Modification of CETP function by changing its substrate preference: a new paradigm for CETP drug design

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Abstract  We previously determined that hamster cholesteryl ester transfer protein (CETP), unlike human CETP, promotes a novel one-way transfer of TG from VLDL to HDL, causing HDL to gain lipid. We hypothesize that this nonreciprocal lipid transfer activity arises from the usually high TG/cholesteryl ester (CE) substrate preference of hamster CETP. Consistent with this, we report here that ~25% of the total lipid transfer promoted by the human Q199A CETP mutant, which prefers TG as substrate, is nonreciprocal transfer. Other CETP mutants with TG/CE substrate preferences higher or lower than wild-type also possess nonreciprocal lipid transfer activity. Mutants with high TG/CE substrate preference promote the nonreciprocal lipid transfer of TG from VLDL to HDL, but mutants with low TG/CE substrate preference promote the nonreciprocal lipid transfer of CE, not TG, and this lipid flow is in the reverse direction (from HDL to VLDL). Anti-CETP TP2 antibody alters the TG/CE substrate preference of CETP and also changes the extent of nonreciprocal lipid transfer, showing the potential for externally acting agents to modify the transfer properties of CETP Overall, these data show that the lipid transfer properties of CETP can be manipulated. Function-altering pharmaceuticals may offer a novel approach to modify CETP activity and achieve specific modifications in lipoprotein metabolism.—Morton, R. E., and L. Izem. Modification of CETP function by changing its substrate preference: a new paradigm for CETP drug design. J. Lipid Res. 2015. 56: 612–619.

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Cholesteryl ester transfer protein (CETP) plays an important role in human lipoprotein metabolism (1–4). CETP alters lipoprotein composition by its ability to promote lipid heteroexchange. This involves the transfer of TG from TG-rich lipoproteins, such as VLDL, to cholesteryl ester (CE)-rich lipoproteins, such as HDL, in return for an equimolar transfer of CE in the opposite direction (5). As a result, the core lipid (CE and TG) composition of lipoproteins is altered, but the amount of this lipid in a lipoprotein particle is unchanged. Changes in core lipid composition alter the biological properties of lipoproteins and modify their catabolism (3, 6–9).

We recently compared the lipid transfer properties of human CETP with CETP from three other species (10). Compared with human CETP, the relative preference for TG as a transfer substrate instead of CE was higher in monkey, rabbit, and hamster CETPs. Like human CETP, these CETP species promoted both lipid homoexchange, where the same lipid species is transferred between two lipoproteins, and the heteroexchange of lipids as described above. However, hamster CETP, which has a markedly higher preference for TG as a substrate compared with all other CETP species, also facilitated a unique lipid transfer event where the transfer of TG from VLDL to HDL was not coupled to the return of lipid to VLDL, causing HDL to have a net gain in core lipid. We refer to this as nonreciprocal lipid transfer.

Hamster CETP has ~80% amino acid homology with human CETP (10). It seems reasonable that the capacity of hamster CETP to promote nonreciprocal lipid transfer arises from a different molecular structure due to the 66 nonconservative amino acid substitutions between these species. However, Qiu et al. (11) demonstrated that a Q199A mutation in human CETP significantly alters its relative preference for TG versus CE as substrate, resulting in a TG/CE preference ratio similar to that of hamster CETP (10, 11). Because it is unlikely that this glutamine to alanine substitution induces global changes in the CETP structure, this observation provides an opportunity to test the hypothesis that human CETP can be induced to promote nonreciprocal lipid transfer activity if its lipid substrate preference is altered to mirror that of hamster CETP. Hence, we propose that nonreciprocal lipid transfer occurs in hamster CETP because of its high preference for TG over CE, not because its many amino acid differences have induced large-scale structural changes that endow it with the unique ability to promote nonreciprocal transfer.

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Data presented here show that multiple human CETP mutants promote nonreciprocal lipid transfer. Depending on whether a mutation increases or decreases the TG/CE substrate preference relative to wild-type CETP, the resulting nonreciprocal lipid transfer activity either promotes the net movement of VLDL-TG into HDL or the net movement of HDL-CE into VLDL. The ability to manipulate CETP’s functionality provides a new and exciting target for lipid lowering therapies.

