The role of apolipoprotein CI in lipid metabolism and bacterial sepsis

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Chapter 9

General Discussion and Future Perspectives

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Cardiovascular disease (CVD) is the major cause of death in the Western society. Dyslipidemia, as characterized by high levels of atherogenic apolipoprotein B (apoB)-containing particles, such as very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), and low levels of anti-atherogenic high-density lipoproteins (HDL), is a crucial risk factor for developing CVD. In the first part of this thesis (**chapters 2-3**) we focused on the role of apoCI and lipoprotein lipase (LPL) in plasma lipid metabolism, while in the second part (**chapter 4**) the association of plasma apoE levels with CVD was investigated. In the final part of this thesis (**chapters 5-8**) we studied the role of lipids and apolipoproteins in bacterial sepsis, the leading cause of death in intensive care units (ICU). Increasing evidence shows that lipoproteins, and in particular their apolipoproteins, modulate the inflammatory response towards bacteria. Here we specifically focussed on the role of apoCI in both murine and human sepsis. The major conclusions and clinical implications of our findings presented in this thesis as well as future perspectives will be discussed in this chapter.

1. Role of ApoCI in Lipid Metabolism

APOC1 transgenic mice develop severe combined hyperlipidemia, in particular hypertriglyceridemia, represented by markedly elevated apoB-containing lipoproteins. Previously it was thought that this apoCI-mediated hypertriglyceridemia was primarily caused by the inhibition of apoE-mediated lipoprotein uptake, either by displacement of apoE on the lipoprotein surface by apoCI[1], or through masking or altering the conformation of apoE[2]. These studies certainly indicate an inhibitory role of apoCI with respect to hepatic lipoprotein recognition. However, apoCI overexpression cannot raise plasma triglycerides (TG) solely by interference with apoE-dependent receptor recognition, since apoE-deficient mice hardly develop hypertriglyceridemia[3]. Indeed, we have now demonstrated that the hyperlipidemic effect of apoCI is not mediated via a direct effect of apoCI on apoE. In fact, the effect of apoCI on plasma lipid levels is even markedly aggravated in apoE-deficient mice (**chapter 2**). Next to apoE, we also excluded apoCIII, VLDL receptor (VLDLr), LDL receptor (LDLr), and LDLr-related protein (LRP), as important players in this hyperlipidemic effect of apoCI, since expression of apoCI still resulted in a marked hypertriglyceridemia in *apoe*$^{-/-}$*apoc3*$^{-/-}$ and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice (**chapter 3**).

The hydrolysis of TG by LPL is a crucial step in the remodelling and the subsequent clearance of apoB-containing lipoproteins. Several proteins, including apolipoproteins (*i.e.* apoCII, apoCIII, apoE, and apoAV) are able to

modulate the hydrolyzing activity of LPL *in vivo*. We now showed for the first time that apoCI is also an important inhibitor of LPL *in vivo* in mice.

Several potential mechanisms may underlie the apoCI-mediated inhibition of LPL activity. ApoCI may interfere with the binding of apoCII (the essential cofactor for LPL activity) to LPL by masking of apoCII on the lipoprotein surface. Displacement of apoCII by apoCI on the lipoprotein surface seems unlikely, since the amount of apoCII on the surface of VLDL from *APOC1* transgenic mice was similar as compared to wild-type controls[4]. In addition, apoCI may act via modulation of the apoAV-mediated stimulation of the LPL activity. However, in our *in vitro* LPL assay apoAV was absent (**chapter 2**), suggesting that apoAV is not involved in the apoCI-mediated inhibition of LPL activity. It is also unlikely that apoCI blocks the binding of apoB-containing lipoprotein particles to heparan sulphate proteoglycans (HSPG), which are able to bring these particles in close proximity to LPL. Not only was HSPG absent in our *in vitro* LPL-activity assay, also the high positive charge of apoCI suggests that apoCI would enhance rather than diminish the binding of TG-rich lipoprotein particles to the negatively charged HSPG. Although not enhanced, it has been shown that VLDL from *APOC1* transgenic mice bound equally well to heparin-Sepharose as VLDL from wild-type controls[4]. Finally, apoCI may directly bind to LPL and hereby interfere with the substrate binding to LPL. ApoE has also been reported to inhibit LPL activity[5]. Its positive arginine residues appeared to be involved in LPL inhibition, probably by bringing about substrate dissociation from LPL. It is conceivable that apoCI acts in a similar manner as apoE. If this is the case, neutralization of the positively charged lysine residues in apoCI may prevent the inhibitory effect on LPL.

Our experimental studies suggest that inhibition of LPL by apoCI may very well occur at physiologically relevant levels in humans, and, therefore, apoCI levels may be a causal determinant for TG levels in men. Indeed we found in a population with 85-year old participants, the Leiden 85-plus Study, that high plasma apoCI levels were strongly associated with high plasma TG levels (**chapter 8**). In addition, expression of the *Hpa*I promotor polymorphism is likely to increase expression of apoCI[6]. Although not entirely conclusive in all human studies, expression of this promotor polymorphism is associated with increased plasma TG levels[6-8]. Taken together, these studies suggest that apoCI is a causal determinant for TG levels in humans by modulating LPL activity.

Modulation of LPL activity not only affects plasma lipid levels, but also the flux of TG-derived free fatty acids into adipose tissue. Indeed, we recently found that mice deficient for apoCI had an increased flux of TG-derived fatty acids into the adipose tissue[9]. Although we did not observe a pronounced effect of

apoCI deficiency or overexpression on body weight on a wild-type (C57Bl/6) background, overexpression of apoCI on a genetically leptin-deficient (ob/ob) background completely protected these mice from diet-induced obesity[10]. These studies are in line with other studies in which LPL activity was modulated. Deletion of the LPL inhibitor apoCIII from mice resulted in increased uptake by adipose tissue of TG-derived fatty acids, but not of albumin-bound circulating free fatty acids (FFA), and a concomitant enhanced diet-induced obesity[11]. Since obesity is also a major risk factor for developing CVD[12,13], apoCI-mediated inhibition of LPL may have a dual role in atherosclerosis. It may be anti-atherogenic by protecting against diet-induced obesity, but on the other hand it may act pro-atherogenic by causing dyslipidemia. Studies by us (Westerterp MW and Rensen PNC *et al*., unpublished results) and others[14] showed that plasma apoCI levels dose-dependently enhanced atherosclerosis development in mice on an *apoe*$^{-/-}$ background, indicating that in mice the apoCI-mediated dyslipidemia is a causal determinant for atherosclerosis development. Since both studies were performed on regular chow diet, the anti-atherosclerotic potential of the apoCI-mediated protection against obesity during diet-induced atherosclerosis development remains to be determined.

Another important aspect of apoCI in lipid metabolism is its role in the remodelling of HDL. ApoCI is the endogenous inhibitor of cholesteryl ester transfer protein (CETP), and *in vitro* data have demonstrated that apoCI can inhibit hepatic lipase (HL) and activate lecithin:cholesterol acyltransferase (LCAT). Interestingly, all of these HDL-modulating properties of apoCI can be expected to increase HDL levels in plasma, which in general is considered anti-atherogenic[15,16]. In early studies apoCI deficiency[17,18] or overexpression[4,19] did not alter plasma HDL levels. However, more recent studies by us (**chapter 3**; Westerterp MW, De Haan W, Rensen PCN, unpublished results) and others[20,21] did show an apoCI gene-dose dependent increase, or trend towards increase, in HDL levels. Also after injection of mice with a recombinant adenovirus expressing human apoCI (Ad*APOC1*) we found a small increase in plasma HDL levels (**chapter 3**). Remarkably, overexpression of human apoCI in CETP transgenic mice did not affect HDL levels, but dramatically increased the atherogenic apoB-containing lipoprotein levels[20]. It appeared that apoCI was able to inhibit CETP activity, but the hyperlipidemic property of apoCI increased the total circulating mass of CETP, hereby counteracting the inhibitory effect of apoCI on CETP activity. However, in human CETP transgenic mice, apoCI deficiency induced a further reduction in plasma HDL levels as compared to CETP transgenic controls[17,21], in line with an apoCI-mediated inhibition of CETP. Taken together, these findings show that both in normal and CETP transgenic mice plasma apoCI level may be

a causal determinant for HDL levels.

Current treatment of subjects at risk for CVD is mainly aimed at reducing the plasma levels of atherogenic apoB-containing lipoproteins. The combination of these lipid-lowering drugs with experimental drugs that increase plasma HDL levels, *e.g.* by reducing CETP activity, is now considered one of the most promising strategies to further reduce CVD risk in humans[22,23]. In this context, apoCI-mediated increase of plasma HDL levels may represent a promising lead for future drug development. The association between plasma levels of apoCI and CETP, HL, and LCAT activity in humans has not been described yet. However, we did find a strong association between high plasma apoCI levels and high plasma HDL levels in subjects of the Leiden 85-plus Study (**chapter 8**), which may be suggestive for an apoCI-dependent remodelling of HDL in humans. Interestingly, in this elderly population we also found that high apoCI levels were associated with decreased mortality from CVD, which is in contrast to the atherosclerosis studies in mice where CETP was absent. This may suggest that in this elderly population the anti-atherogenic effect of apoCI-mediated CETP-inhibition may outweigh the pro-atherogenic effect of apoCI-associated hyperlipidemia, and plasma apoCI in general may thus be regarded as an anti-atherogenic protein.

To further explore and enhance the potentially anti-atherogenic property of apoCI, future studies should be aimed at identifying peptides derived from apoCI able to inhibit CETP activity and thereby increase HDL levels, but do not cause hyperlipidemia by inhibiting LPL activity. In order to achieve this, the domains within apoCI responsible for the inhibition of CETP and LPL should first be identified. No reports have been published yet in which the domain of apoCI was studied that is responsible for the inhibition of LPL. In addition, the few studies addressing the domain within apoCI that inhibits CETP are inconsistent. ApoCI consists of two amphipathic α-helices, an N-terminal helix (residues 7-29) and a C-terminal helix (residues 38-52), which are separated by a flexible, unstructured linker region (residues 30-37). One group of researchers identified the N-terminal fragment ($apoCI_{1-38}$) of baboon[24] and human[25] apoCI as the CETP inhibitory domain of apoCI. However, Dumont *et al.*[26] showed that a C-terminal fragment of apoCI ($apoCI_{34-54}$) inhibits CETP, while an N-terminal fragment ($apoCI_{4-25}$) was not able to inhibit CETP. Combined, these findings suggest that part of the linker region within apoCI that is present in both $apoCI_{1-38}$ and $apoCI_{34-54}$ may be involved in CETP inhibition. If this region is indeed involved in the inhibition of CETP, and appears not to overlap with the domain that inhibits LPL, this might either be considered as a potential drug or as a lead to design new CETP inhibitors.

2. Role of ApoCI in Bacterial Infection

Lipoproteins have been shown to strongly influence inflammatory responses towards lipopolysaccharide (LPS), the main inflammatory component of Gram-negative bacteria, and during sepsis (reviewed in **chapter 1**). Increasing evidence supports the hypothesis that not the lipid components of the lipoproteins, but the apolipoproteins are responsible for the effects of the lipoproteins on inflammatory processes. Whereas other apolipoproteins described so far (*e.g.* apoE, apoAI, and apoAIV) decrease inflammatory responses, we now identified apoCI as the first apolipoprotein to markedly enhance inflammatory responses towards LPS and to be crucial in the protection against bacterial infection in mice (**chapter 5** and **6**).

Based on our data presented in this thesis, we propose the following model for the protective effects of apoCI in Gram-negative sepsis (**Figure 1**). On the entry and proliferation of bacteria in the blood, LPS is released from the bacterial membrane into the circulation and binds to apoCI. This binding involves the interaction between the positively charged LPS binding motif *KVKEKLK* (residues 48-54; **chapter 5**) together with other cationic/hydrophobic motifs (**chapter 6**) within apoCI and presumably the negatively charged phosphate groups within the lipid A domain of LPS, the shared moiety of all LPS species. ApoCI may bind LPS both in the lipid-free and lipid-bound state, thereby effectively presenting the LPS to responsive cells, such as macrophages, leading to an enhanced production of proinflammatory cytokines. These cytokines are essential for

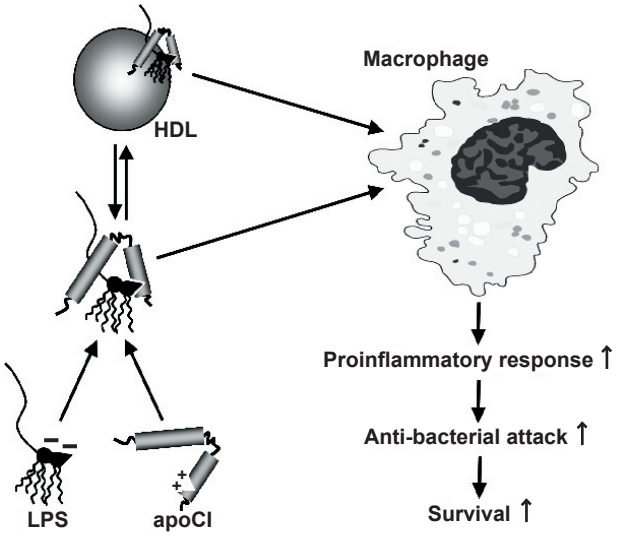

Figure 1. Mechanism underlying the protective effect of apoCI in Gram-negative sepsis. See text for explanation.

effective eradication of the bacterial infection thereby preventing infection-related mortality. Therefore, plasma apoCI protects against fatal sepsis by effectuating an early and adequate antibacterial response towards Gram-negative infections.

To further understand how apoCI stimulates the LPS-induced inflammatory response, it would be interesting to elucidate the molecular mechanism at the cellular level. The Toll-like receptor 4 (TLR4) pathway is likely to be involved in this mechanism, since it is commonly assumed that LPS activates cells by binding to the MD2-TLR4-complex on the cell membrane. ApoCI could be directly involved in enhancing the presentation of LPS to membrane-bound CD14 (mCD14) and/or the MD2-TLR4-complex, leading to increased signalling via TLR4. It is less likely that apoCI increases the binding of LPS to soluble CD14 (sCD14) and/or LBP, since we observed that apoCI enhanced the inflammatory response by macrophages in *in vitro* assays in which sCD14 and LBP were both absent (**chapter 5**). Alternatively, apoCI may improve the stabilization of the LPS-MD2-TLR4-complex.

It is important to realize that during bacterial infection the inflammatory response is multifaceted, complex, highly regulated, and varies in time. To date many different experimental models for sepsis are being used, ranging from simple intravenous injections of LPS or bacteria to more clinically relevant models such as intra-abdominal infection (*e.g.* cecal ligation and puncture, CLP) and the bacterial pneumonia model. It should be realized that in the models using intravenous administration of LPS often an unphysiologic overload of LPS is given, resulting in an acute and excessive host's response, followed by organ failure and death. The more clinically relevant models use a low dose of bacteria residing in cavities or organs where an infectious source or abscess is formed in which the bacteria multiply. From these hazards slow dissemination of bacteria in the circulation occurs, hereby gradually and persistently triggering the immune system. Finally, enhanced release of bacteria will result in increased proinflammatory mediators, organ failure, and subsequently death. Therefore, agents aimed at preventing the onset of sepsis by enhancing the inflammatory response should be tested in actual infection models, whereas acute endotoxemia models suffice for evaluation of agents aimed at reducing the LPS-induced inflammation in a late stage of (severe) sepsis.

This important issue is nicely illustrated by our findings that apoCI has opposite effects in the outcome of acute endotoxemia versus a clinically relevant bacterial pneumonia, even when using the same bacterial strain. As outlined above, in the *Klebsiella pneumoniae*-induced pneumonia model, apoCI is able to effectuate an efficient anti-bacterial attack and surmount the infection in the early phase (**chapter 5**). However, apoCI appeared to be deleterious in an acute

endotoxemia model in which a large bolus of *K. pneumoniae* had been infused intravenously, thus showing an opposite survival pattern as for the pneumonia model (**Figure 2**). Although we did not determine the inflammatory mediators in the acute endotoxemia model, it is likely that apoCI gene-dose dependently aggravated the inflammatory response towards these bacteria and hereby induced organ failure and subsequent death.

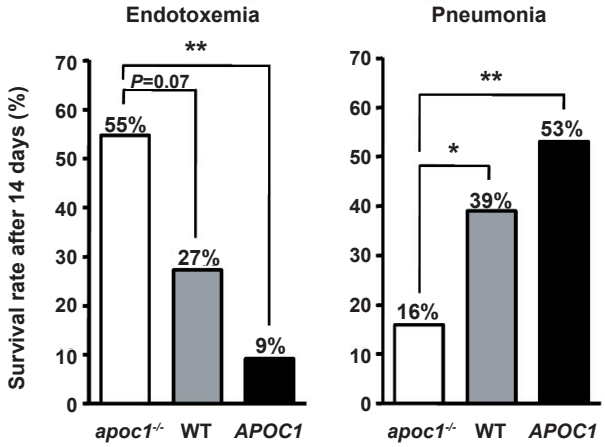

Figure 2. Opposite associations between apoCI and survival of mice in which *K. pneumoniae* was either injected intravenously (acute endotoxemia model; 5000 cfu/mouse; *left*) or inoculated pulmonally (pneumonia model; 500-750 cfu/mouse; *right*). The two-week survival was assessed in apoCI-deficient (*apoc1*$^{-/-}$), wild-type (WT), and human apoCI transgenic mice (*APOC1*). See text for explanation. *P<0.05; **P<0.01.

In this thesis we mainly focussed on the interplay between apoCI and responses as induced by LPS or Gram-negative bacteria. However, our data from three human studies indicate that the effects of apoCI on infection and/or inflammation go beyond that of LPS modulation in Gram-negative sepsis alone. In **chapter 7** we showed that patients with severe sepsis had markedly decreased plasma apoCI levels upon hospitalisation. In the survivors apoCI levels gradually recovered to healthy control levels within 4 weeks, whereas in the non-survivors plasma apoCI levels remained low. However, only 9 of the 17 subjects suffered from Gram-negative infection. Discarding these subjects from the analysis did not affect the inverse association between apoCI levels and mortality outcome in these patients, indicating that apoCI may also predict the outcome of other causes of sepsis such as Gram-positive bacteria. In addition, in a study with 307 patients hospitalized with febrile illness we determined the plasma apoCI levels upon hospitalization and the mortality outcome (Berbée JFP, Schippers EF, Van Dissel JT, Rensen PCN, unpublished results). Plasma apoCI levels were significantly lower in patients that deceased during their hospital stay as

compared to patients that survived the febrile illness. Only 8 percent (26 cases) of these patients suffered from Gram-negative infection. After discarding these patients from the analysis, low plasma apoCI levels were still associated with increased mortality, supportive for an effect of apoCI independent of Gram-negative bacteria. Finally, we measured plasma apoCI levels in 85-year old participants of the Leiden 85-plus Study and determined the cause of mortality during follow-up (**chapter 8**). In this elderly population we found that high plasma apoCI levels associate with decreased mortality from infection. Although in this study the microorganisms responsible for infection were not determined, it is plausible that these associations are not caused by Gram-negative infection only.

Taken together, these human association studies strongly indicate that apoCI, besides being a crucial mediator in Gram-negative infection, may affect infection with other microorganisms as well. In fact, this is also the case for apoE, which is encoded by the same gene cluster as apoCI, and is primarily known for its LPS-neutralizing actions (reviewed in **chapter 1**). ApoE-deficient mice have impaired innate immune responses to Gram-negative bacteria, however, these mice have also impaired responses to Gram-positive bacteria and fungi. Preliminary data suggest that apoE also binds to lipoteichoic acid (LTA), an inflammatory moiety of Gram-positive bacteria, which may result in an attenuated response (Van Amersfoort ES and Rensen PCN *et al.*, unpublished results). Future research should confirm these initial findings, and demonstrate whether apoCI may also augment the response to Gram-positive bacteria by binding to LTA.

It has already been known for a long time that apoE and apoCI have opposite effects in lipid metabolism. Whereas apoE is necessary for the uptake of TG-rich lipoprotein particles by the liver and other organs and tissues, apoCI inhibits the clearance of these lipoproteins by inhibiting the LPL-mediated TG-lipolysis and blocking the apoE-mediated uptake by lipoprotein receptors. We have now demonstrated that apoE and apoCI also have opposite effects in LPS-induced inflammation. Whereas apoE diminishes the LPS-induced inflammatory response by mediating its uptake and subsequent inactivation by the liver, apoCI efficiently increases this response as a consequence of enhancing the presentation to LPS-responsive cells directly as well as indirectly, by inhibiting the uptake and inactivation of LPS.

Our data also show an inverse relation between apoCI and apoE during the time course of infection and severe sepsis. High plasma apoCI levels were associated with low mortality from infection (**chapter 8**), whereas low plasma apoE levels tended to associate with low mortality from infection (**chapter 4**). Moreover, plasma apoCI levels markedly decreased during severe sepsis (**chapter 7**),

whereas plasma apoE levels increased[27] (Berbée JFP, Van Leeuwen HJ, Rensen PCN, unpublished results). These findings suggest that a high apoCI/apoE ratio is protective against the development of infections, most likely via enhancing the inflammatory response towards invading microorganisms. However, during (severe) sepsis a low apoCI/apoE ratio may be protective against septic shock and endotoxemia via reducing the inflammatory response.

It should be realized that besides apoCI and apoE also other apolipoproteins are involved in inflammatory responses. Therefore, the balance between the various apolipoproteins, in particular between apoCI and LPS-neutralizing apolipoproteins, on the lipoprotein surface may eventually determine the overall inflammatory properties of these lipoproteins. Indeed, the apolipoprotein content of acute phase HDL is different as compared to circulating HDL from healthy subjects[28], but also differs in the inflammatory response towards LPS. Whereas HDL from healthy subjects was able to augment the inflammatory response towards LPS by monocytes, this ability was lost for acute phase HDL[27]. Since one of the most distinct differences between normal and acute phase HDL is the almost complete depletion of apoCI[28], it is still tempting to speculate that the content of apoCI on HDL is a main determinant of the inflammatory property of HDL and concomitantly of the inflammatory response towards LPS.

3. Clinical Implications for ApoCI during Sepsis

In the last decade it has been generally accepted that precise staging of the infection process is necessary to effectively identify and accurately treat patients at high risk for developing severe sepsis and patients at high risk of dying. Clinical symptoms (*e.g.* fever, leukocytosis) and conventional biomarkers (*e.g.* procalcitonin, C-reactive protein (CRP), cytokines, cholesterol) are not always reliable. Measurement of the procalcitonin level in plasma has been the most established and valuable biomarker of sepsis for several years now, but as with all biomarkers it has its shortcomings. Whereas some studies show that the procalcitonin level in plasma is a prognostic factor for survival[29-31], this has not been confirmed by other studies[31,32]. Moreover, procalcitonin levels vary between patient groups, for example between patients with surgical infections as compared to patients with community-acquired infections[33], and therefore different diagnostic and prognostic cut-off values of procalcitonin have to be used for different patient groups. A variety of other potential new biomarkers has come up, including triggering receptor expressed on myeloid cells-1 (TREM-1), mannan binding lectin (MBL), and mid pro-atrial natriuretic peptide (mid pro-ANP). However, data on their prognostic value are still limited. A disadvantage of these (and most conventional) biomarkers is that they offer only one-dimensional

information, focussing either on diagnosis of infection, severity of inflammation, or the survival outcome. Therefore, the demand for new better biomarkers of sepsis, or combinations of biomarkers, for clinical application is still rising.

We have now identified plasma apoCI as a very promising biomarker of several stages of the process from infection to the development of sepsis, septic shock and death.

First, plasma apoCI levels can be used to identify apparently healthy subjects with increased risk of dying of infection. Elderly have a higher risk of dying from infection-related causes than younger subjects, since the immune system of elderly has a reduced ability to combat invading microorganisms[34]. In elderly of the Leiden 85-plus Study mortality from infection was the second cause of death after CVD mortality (17% and 41% respectively; **chapter 8**). In this population-based prospective follow-up study low apoCI levels at baseline were a strong and specific predictor of mortality from infection during 5 years of follow-up. In this study only data on the cause of mortality have been collected and data on the incidence of suffering infection are lacking. However, we hypothesize that the observed association is a direct result of a reduced risk of onset of infection in subjects with high apoCI levels.

Second, plasma apoCI levels can also be used as a prognostic factor to identify patients at high risk of dying during acute human endotoxemia. An exaggerated inflammatory response during endotoxemia and septic shock can result in organ failure, subsequently causing an extended intensive care unit (ICU) stay or even death[35,36]. In line with the observation that apoCI aggravated the inflammatory response during endotoxemia in mice (**Figure 2**), we also found in patients who underwent cardiac surgery with cardiopulmonary bypass (CPB) that pre-operative plasma levels of apoCI positively correlated with TNFα levels during the peri-operative endotoxemic episode (Berbée JFP, Schippers EF *et al.*, manuscript in preparation). In fact, pre-operative apoCI levels were a better predictor of the inflammatory response than other lipid parameters (*i.e.* TG and TC), apolipoproteins (apoE and apoCIII), and previously determined candidates in this patient group, such as polymorphisms in the TNFα promotor, Nod2, and TLR4[37]. Therefore, apoCI can be used to identify patients at high risk of developing severe endotoxemia to subsequently treat them with anti-inflammatory agents.

Third, apoCI levels can be used to diagnose systemic inflammatory response syndrome (SIRS) and (severe) sepsis. ApoCI levels are markedly lower in patients with SIRS, sepsis, (Berbée JFP, Kitchens RL, Rensen PCN, unpublished results), and severe sepsis (**chapter 7**) as compared to healthy controls. To eventually standardize the use of apoCI as a biomarker during infection, it is

crucial to know whether and how plasma apoCI levels are affected by other diseases than sepsis. For instance, apoCI levels are higher in hyperlipidemic subjects as compared to normolipidemic subjects, which should be taken into account during SIRS and (severe) sepsis.

Finally, our data also indicate that plasma apoCI levels can be used to predict survival outcome during infection and (severe) sepsis. Both in patients hospitalized with febrile illness (Berbée JFP, Schippers EF, Van Dissel JT, Rensen PCN, unpublished results) and in patients with severe sepsis (**chapter 7**) apoCI levels were lower in non-survivors as compared to survivors. During the time course of severe sepsis apoCI levels remained low in non-survivors, whereas apoCI levels gradually increased to healthy control levels in survivors, hereby enlarging the difference between non-survivors and survivors. Adjustment for lipid levels enlarged these differences in both groups of patients, further indicating the potential of apoCI as a prognostic factor for survival outcome during infection and (severe) sepsis.

In conclusion, our studies identify apoCI as a promising biomarker for precise staging of the infection process, and identifying patients at high risk for developing severe sepsis and patients at high risk of dying. However, confirmation in other populations is essential. To eventually standardize the use of apoCI as a biomarker in the clinic, it is likely that plasma apoCI levels should be corrected for lipid levels in order to adjust for the basal lipid differences between normolipidemic and hyperlipidemic subjects. In addition, plasma apoCI should be studied in combination with other (conventional) biomarkers, such as procalcitonin, which is most commonly used at the moment.

Current treatment of sepsis is primarily directed against the symptoms of sepsis and septic shock. The use of corticosteroids, early goal-directed resuscitation therapy, tight glycemic control using insulin treatment, activated protein C (APC), and immunomodulatory therapies improves survival in selected populations of septic patients[38-41]. However, the prognosis for patients with sepsis is still very poor. The mortality rate is still high and also the survivors of sepsis often have a reduced quality of life as compared to other critically ill patients.

In septic patients at low risk of dying, treatment with anti-inflammatory agents, such as anti-TNFα antibodies, has minimal effect and even aggravated survival outcome[38]. However, in septic patients at high risk of dying, treatment with anti-inflammatory agents critically reduced the inflammatory status of these patients and concomitantly improved survival outcome. In a similar manner neutralization of apoCI (and thus its proinflammatory properties) may be an effective (additional) treatment during human endotoxemia and septic shock. This is schematically represented in **Figure 3**. Neutralization of apoCI in subjects with an exaggerated

host inflammatory response may decrease the inflammatory response towards a more physiologic response, and as such, decrease the risk of mortality. However, neutralization of apoCI in patients with septic shock may have limited effect, since during septic shock apoCI levels are likely to be already decreased, similar as observed in severely septic patients (**chapter 7**). Nevertheless, neutralization of apoCI in patients with acute endotoxemia, for example by antibodies directed against apoCI, may have a high impact on reducing the inflammatory response, and subsequently organ failure and death.

Since apoCI levels before pneumonia infection are associated with an early and adequate anti-bacterial response resulting in reduced mortality (**Figure 1** and **chapter 5**), and high apoCI levels in humans predicted lower mortality from infection (**chapter 8**), apoCI may be a promising prophylactic agent against the development of bacterial infection. Critically ill patients often develop nosocomial infections. This is mostly related to the use of devices that are needed for patient life support, but that are at the same time responsible for complications as hospital-acquired pneumonia, surgical site infections, and urinary tract infections. Therefore, prophylactic treatment of these critically ill patients, or other immunodepressed subjects at high risk of infection, with apoCI may enhance the host inflammatory response towards a more physiologic adequate response to combat invading microorganisms, resulting in reduced infection susceptibility (**Figure 3**). Such a prophylactic treatment with apoCI is thus aimed at preventing the infection to develop into sepsis, which opposes the current treatments aimed at reducing the symptoms of sepsis and septic shock once the infection has developed into sepsis.

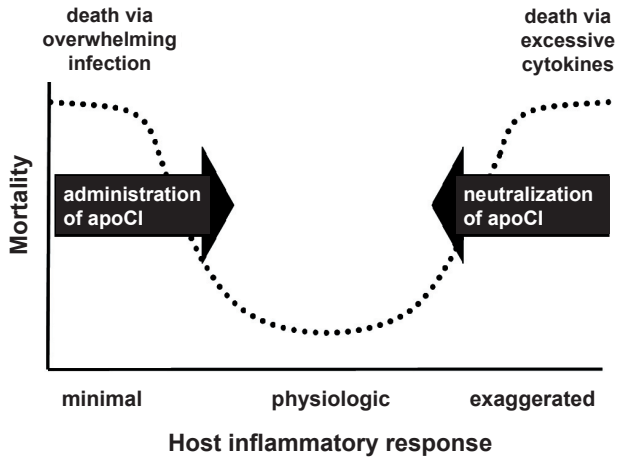

Figure 3. The effect of apoCI administration or neutralization in the context of the U-shape relationship between the host inflammatory response and mortality. See text for explanation. Modified from Cross *et al.* (*Cross AS, International Endotoxin Society Meeting, Kyoto, Japan, 2004*).

Prophylactic administration of apoCI in patients at high risk of infection is likely to be a complex treatment and requires much more investigation. It should be elucidated at which plasma levels apoCI enhances the inflammatory response, and which dose of apoCI is necessary to reach this plasma level. Also the effect of the variation in the inflammatory response between individuals, and between different groups of patients, should be investigated. Finally, since many patients at high risk of infection are critically ill patients and elderly, the effect of apoCI administration in combination with other drugs must be studied. Therefore, many studies have to be performed in order to understand how apoCI can be effectively used to prevent bacterial infection.

4. Concluding Remarks

In this thesis we showed that apoCI is a potent inhibitor of LPL, and therefore may have a dual role in atherosclerosis. Inhibition of LPL will be pro-atherogenic by inducing hypertriglyceridemia, but on the other hand apoCI could be anti-atherogenic by preventing obesity and increasing HDL levels via modulation of enzymes involved in HDL remodelling, such as CETP. Identifying a domain within apoCI that is capable of increasing HDL by inhibiting CETP activity without inducing hypertriglyceridemia via inhibition of LPL activity, may be a promising agent or lead in the treatment against CVD.

Furthermore, we identified an up till now unrecognized role of apoCI in infection. ApoCI may be a main determinant of the inflammatory response *in vivo*. Both murine and human studies showed that apoCI protects against infection, but the exact mechanism requires further investigation. Based on the studies presented in this thesis, apoCI (or its derivates) have potential enter the clinic, as a biomarker for sepsis, as a prophylactic agent to protect against infection and sepsis, and as a therapeutic target to reduce excessive inflammation in endotoxemia and septic shock.

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