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学　位　論　文　名	Expression of cholesteryl ester transfer protein in human atherosclerotic lesions and its implication in reverse cholesterol transport （ヒト粥状動脈硬化巣におけるコレステロールエステル転送蛋白の発現とその意義）
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論　文　内　容　の　要　旨

[Aim]

Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein with a molecular weight of 74 kD, which is synthesized by the liver, small intestines and adipose tissues. In the reverse cholesterol transport (RCT), CETP is known to facilitate the transfer of cholesteryl ester (CE) from high density lipoprotein (HDL) to very low density lipoproteins (VLDL) and low density lipoproteins (LDL). The transferred cholesterol by CETP is finally taken up by LDL receptor in the liver, a terminus of RCT. Our previous studies demonstrated that CETP may play an important role in remodeling large HDL particles into smaller ones which are active for cholesterol efflux. The aim of present study is to investigate the roles of CETP in the initial step of RCT, namely macrophages (Mφ) in the blood vessels.

[Materials and Methods]

1. Immunohistochemistry

Human aortic and coronary tissues were obtained from 19 autopsied cases. Fatty streaks were observed in 5 out of 19 cases, and plaques were observes in all of the cases. Mononuclear cells were isolated from healthy volunteers and homozygous CETP-deficient patients. CETP-LT-A4, anti-human CETP monoclonal antibody, was used to detect immunoreactive CETP with labeled streptavidin biotin (LSAB)-peroxidase method.

2. Transient transfection

Ten μg of plasmid DNA was transfected into Cos7 cells using FuGene 6 reagent. In order to confirm the efficiency of the transfection, we measured the concentration of CETP in culture medium of transfected cells. The concentration of CETP in the medium of CETP transfected cells (0.25 ng/ml) was much higher than that of mock transfected cells (below 0.02 ng/ml), showing a successful transfection.

3. Cholesterol efflux

Cells were labeled with ^{3}H cholesterol, and 100 μg/ml of HDL3 was used as acceptor.

[Results]

1. Immunocyto- and immunohisto- chemical analyses of CETP

(1) Immunocytochemical analysis of CETP in human monocyte-derived Mφ

The immunoreactive mass of CETP was clearly detected in the Mφ from controls, whereas control IgG did not show any signals. Furthermore, we could not detect any immunoreactive CETP in the Mφ from CETP-deficient patients. These data demonstrated the specificity of the CETP antibody used in the following procedures.

(2) Immunohistochemical analysis of CETP in human aorta

We could not detect CETP in the normal regions of aorta, but the immunoreactive mass of CETP was detected in fatty streaks and atherosclerotic plaques. Along with the progression of atheroclerosis, from fatty streaks to advanced lesions, the intensities of immunoreactive CETP appeared to be increased.

(3) Immunohistochemical analysis of CETP in human coronary arteries

In coronary arteries, the staining patterns of CETP were similar to those observed in the aorta. CETP-positive cells were detected in the plaque, whereas it was not detected in diffusely thickened intima.

(4) Identification of CETP-positive cells by double immunostaining

In order to know the cell types of CETP-positive cells, double immunostaining was performed. No immunoreactive CETP was detected in the Mφ and smooth muscle cells (SMCs) of non-atherosclerotic lesions. In contrast, a positive immunostaining for CETP could be detected in the regions of atherosclerotic plaques. The majority of CETP-positive cells appeared to be the Mφ and some of CETP-positive cells appeared to be SMCs.

2. Functional assay of CETP

(1) Effect of the transfection of CETP cDNA on cholesterol efflux

We tansfected CETP cDNA into Cos7 cells and analyzed the cholesterol efflux from these cells. The transient transfection of CETP cDNA led to a significant increase in free cholesterol efflux. The increased efflux was not inhibited by the neutralizing antibody against CETP.

(2) Function of endogenous CETP

In order to know the function of endogenous CETP, we also examined the cholesterol efflux from the Mφ of subjects with or without CETP deficiency. The free cholesterol efflux from CETP deficient Mφ was decreased compared with normal control, suggesting that endogenous CETP also increased free cholesterol efflux.

[Summary]

(1) CETP was abundantly expressed in *in vitro* differentiated Mφ and the foam cells in human atherosclerotic lesions.

(2) The majority of CETP-positive cells were Mφ and a minor population was SMCs.

(3) Overexpression of CETP led to a significant increase in free cholesterol efflux.

(4) Free cholesterol efflux was significantly decreased in CETP-deficient Mφ compared with control Mφ.

[Conclusion]

CETP may possess an anti-atherogenic function to remove free cholesterol from the peripheral cells, suggesting a role of CETP at the initial step of RCT.

論文審査の結果の要旨

高比重リポ蛋白（HDL）を介した動脈硬化防御機構として、動脈硬化巣の泡沫細胞に蓄積したコレステロールをくみ出し、最終的に肝臓へと輸送するいわゆるコレステロール逆転送系（RCT）が最も重要である。コレステリルエス

テル転送蛋白（CETP）は、この経路において HDL からコレステロールをアポ B 含有リポ蛋白に転送することにより、泡沫細胞からのコレステロール引き抜き活性の強い小粒子の HDL の形成に関与していることが明らかとなっている。本研究では、CETP が培養マクロファージおよびヒト冠状動脈、大動脈の粥状動脈硬化巣の泡沫細胞に強く発現していることを見出した。さらに、CETP の過剰発現細胞ではコレステロール引き抜きが増加していること、逆に CETP 欠損症患者から得たマクロファージでは、コレステロール引き抜きが有意に低下していることも見出した。以上、本研究によって、CETP は RCT の第 1 段階として泡沫細胞からのコレステロール引き抜きに重要な役割を果たしていること明らかにした。

本研究は、動脈硬化防御機構における CETP の新しい意義を明らかにしたもので学位に充分、値すると考えられる。