METHODS

Materials

Mouse monoclonal antibody to human CETP (TP2) was purchased from the Ottawa Heart Institute (Ottawa, Ontario, Canada). TP2 Fab fragments were generated by immobilized papain (Thermo Fisher Scientific, Waltham, MA). The [9,10-3H(n)]triolein and cholesteryl [1-14C]oleate were from Perkin-Elmer (Waltham, MA). Buffers, salts, and detergents were purchased from Sigma (St. Louis, MO).

CETP preparation

Plasma-derived human CETP was purified as previously described (12). cDNAs for wild-type human CETP (M30185.1) and single amino acid mutants (Q199A, R201S, H232A, M433A, D442G, and R451Q) were synthesized (GenScript, Piscataway, NJ), subcloned into pcDNA3, and sequence verified (10). The cDNAs for rabbit (Oryctolagus cuniculus, XM_002711536), monkey (Macaca fascicularis, M86343), and hamster (Cricetulus griseus, XM_003503614) CETPs were synthesized as previously described (10). All constructs contained native start and stop codons including the codon sequence for a 17 amino acid signal peptide. pcDNA5-CETP constructs were transfected (20 μg/~106 cells) into HEK293 cells containing no added CETP were incubated under identical conditions to determine the assay blank value. When the effect of anti-CETP antibody was examined, CETP was preincubated with 10% donor and acceptor lipoproteins. Transfer was initiated by adding 20% donor and acceptor lipoproteins. Transfer was initiated by adding lipoproteins and incubating at 37°C.

Lipoprotein substrates

Fresh plasma obtained from the Cleveland Clinic Blood Bank was fractionated by sequential density ultracentrifugation (13) to yield VLDL (d < 1.006 g/ml), LDL (1.019 < d < 1.063 g/ml), and HDL (1.063 < d < 1.21 g/ml). Double-labeled (3H-TG and 14C-CE) lipoproteins were prepared by two methods. In one instance, prior to their isolation from plasma, lipoproteins were labeled by the lipid dispersion method previously described (14), then isolated by ultracentrifugation. Alternatively, isolated VLDL or HDL were incubated with partially purified human plasma CETP and phosphatidylcholine-cholesterol liposomes containing tracer quantities of 3H-TG and 14C-CE (15), followed by isolation of the lipoprotein within its original density limits (13). This method produced radiolabeled lipoproteins with minimal change to their native composition. Unlabeled VLDL and HDL were prepared in the same way, except that the phosphatidylcholine-cholesterol liposomes contained no radiolabel.

CETP transfer assays

Except when noted, transfer assays followed the general design previously described (16, 17). In brief, 3H-TG- and 14C-CE-labeled donor and unlabeled acceptor lipoproteins were incubated in Tris-buffered saline (pH 7.4), 0.5% BSA, and a source of CETP at 37°C for the indicated time. In assays containing HDL, transfer reactions were stopped by precipitating LDL or VLDL with MnCl2 in the presence of sodium phosphate (17). When lipid transfer was measured between VLDL and LDL, samples were cooled to 4°C and lipoproteins were separated by ultracentrifugation (d = 1.019 g/ml). In all assays, radioactivity in the acceptor lipoprotein was determined by scintillation counting. The percent of lipid transferred was typically calculated as previously described (16). However, when determining the mass transfer of TG and CE between VLDL and HDL, a two-compartment closed model calculation was used. This calculation takes into account the “return” of labeled lipid to the donor from the acceptor during the transfer reaction, which can be significant when the acceptor TG or CE pool is small compared with the donor pool. The mass of TG or CE transferred was calculated as:

\[
\text{mass transferred} = -\frac{(M_1 \times M_2) \times \ln(1 - \frac{[Rt - \text{blank}]}{M_1})}{(M_2 \times \text{Ro})};
\]

where M1 is the mass of lipid in the donor lipoprotein, M2 is the mass of lipid in the acceptor lipoprotein, M1 is M1 + M2, Ro is the radioactivity in the donor lipoprotein at time zero, and Rt is the radioactivity in the acceptor lipoprotein at time t.

Previous studies have shown that a two-pool model adequately describes CETP activity between a LDL and HDL (18). This calculation assumes that only homoechange occurs, which is not the case between VLDL and HDL. Although homoechange is the predominant CETP reaction between these lipoproteins (15), to minimize the effects of heteroexchange and nonreciprocal lipid transfer on the lipid pool sizes, we limited the extent of lipid transfer to ≤20%.

Lipid transfer assays were linear with respect to assay time and CETP concentration. Varying the ratio or concentration of donor and acceptor lipoproteins altered CETP activity, but had the same effect on both CE and TG lipid transfers, resulting in no change to the ratio of TG to CE transferred. Assay samples containing no added CETP were incubated under identical conditions to determine the assay blank value. When the effect of anti-CETP antibody was examined, CETP was preincubated with TP2 Fab fragments for 1 h at room temperature in the absence of donor and acceptor lipoproteins. Transfer was initiated by adding lipoproteins and incubating at 37°C.

Other analyses

Protein was quantified by a modification of the Lowry et al. (19) method with BSA as standard. Total cholesterol and TG were determined by enzymatic kits from ThermoFisher Scientific (Middleton, VA), and free cholesterol was measured by an enzymatic kit from Wako Chemical (Richmond, VA). CE was calculated from the difference between total and free cholesterol measurements times 1.69 to correct for its fatty acid content. For electrophoresis, conditioned media from transfected HEK cells were subjected to RIPA buffer, treated with 5× SDS-β mercaptoethanol (20), and fractionated by SDS electrophoresis on 4–20% polyacrylamide gels (Lonza, Rockland, ME). Following transfer of proteins to polyvinylidene difluoride, blots were reacted sequentially with mouse TP2 antibody and horseradish peroxidase-conjugated goat-anti mouse IgG (EMD Millipore, Billerica, MA). Immune complexes were visualized by ECL (PerkinElmer, Waltham, MA). Data shown are the mean ± SD. Statistical significance was determined by Student’s t-test.

RESULTS

Effect of CETP mutations on lipid transfer between various lipoproteins

Qiu et al. (11) described multiple human CETP mutants that have altered ability to transfer TG and/or CE.
For example, the Q199A CETP mutant strongly prefers TG as a substrate, much like that observed for hamster CETP (10). We have hypothesized that the unique ability of hamster CETP to promote the nonreciprocal transfer of TG from VLDL to HDL (10) does not arise from an underlying different transfer mechanism in this species of CETP, but is due to its unusually high preference for TG versus CE as a substrate. To test this hypothesis, we examined the lipid transfer properties of human CETP mutants that have markedly higher preference for TG, or CE, as a substrate compared with wild-type. Because the lipid transfer properties of the mutants described by Qiu et al. were measured only by their capacity to transfer these lipids from radiolabeled LDL to HDL, we initially sought to verify that the altered TG and CE activity of these mutants was also observed with other lipoprotein substrates. Although some small differences were noted, in general the altered preference for TG or CE by mutants was observed independent of the donating or accepting lipoprotein (Fig. 1). Recombinant Q199A CETP consistently showed a 3- to 4-fold higher preference for TG than CE compared with wild-type CETP, and recombinant H232A CETP had a ∼3-fold higher preference for CE over TG (lower TG/CE preference ratio).

**Impact of altering the substrate preference of CETP on lipid movement between lipoproteins**

To assess the lipid transfer properties of CETP variants, we measured the transfer of TG and CE from VLDL to HDL, and from HDL to VLDL. As expected, due to the higher TG content of VLDL, wild-type CETP promoted greater TG transfer from VLDL to HDL than from HDL to VLDL (Fig. 2A). Similarly, CE transfer from HDL to VLDL exceeded that from VLDL to HDL (Fig. 2B). As shown in Fig. 2C, the net gain of TG by HDL and its net loss of CE were equimolar. Changes in VLDL composition were the opposite of those noted in HDL (not shown). These data demonstrate that wild-type CETP facilitates only lipid exchange, resulting in changes in the amounts of TG and CE present in lipoproteins, but no change in the sum of TG + CE.

Compared with wild-type CETP, and consistent with its higher TG preference, Q199A CETP facilitated higher TG transfer between VLDL and HDL (Fig. 3A) and lower CE transfer (Fig. 3B). In contrast to wild-type CETP, the transfer of TG into HDL by Q199A CETP significantly exceeded the loss of CE from this lipoprotein (Fig. 3C). Thus, like hamster CETP, in addition to lipid exchange, Q199A CETP also promoted the unidirectional flow of TG into HDL causing HDL to gain lipid and VLDL to lose lipid. For each CETP molecule leaving HDL, approximately two molecules of TG entered.

Studies with H232A CETP, which has a much higher preference for CE versus TG compared with wild-type CETP, provided further insight into how the function of CETP can be manipulated. TG transfer between VLDL and HDL was markedly reduced, whereas CE transfer was not markedly different from wild-type (Fig. 4A, B). However, with H232A CETP, the loss of CE from HDL exceeded its gain of TG (Fig. 4C). For each TG molecule gained by HDL, ∼2 molecules of CE were transferred to VLDL, causing HDL to lose core lipid. Therefore, H232A CETP, like Q199A, promotes nonreciprocal lipid transfer, but the direction of this lipid flow is opposite and it involves CE not TG. Nonreciprocal lipid transfer accounted for up to 23% of total lipid transferred by these CETP mutants (Table 1). The extent of TG-CE heteroexchange was also impacted by alterations in CETP’s substrate specificity, further modifying how these CETP mutants change lipoprotein composition.

These data suggest that there may be a direct correlation between the TG versus CE preference of CETP and its effect on lipoprotein lipid composition. To further investigate this relationship, we conducted similar studies with other human CETP mutants and with CETP from other species that naturally have different TG/CE substrate preferences than wild-type human CETP. With the eight CETPs studied, there was a strong linear correlation (r = 0.979) between the preference of these CETPs for TG as a substrate and the extent to which they promoted TG gain versus CE loss in HDL (Fig. 5). CETPs with TG/CE substrate preference ratios higher than wild-type human CETP promoted an increase in HDL core lipid by facilitating greater TG influx than CE efflux. Conversely, CETPs with lower TG/CE substrate preference ratios than wild-type CETP decreased HDL core lipid by driving greater CE efflux from HDL than TG influx.

**Substrate preference of natural CETP variants**

Multiple variants of CETP have been identified and their association with lipoprotein levels and cardiovascular risk has been extensively studied (21). CETP mutations affecting the coding region have typically been assayed only for their CE activity. In view of our observation above, we measured the TG versus CE substrate preference for two common CETP variants, D442G and R451Q. Whereas the

![Fig. 1. Preference of CETP variants for TG and CE as substrate from different lipoprotein donors. Recombinant human wild-type (Wt) or the indicated variant was incubated with 3H-TG, 14C-CE-labeled VLDL (V), 3H-TG, 14C-CE-labeled LDL (L), or 3H-TG, 14C-CE-labeled HDL (H) and the transfer of these lipids to unlabelled V, L, or H was measured as described in the Methods. Assays contained 9 μg V, 7 μg L, or 38 μg H (protein) as indicated. Data are expressed relative to the activity of wild-type CETP in each assay. Inset: Immunoblot of CETP fractionated by SDS polyacrylamide electrophoresis. The position of molecular mass standards in kilodalton units is shown on the left.](image-url)
Alteration of CETP functionality

fragments of the TP2 monoclonal antibody inhibit TG transfer by CETP greater than CE transfer. We have verified a similar selective effect in HDL to LDL transfer assays, as reported by these investigators, and also found that the preferential inhibition of TG transfer by TP2 Fab is also observed when lipid transfers are measured between other lipoproteins (data not shown).

To optimize conditions for assessing the impact of TP2 Fab fragments on CETP function, we evaluated the effect of these antibody fragments on CETP from different species. The C-terminal 26 amino acids identified to contain the TP2 epitope (23) are highly similar between human, monkey, rabbit, and hamster (Fig. 6A). Subsequent studies identified a smaller 15 amino acid sequence in this epitope directly involved in TP2 binding (24). This Phe463-Leu475 sequence is completely retained in monkey and hamster CETP; rabbit CETP has a single amino acid different in the binding region. Using sufficient TP2 Fab to elicit ~50% TG transfer inhibition, we found that

substrate preference for R451Q CETP did not differ from wild-type CETP, the substrate preference ratio for D442G CETP was significantly reduced (Table 2). Because most CETP mutants cause a loss of function (11), these data suggest that the D442G mutation reduces TG transfer by CETP. Thus, the D442G mutation is sufficient to induce a small, but perhaps metabolically important, shift in the lipid transfer properties of CETP.

TP2 Fab as a model for control of CETP function by an externally acting agent

The above studies indicate that the biological function of CETP can be modified by alteration of its primary structure. Manipulating the function of CETP may have therapeutic value. Such alterations in CETP function would need to be achieved by agents that interact with CETP and induce conformational changes and/or modify specific amino acid residues. We have taken advantage of the observation of Swenson et al. (22) that limiting doses of Fab
TP2 Fab can reduce the TG/CE substrate preference of all four CETP species (Fig. 6B). This reduction was the largest for hamster, presumably because the native hamster CETP has a very high preference for TG.

Having observed that the substrate preference of hamster CETP is most easily manipulated by TP2 Fab, we then determined whether TP2 Fab could modify the natural ability of hamster CETP to alter HDL composition. As previously reported (10) and also shown in Fig. 5, native hamster CETP promotes greater TG flux into HDL than CE loss from this lipoprotein, leading to an increase in HDL core lipid. However, pretreating this CETP with TP2 Fab caused a dose-dependent decrease in its ability to enrich HDL with TG with minor changes in the CE loss (Fig. 7). Consequently, the extent of nonreciprocal transfer of TG into HDL, which is calculated from the imbalance in TG gain and CE loss from HDL, decreased by 63%. These data illustrate the potential for modifying CETP by externally acting agents such that it remains active but its function is changed.

**DISCUSSION**

We previously observed that most lipid movement by hamster CETP can be explained by a mechanism that promotes the equimolar exchange of CE for CE, TG for TG, or CE for TG (10). However, hamster CETP also promotes the transfer of TG from VLDL to HDL by a process that is not coupled to the return of TG or CE. We call this process nonreciprocal lipid transfer. When HDL and VLDL are incubated with hamster CETP, nonreciprocal lipid transfer causes a net increase in HDL core lipid (CE + TG) by the net movement of VLDL-TG to HDL. Conversely, this process decreases VLDL core lipid. Wild-type human CETP does not mediate nonreciprocal lipid transfer between VLDL and HDL.

Here, we investigated the possibility that hamster CETP’s ability to promote nonreciprocal lipid transfer arises from its markedly different lipid substrate preference compared with human CETP, and not because its many amino acid differences from human CETP result in a unique global structure that creates the potential for this type of lipid transfer. To test this, we investigated the lipid transfer properties of multiple human CETP mutants that were originally described by Qiu et al. (11). These mutations modify the internal hydrophobic lipid transfer tunnel of CETP and are unlikely to have altered lipoprotein binding (11). They are well-secreted and appropriately glycosylated, based on their apparent molecular weight, suggesting they are correctly folded. We observed that human CETP with an amino acid mutation that induces markedly higher TG/CE substrate preference than wild-type CETP was capable of mediating the nonreciprocal lipid transfer of TG from VLDL to HDL, just as observed with hamster CETP. Conversely, we also found that human CETP mutants with higher preference for CE versus TG than

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**TABLE 1.** Homoechange, heteroexchange, and nonreciprocal lipid transfer mediated by CETP mutants

| CETP   | TG-TG (% of total) | CE-CE (% of total) | TG-CE (% of total) | NR Transfer (%) | Direction of NR Transfer |
|--------|--------------------|--------------------|--------------------|----------------|--------------------------|
| Wild-type | 10.3               | 60.9               | 28.5               | 0.2            | TG into HDL              |
| Q199A  | 25.6               | 26.9               | 24.1               | 23.4           | CE into VLDL             |
| H232A  | 3.7                | 69.5               | 9.2                | 17.5           |                          |

Values were calculated from the lipid transfer mediated by the highest CETP concentration shown in Figs. 2–4. For example, if TG transfer from VLDL to HDL = A and from HDL to VLDL = B, and CE transfer from VLDL to HDL = C and from HDL to VLDL = D. Then, TG-TG homoechange is the smaller of A or B; CE-CE homoechange is the smaller of C or D; TG for CE heteroexchange is the smaller of the absolute values for A-B or C-D; and nonreciprocal lipid transfer (NR transfer) is the difference between the absolute values of (A-B) and (C-D).
wild-type CETP also promoted nonreciprocal lipid transfer, but this occurred for CE instead of TG and this lipid movement was in the opposite direction, i.e., from HDL into VLDL. Results with multiple human CETP mutants confirmed a linear relationship between the substrate preference of CETP and the extent and direction of nonreciprocal transfer. CETPs from hamster, monkey, and rabbit, whose native substrate preferences differ from human CETP, promoted nonreciprocal lipid transfer to the extent expected based on the relationship defined by human mutants. Overall, these data conclusively demonstrate that the capacity of CETP to facilitate nonreciprocal lipid transfer, and the direction of this net lipid flow, is controlled by the relative preference of a CETP for TG versus CE as a substrate.

Previous studies have shown that CETP binds TG or CE contained within the lipoprotein phospholipid surface (25). Further, the concentration of this lipid in the lipoprotein surface, not CETP binding to the lipoprotein, is rate limiting to the transfer process. The prevailing mechanism for CETP-mediated lipid transfer is via a carrier mechanism where CETP containing lipid cargo binds to a lipoprotein, releases this lipid, and then picks up another lipid prior to dissociating (11, 18, 26, 27). By this mechanism CETP facilitates either the exchange of like lipid molecules (homoexchange) or the exchange of different lipids (heteroexchange). However, we previously reported that under nonphysiologic conditions where the acceptor particle lacks neutral lipid substrate, that CETP-mediated lipid transfer is greatly reduced, but there remains a low level of lipid transfer to these neutral lipid-null particles (5, 10). These results show that CETP is capable of delivering CE or TG to an acceptor particle and leaving the particle surface without picking up any TG or CE, thus mediating a one-way transfer. Hence, nonreciprocal transfer activity

TABLE 2. Substrate preference for natural CETP variants

| CETP Variant | TG/CE Specificity | Relative Specificity |
|--------------|------------------|---------------------|
| Wild-type    | 0.48 ± 0.03      | 1                   |
| R451Q        | 0.46 ± 0.03      | 0.96                |
| D442G        | 0.36 ± 0.02*     | 0.75*               |

*Significantly different from wild-type, *P < 0.01.

Fig. 5. Relationship between CETP substrate preference and the net flux of lipid. The indicated recombinant human CETP mutant or CETP from other species was assayed as described in Fig. 2 to determine the extent to which it promotes the gain of TG by, and the loss of CE from, HDL when incubated with VLDL. The ratio of TG gain versus CE loss was calculated from the ratio of the slopes of the TG loss and CE gain curves determined in CETP dose response studies, such as those shown in (C) of Figs. 2–4. The x-axis units are TG/(TG + CE). When expressed this way, the substrate preference ratio for human CETP is 0.33 instead of the ~0.5 value shown in Table 2 and also previously reported (10). Results are the mean ± SD of multiple experiments as follows: R201S (two), M435A (one), H232A (five), wild-type human (seven), monkey (two), rabbit (two), Q199A (six), hamster (two). In each experiment the indicated CETP was assayed in triplicate at three concentrations.

Fig. 6. Effect of TP2 Fab fragments on TG versus CE preference of human, monkey, rabbit, and hamster CETP. A: The C-terminal 26 amino acids of each CETP species. The underlined sequence in human CETP (amino acids 463-475) is the binding site for TP2. B: TP2 Fab concentrations were adjusted to produce approximately 50% inhibition of TG transfer for each CETP species. Shown are the ratios of TG and CE transfer activities of native and Fab-treated CETP measured in LDL to HDL transfer assays. See Fig. 1 for details.

Fig. 7. Alteration of CETP function by TP2 Fab. Hamster CETP was pretreated with the indicated amount of TP2 Fab and then used in assays identical to those described in Fig. 2. Shown are the net TG gain and CE loss from HDL caused by CETP. Numerical values within the figure are the mole ratio of TG gained to CE lost. *P < 0.05 versus no TP2 Fab; **P < 0.05 versus 4.5 ng TP2 Fab.
appears to be an innate property of native CETP. We propose that nonreciprocal lipid transfer is amplified when CETP mutants with altered preference for TG versus CE interact with lipoprotein substrates that contain widely differing amounts of TG or CE. For example, when Q199A CETP, which has a markedly elevated preference for TG as a substrate, binds TG in VLDL then dissociates from VLDL and binds to a HDL, it encounters very little of its preferred substrate because of the low TG content of HDL. As a result, the chance that CETP will deliver its TG cargo then dissociate from HDL before binding any lipid is enhanced. The converse process occurs when the CETP’s substrate preference is altered to greatly prefer CE over TG. In this case, the limiting events occur on VLDL due to its low CE content, leading to an enhanced probability that CETP will deliver CE to VLDL and dissociate without binding any substrate lipid in VLDL. A competing mechanistic view of CETP-mediated lipid transfer is the ternary complex model where CETP forms a bridge between donor and acceptor lipoproteins (28). Assuming that such a model allows for the bidirectional flow of lipids between the two lipoproteins in the complex, then the concepts described above for the carrier model still hold if these transfer complexes are short-lived relative to the rate of lipid movement.

