activity and Lipoprotein Lipid Hydrolysis in Vivo and in vitro**

Robert J Brown, Gwen C Miller, Nathalie Grifflon, Christopher J Long, Daniel J Rader; Univ of Pennsylvania, Philadelphia, PA

We previously identified that four of five putative N-linked glycosylation sites of human endothelial lipase (EL) are utilized, and showed that substitution of the carbohydrate-linked Asn-116 with Ala (N116A) increased the hydrolytic activity of EL. We expanded this observation by first assessing the catalytic activities of additional mutants of the Asn-Asn-Thr glycosylation sequence: Asn-116 to Thr (N116T), Asn-117 to Ala (N117A), and Thr-118 to Ala (T118A). The specific activities of N116T- and T118A-EL were significantly enhanced toward dipalmitoylethanolamine (25% and 57% above, respectively, p<0.001) while both wild-type EL and N117A-EL had 25% and 12% 116 and 117, respectively, and the specific activities of N116A and N117A-EL toward triolein were also significantly greater (29% and 26% above, respectively, p<0.001) than WT- and N117A-EL (100% and 26%, respectively). These data demonstrate that it is the loss of glycosylation at Asn-116, and not a structural effect of specifically the N116A mutant, that results in enhanced catalytic activity of EL. We next assessed the hydrolysis of native lipoprotein lipases by N116A-EL. Compared to WT-EL, the N116A mutant exhibited a significant 5-fold increase in low density lipoprotein (LDL) hydrolysis and a 1.5-fold increase in high density lipoprotein (HDL) 2 hydrolysis. HDL hydrolysis was unchanged. Consistent with these observations, adenoviral-mediated expression of N116A-EL (3 x 10^11 virus particles per mouse) in LDL-receptor-null mice significantly increased levels of both HDL cholesterol (8.3 ± 1.9 mg/dl, p<0.03) and non-HDL cholesterol (37.8 ± 9.7 mg/dl, p<0.03) beyond the reductions observed by the expression of WT-EL alone (30.8 ± 9.7 and 81.8 ± 13.8 mg/dl, respectively). Finally, we introduced Asn-116-EL in the aorto-proximal arteries, the aortic arch, root, and thoraco-abdominal aorta compared to LDLR single-deficient controls in mice that consumed an atherogenic diet for 8 and 16 weeks. Lesions in these two groups of mice were compared with lesions in C57BL/6 background individuals and were also compared with lesions in LDLR−/− mice. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated atherogenesis. This novel mechanism expands understanding of inflammatory signaling during atherogenesis and has implications regarding novel anti-inflammatory therapies.

**Structure-Function of Apolipoprotein A-IMilano and Related Variants**


Human apolipoprotein A-V (apoA-V) is a potent modulator of plasma triacylglycerol (TG) levels. To probe different regions of this 343 amino acid protein, 4 single Trp apoA-V variants were engineered: R173C apoA-V, R173K apoA-V, R173S apoA-V, and 269 (e.g. mouse) that display a monophasic profile. HDL2 is thought to be more atheroprotective than HDL3 because of a superior ability of A-IM HDL to protect the endothelium. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM carriers showed that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells.