Plasma PLTP (phospholipid-transfer protein): an emerging role in ‘reverse lipopolysaccharide transport’ and innate immunity

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Abstract
Plasma PLTP (phospholipid-transfer protein) is a member of the lipid transfer/LBP [LPS (lipopolysaccharide)-binding protein] family, which constitutes a superfamily of genes together with the short and long PLUNC (palate, lung and nasal epithelium clone) proteins. Although PLTP was studied initially for its involvement in the metabolism of HDL (high-density lipoproteins) and reverse cholesterol transport (i.e. the metabolic pathway through which cholesterol excess can be transported from peripheral tissues back to the liver for excretion in the bile), it displays a number of additional biological properties. In particular, PLTP can modulate the lipoprotein association and metabolism of LPS that are major components of Gram-negative bacteria. The delayed association of LPS with lipoproteins in PLTP-deficient mice results in a prolonged residence time, in a higher toxicity of LPS aggregates and in a significant increase in LPS-induced mortality as compared with wild-type mice. It suggests that PLTP may play a pivotal role in inflammation and innate immunity through its ability to accelerate the ‘reverse LPS transport’ pathway.

Introduction
Lipoproteins are macromolecular complexes that transport a number of hydrophobic and amphipathic compounds in the bloodstream. Because of the large variety of transported molecules, including lipids (non-esterified and esterified cholesterol, phospholipids and triacylglycerols), LPS (lipopolysaccharides), liposoluble vitamins and drugs, alterations in lipoprotein metabolism can produce a number of biological consequences. For instance, the lipoprotein transport of cholesterol has a crucial impact on vascular biology, the lipoprotein-mediated distribution of vitamin E in the body can influence the brain, reproductive and vascular functions, whereas the association of LPS with lipoproteins is a key step in the prevention of inflammation and innate immunity.

Lipoprotein particles do not constitute stable entities. During their intravascular transport, they are modified under the action of specific factors, including the plasma lipid transfer proteins [CETP (cholesteryl ester-transfer protein) and PLTP (phospholipid-transfer protein)]. Plasma lipid transfer proteins can produce their effects either directly through their molecular binding and transfer properties or indirectly through their ability to modify the structure, metabolic properties and behaviour of the lipoprotein carriers. CETP and PLTP are related to LBP (LPS-binding protein) and BPI (bactericidal/permeability-increasing protein). They derive from a common ancestral gene and constitute the LT/LBP gene family. Recently, the LT/LBP family was found to be part of a superfamily including the short and long PLUNC (palate, lung and nasal epithelium clone) proteins. Although PLTP was studied initially for its involvement in metabolism of HDL (high-density lipoprotein) [2–4]. Recently, PLTP was shown to display a number of additional effects in direct link with its ability to exchange a number of amphiphatic compounds (diacylglycerols, LPS, non-esterified cholesterol, vitamin E, etc.) (Table 1). Because of such a large variety of binding components, PLTP displays a number of biological properties, and it was found to modulate atherosclerosis, thrombosis, reproductive biology, brain physiology and innate immunity. Indeed, PLTP deficiency significantly decreases atherosclerosis in hyperlipidaemic mice [23], while PLTP overexpression increases atherosclerosis susceptibility [43]. PLTP is present in human atherosclerotic lesions [30,31]. It is positively and independently associated with coronary atherosclerosis in high-risk patients [44]. In animal models, the PLTP-mediated increase in cardiovascular risk was explained at least in part by the ability of PLTP to increase cholesterol absorption in the intestine and apoB.
Table 1 | PLTP-mediated molecular transfers and pathophysiological consequences

(a) Transferred molecules

<table>
<thead>
<tr>
<th>Class</th>
<th>Biological effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>Phosphatidylcholine transfer to HDL in vitro</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td>Exchange between HDL in vitro</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>From VLDL to HDL in vitro</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>From VLDL to HDL in vivo</td>
<td>[8]</td>
</tr>
<tr>
<td>Diacylglycerols, phosphatidic acid, phosphatidylglycerol</td>
<td>Exchange between lipoprotein subclasses in vitro</td>
<td>[9]</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>Exchange between lipoprotein subclasses in vitro</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>From liposomes to HDL in vivo</td>
<td>[8]</td>
</tr>
<tr>
<td>Non-esterified cholesterol</td>
<td>From liposomes to HDL in vitro</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>From fibroblasts to HDL in vitro</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>From liposomes to HDL in vivo</td>
<td>[8]</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>Between lipoproteins and towards red blood cells in vitro</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>Between lipoproteins and towards endothelial cells in vitro</td>
<td>[13]</td>
</tr>
<tr>
<td>LPS</td>
<td>From LPS aggregates to HDL in vitro</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>From HDL to LDL in vitro</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>From bacterial blebs to HDL in vitro</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>From LPS aggregates to HDL in vivo</td>
<td>[17]</td>
</tr>
</tbody>
</table>

(b) Biochemical/biological pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Related pathophysiology</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL remodelling</td>
<td>Atherosclerosis</td>
<td>[2,8,18–22]</td>
</tr>
<tr>
<td>VLDL assembly/secretion</td>
<td>Atherosclerosis</td>
<td>[22–24]</td>
</tr>
<tr>
<td>Cholesterol absorption</td>
<td>Atherosclerosis</td>
<td>[25,26]</td>
</tr>
<tr>
<td>Macrophage biology</td>
<td>Atherosclerosis</td>
<td>[27–35]</td>
</tr>
<tr>
<td>Reverse cholesterol transport</td>
<td>Atherosclerosis</td>
<td>[26,36,37]</td>
</tr>
<tr>
<td>Vitamin E distribution</td>
<td>Endothelial function</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>Thrombosis</td>
<td>[38,39]</td>
</tr>
<tr>
<td>Reverse LPS transport</td>
<td>Innate immunity</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>Cancer treatment</td>
<td>[42]</td>
</tr>
</tbody>
</table>

(apolipoprotein B)-containing lipoprotein production by the liver [23,25,26,45]. In addition, vitamin E redistribution between lipoproteins and the vascular wall could contribute to PLTP atherogenicity [13,43,46]. Finally, beyond lipid homoeostasis and atherogenesis, PLTP could influence other processes such as brain physiology [40], reproductive biology [47] and the LPS-mediated immune response [14,16]. The last-named property is directly related to PLTP being a member of the LT/LBP gene family.

PLTP and LPS binding

LPS are complex molecules located at the surface of Gram-negative bacteria. They bind to LBP and activate the TLR4 (Toll-like receptor 4) at the surface of immune cells, thus leading to the release of pro-inflammatory cytokines and to inflammation. Alternatively, bacterial blebs forming large LPS aggregates can be disrupted and molecular transfer of LPS towards HDL can occur. It results in its neutralization and elimination back to the liver.

Thus an important question is to know the molecular mechanism for the association of disaggregated LPS with HDL because it may constitute an important, limiting step in LPS metabolism and may accelerate elimination and decrease toxicity.

Earlier studies by Wright and co-workers reported that PLTP may be a key factor in mediating lipoprotein binding of LPS [14]. Indeed, it was demonstrated that incubation of LPS and HDL in the presence of increasing amounts of purified PLTP leads to the shift of LPS from an unbound form towards an HDL-bound form (Figure 1A). In subsequent in vivo studies, PLTP deficiency in mice was found to be associated with the accumulation of LPS in the lipoprotein-free fraction at the expense of HDL, whereas, in contrast, LPS did not accumulate in lipoprotein-deficient plasma of wild-type mice with naturally high PLTP expression [17].

The reverse LPS transport

As far as cholesterol metabolism is concerned, there are three main pathways in vivo: (i) the absorption and transport...
Figure 1 | PLTP promotes the transfer of LPS to HDL and enhances LPS excretion into the bile

(A) Recombinant PLTP (rPLTP) is able to dissociate LPS aggregates and to transfer LPS to isolated HDL in a dose-dependent manner in vitro. Figure adapted from [14] with permission. © 1996 The American Society for Biochemistry and Molecular Biology. (B) Gene dose-effect of PLTP on the magnitude of LPS biliary secretion. Wild-type mice (PLTP^{+/+}, n = 10) and PLTP-deficient heterozygous (PLTP^{+-}, n = 6) or homozygous mice (PLTP^{-/-}, n = 10) were injected with LPS, and LPS content of the bile was measured 24 h later by a specific β-hydroxymyristic acid assay. Results are means±S.E.M., *significantly different from PLTP^{+/+} mice, P < 0.05; **significantly different from PLTP^{+/+} mice, P < 0.005; †significantly different from PLTP^{+-} mice, P < 0.05; Mann–Whitney U-test.

of dietary cholesterol through the chylomicron pathway during the postprandial phase, (ii) the transport of cholesterol from the liver towards peripheral tissues through the VLDL (very-low-density lipoprotein) and LDL pathway, and finally (iii) the reverse transport of cholesterol from peripheral tissues to the liver through the HDL pathway. In the last step of reverse cholesterol transport, cholesterol can be excreted in the bile, either as non-esterified cholesterol or after conversion into bile acids. Similar to cholesterol transport, it is proposed here that HDL and PLTP might be the leading components of a new pathway for LPS detoxification in vivo, namely the ‘reverse LPS transport’ pathway. PLTP expression in wild-type mice was found to promote the disaggregation of LPS and to favor its binding to lipoproteins (in particular HDL), thus reducing its interaction with leucocytes and decreasing its pro-inflammatory property. Most importantly, the ‘reverse LPS transport’ mediated by HDL and PLTP was found to enhance the biliary elimination of LPS as the ultimate and irreversible step (Figure 2). Interestingly, an intermediate phenotype was found in PLTP-KO (knockout) heterozygous mice (with moderate PLTP activity) as compared with wild-type mice (with naturally high PLTP activity) and PLTP-KO homozygous mice (with no detectable PLTP activity) (Figure 1B), indicating that the reverse LPS transport might strictly rely on the circulating level of active PLTP. Finally, as the slower biliary elimination in PLTP-KO than in wild-type mice was found to be associated with a weaker resistance to endotoxic shock and with a lower survival rate [17], the combination of PLTP and HDL carriers arises here as a promising way in preventing endotoxic shock.

Towards a new strategy for prevention and treatment of endotoxic shock?

Endotoxic shock is an excessive/dysregulated inflammatory response to LPS. It leads to multiple organ failure and death and it is a frequent complication of sepsis. About 18 million cases might occur worldwide each year, with a high mortality rate [48,49]. Whereas a number of therapeutic strategies have been investigated over the last decade (including the use of anti-LPS antibodies, anti-tumour necrosis factor α antibodies, lipid A analogues, recombinant HDL, antithrombin agents, modulators of TLR signalling, etc.), no efficient treatment of sepsis and related SIRS (systemic inflammatory response syndrome) has yet been identified. It indicates that additional, new therapeutic strategies are needed. Because plasma PLTP can neutralize LPS, thus producing a marked, highly significant decrease in endotoxic shock, it raises the hypothesis that the plasma concentration of PLTP, in combination or not with the anti-inflammatory

Figure 2 | Schematic representation of the co-ordinated action of PLTP, lipoproteins and liver in the reverse LPS transport process

LPS aggregates originate from the outer membrane of Gram-negative bacteria and may accumulate in the intravascular compartment. These LPS aggregates are susceptible to induce leucocyte activation via the LBP/CD14/MD2/TLR pathway, thereby triggering the inflammatory process. The first step of reverse LPS transport is mediated by PLTP which promotes the disaggregation of LPS and their binding to HDL. In the second step, HDL, in addition to their LPS-neutralizing properties, serve as carriers bringing LPS molecules back to the liver. In the last step, LPS molecules taken up by the liver are eventually eliminated from the body via biliary secretion.
HDL may predict the occurrence of endotoxic shock, and in this context up-regulation of PLTP expression might constitute a promising strategy against endotoxic shock.

Besides their roles in reverse cholesterol transport, LXR (liver X receptor), CAR (constitutive androstane receptor) and PXR (pregnane X receptor) nuclear receptors are potential targets for the reverse LPS transport and prevention of the LPS-induced inflammatory response.

LXRα and LXRβ exert anti-inflammatory effects per se and are able to modulate LPS-dependent pathways in macrophages. A protective role of LXR agonists against LPS-mediated tissue injury has been demonstrated in vivo in the liver and the lung [50,51]. LXR is also able to regulate the expression of proteins potentially involved in LPS detoxification such as PLTP and CETP [52,53].

Both PXR and CAR are potential modulators of LPS catabolism in liver through the co-ordinated induction of phase I (Cyp3A, Cyp2B, etc.), II (Sult2A, UDPGT) and III (MRP2 (multidrug-resistance protein 2), MRP3, MRP4) detoxification enzymes that are prototypical CAR and PXR target genes. Recently, a protective role for PXR was demonstrated against LPS-mediated liver injury, and PXR-deficient mice have greater increases in ALAT (alanine transaminase), hepatocyte apoptosis, necrosis and haemorrhagic liver injury as compared with wild-type mice after LPS administration [54]. Interestingly, both CAR and PXR interfere with bile acid metabolism and CAR activation was recently shown to induce a marked increase in secretion of bile acids [55], which are known to constitute effective compounds to complex LPS into an inactive form [56]. Whether CAR and PXR activation could modulate the final step of the ‘reverse lipopolysaccharide transport’ through their ability to modulate biliary acid synthesis and excretion in the liver is unknown at present.

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