Although observed with artificial substrates, one may ask why wild-type human CETP doesn’t promote nonreciprocal lipid transfer when interacting with plasma lipoproteins, even though it naturally prefers CE as a substrate? Because nonreciprocal lipid transfer depends on the lipid substrate preference of CETP and the lipid composition of lipoproteins, we speculate that the lack of nonreciprocal lipid transfer by wild-type human CETP occurs because our assays use lipoproteins that have already been modified by CETP in vivo. As a result, the potential of these lipoproteins to support nonreciprocal lipid transfer may have been largely dissipated. In vivo, we speculate that nascent VLDL, or HDL that is continuously being enriched with CE by the action of lecithin:cholesterol acyltransferase, may support nonreciprocal lipid transfer driven by human CETP. If correct, then the in vivo function of CETP in lipoprotein metabolism may be more complex than currently envisioned.

CETP is a major regulator of human lipoprotein metabolism (1, 29–32). For example, cholesterol removed from peripheral tissues by HDL is delivered to the liver for excretion either directly by HDL or indirectly after the cholesterol (as CE) is transferred to apolipoprotein-B-containing lipoproteins (33). In humans, more than 70% of cholesterol clearance occurs by the indirect pathway, which is completely dependent on CETP (34, 35). Humans genetically deficient in CETP have multiple lipoprotein abnormalities, including marked hyperalphoproteinemia (36). Because CETP deficiency increases HDL levels, there has been much interest in developing pharmacologic CETP inhibitors. Thus far, CETP inhibitors studied in clinical trials equally inhibit the CETP-mediated transfer of both TG and CE (37). To date, this approach has not shown any clinical benefit (38), suggesting that global suppression of CETP activity may not be a viable therapeutic approach. However, the data presented here show that it is possible to modify the lipid transfer properties of CETP by altering its preference for CE versus TG as substrate. In light of this, and given the capacity of CETP to modify lipoproteins and alter their metabolism, we propose a novel pharmacologic approach where drugs are designed to modify the lipid substrate preference of CETP instead of simply blocking its activity. This approach provides an opportunity to tailor the lipid transfer properties of CETP and to harness its capacity to alter lipid metabolism to achieve specific outcomes. As a proof of principle, we demonstrate with CETP antibody Fab fragments that the substrate preference of CETP can be modified by a compound interacting with the CETP surface and that this changes how CETP modifies lipoprotein lipid composition.

In conclusion, we demonstrate that the unique capacity of hamster CETP to facilitate nonreciprocal lipid transfer can be completely recapitulated in human CETP by a single amino acid substitution that causes it to have a preference for TG versus CE that is similar to that of hamster CETP. We further demonstrate with other CETP mutants that both the magnitude and the direction of this nonreciprocal lipid transfer are controlled by the lipid substrate preference of CETP. CETP mutants with high TG preference cause a net unidirectional flow of TG from VLDL into HDL, whereas CETP mutants with high CE preference promote the net flux of CE out of HDL and into VLDL. These findings clearly show that the lipid transfer properties of CETP are malleable. Because CETP is a major regulator of lipoprotein metabolism, we propose that these new findings lay the foundation for a novel therapeutic approach where the function of CETP is manipulated to enhance its antiatherogenic properties. Conceptually, it seems that increasing the preference of CETP for CE over TG would be desirable, because we observed that such changes increase the net flux of CE out of HDL, which should allow it to accept more cholesterol from peripheral tissues. However, whether enhancing CETP’s CE preference or its TG preference produces the greatest benefit will require detailed investigation into the consequences of these changes on lipid and lipoprotein metabolism.

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