Lipoproteins in Innate Immunity
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Chapter IX

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Summary
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Inflammatory acute phase responses are characterized by fever, leukocytosis, tachycardia, and tachypnea, and may progress to septic shock and multiple organ failure. These clinical symptoms are mediated by marked changes in plasma and tissue concentrations of pro- and anti-inflammatory proteins. In addition, and the major subject of this thesis, drastic changes in lipid homeostasis occur during acute phase responses during bacteremia and endotoxemia. These changes are functionally of great importance, and lipoproteins are thought to be a part of the innate immunity because they are capable of "neutralizing" many of the toxic or inflammatory agents which include bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA).

In chapter 2 - 4, we have investigated the capacity of the main lipoprotein classes very low-density lipoproteins (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) to bind bacterial LPS and LTA.

In Chapter 2 and 3, the distribution, specificity, binding capacity and kinetics of binding of LPS (three different chemotypes) and LTA (Staphylococcus aureus) to lipoproteins (ex vivo) were characterized. We observed a total endotoxin binding capacity that far exceeds the concentrations observed in clinical situations, and that HDL apparently had the highest binding capacity for all endotoxins. In addition, a chemotype dependent redistribution of LPS or LTA among the lipoproteins was observed, and rough LPS chemotypes (Re and J5) were more rapidly redistributed than smooth LPS chemotypes. The smooth *E. coli* O111:B4 LPS, showed no significant redistribution. Our data also provided evidence for the notion that LPS redistribution amongst lipoproteins is an active process.

In chapter 4 the LPS and LTA sequestering characteristics of different HDL subtypes are presented. We found that HDL subtypes with α-mobility accounted for the bulk of LPS binding or neutralization, and a direct relationship of LPS binding and HDL cholesterol content was demonstrated. We propose that this phenomenon may be explained by differences in the composition of the phospholipid monolayer in the different lipoprotein subtypes, in which endotoxin is incorporated. Additionally, we found a PEG-precipitable lipid-poor particle with a very low endotoxin binding capacity. However, protein analysis revealed that this particle indeed contained many (apolipo)proteins characteristic of α-HDL. The fact that these particles were found in the plasma of healthy
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subjects suggests that the lipid poor HDL-like particles may be an integral part of the HDL catabolic pathway.

In chapter 5, we investigated the lipoprotein homeostasis and endotoxin binding capacity of plasma and lymph (obtained from the thoracic duct) from patients with systemic inflammatory response syndrome or multiple organ failure. The lymph serves as a transport route for lipoproteins and associated endotoxin from the gut and peripheral tissues to the systemic circulation. Hence, lymph composition more closely reflects inflammatory processes at the tissue level. The apolipoproteins apo A-I, apo B, as well as total plasma and lymph cholesterol and triglyceride lymph levels were decreased compared to the control group. Plasma triglyceride levels however were slightly increased. These observations reveal that alterations in the lipid profile in lymph reflects that of plasma with the exception of triglyceride levels. Addition of fluorescent-LPS to plasma and lymph revealed that the LPS-binding capacity in these patients showed a relative shift toward LDL in plasma and lymph. In addition, low LPS binding lipid-poor particles were found. We assume that these particles are the consequence of the acute phase reaction which causes dramatic changes not only in lipid levels but also in lipid composition especially that of HDL. Compared to the lipid-poor particles described in chapter 4 it is more likely to assume that these HDL particles appear to represent acute phase HDL.

The route of LPS clearing is currently thought to be via lipoproteins that deliver LPS to the hepatocytes which subsequent results in secretion into the bile. Other investigators have reported that VLDL and chylomicrons are responsible for LPS excretion via the bile. We hypothesized that the lipid/LPS transport proteins. LPS binding protein (LBP) and phospholipid transfer protein (PLTP), were not only involved in the LPS loading of HDL but also in the LPS redistribution from HDL to other lipoproteins. In chapter 6, we demonstrated that both LBP and PLTP transport LPS from HDL to LDL and VLDL in vitro, and that this process is associated with the remodeling of HDL. BIAcore analysis confirmed this observation and showed a PLTP and LBP-dose dependent binding of HDL and LDL. This phenomenon was further enhanced when HDL was pre-loaded with LPS, which under influence of LBP resulted in the formation of a HDL/LDL fusion particle. It is likely that redistribution under these conditions is extremely efficient.

Sepsis is often accompanied by cholestasis in which low HDL levels and high susceptibility to endotoxin is observed. One option for therapy is to
neutralize LPS by administration of reconstituted HDL (rHDL). In chapter 7, the effects of rHDL infusion on the outcome of LPS-induced inflammatory responses in cholestatic rats was investigated. rHDL infusion resulted in a reduced mortality after LPS challenge. However, paradoxically rHDL infusion led to accumulation of LPS in the liver and increased LPS-induced mortality in cholestatic rats. These observations indicate that the protective effects of rHDL are dependent on an intact LPS clearing mechanism via the bile. In the absence of such clearing, rHDL treatment may increase septic mortality.

Sepsis and the acute phase inflammatory response is accompanied by drastic changes in lipid and apolipoprotein composition of all lipoproteins. In Chapter 8, the sequential changes of the (apo)lipoprotein concentrations and composition are demonstrated in two experimental endotoxemia models (one in baboons and one in humans) and during clinical sepsis in humans. Further, the influence of rHDL administration on the lipid changes during experimental endotoxemia were studied. An overall decrease of the lipoprotein lipid constituents and apolipoproteins were observed combined with an increase in the acute phase proteins CRP and LBP in baboons and humans. However, triglyceride changes were species-dependent showing and increase in baboons and a decrease in humans. In time during recovery an overall rise in (apo)lipoproteins levels was observed and a decrease in the acute phase markers in patients with clinical sepsis. rHDL infusion did not alter the LPS-dependent lipid changes, with the exception of increased cholesterol levels combined with a high initial turnover of phosphatidylcholine indicating extensive remodeling of rHDL. The concentrations of the lipid transfer proteins CETP and LCAT were all decreased during sepsis with the exception of PLTP of which two-fold higher activities were found.

Concluding remarks
Our results indicate that lipoproteins play a pivotal protective function in innate immunity. HDL has a very high capacity for LPS or LTA sequestration thereby neutralizing the factors responsible for the primary inflammatory host response. The acute phase reaction during experimental endotoxemia, SIRS or clinical sepsis is accompanied by dramatic changes in lipoprotein concentration and composition. These alterations have the greatest impact on concentration and composition of HDL resulting in appearance of smaller HDL particles with low endotoxin binding capacity. HDL is probably the first buffer in innate
immunity against toxic bacterial agents in vivo. Administration of rHDL appears to attenuate the acute phase reaction. It should be noted that binding of LPS to HDL does not result in a stable complex during the acute phase. As demonstrated in this thesis, active redistribution of endotoxin towards other lipoprotein classes occurs in vitro and in vivo. When clearing of these particles is impaired, an enhanced inflammatory response may occur.

Our data may guide the development of novel intervention strategies in sepsis. Clearly, substitution of lipoproteins, such as rHDL, or LPS-binding lipid particles that do not contain proteins, may result in neutralization and protection against overwhelming inflammatory injury. However, our findings also indicate that binding of LPS to lipoproteins or lipid particles is only a first step in a complex scavenging pathway that involves exchange of LPS between lipoproteins and removal by the liver. Impairments in the function of these downstream mechanisms may importantly affect the efficacy of the initial intervention, and need to be studied before initiation of clinical studies.