Lipoproteins in Innate Immunity
Levels, J.H.M.

Citation for published version (APA):
Levels, J. H. M. (2002). Lipoproteins in innate immunity

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Lipid composition and lipopolysaccharide binding capacity of lipoproteins in plasma and lymph of patients with the systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF)


1 Department of Vascular Medicine, 2 Department of Surgery, 3 Department of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands.

(Accepted for publication in Critical Care Medicine)
LPS binding capacity in lymph and plasma during SIRS

ABSTRACT

Lipopolysaccharide (LPS), the major glycolipid component of Gram-negative bacterial outer-membranes, is a potent endotoxin responsible for many of the directly or indirectly induced symptoms of infection. Lipoproteins (in particular HDL) sequester LPS, thereby acting as a humoral detoxification mechanism.

Differences in the lipoprotein composition in human plasma and lymph of a control patient group (n=5) without systemic inflammatory response syndrome (non-SIRS/MOF) and patients with SIRS and multi organ failure (MOF) (n=9) (SIRS/MOF) were studied. The LPS binding capacity of the lipoproteins in SIRS/MOF and non-SIRS/MOF patients was investigated by rechallenge of the plasma and lymph with fluorescently labeled LPS \textit{ex vivo}. The lipoprotein composition was analyzed using immunochemical techniques and high performance gel-permeation chromatography.

In the non-SIRS/MOF patient group, plasma and lymph levels of apo A-I (600 and 450 mg/L resp.), apo B (440 and 280 mg/L resp.), total cholesterol (2.88 and 1.05 mM resp.) and total triglycerides (0.67 and 0.97 mM resp.) were observed. In the SIRS/MOF group a decrease of apo A-I (-55% in plasma and lymph), a decrease of apo B (-43% in plasma and -38% in lymph) and a decrease of total cholesterol levels (-54% in plasma and -37% lymph) were demonstrated. However, the triglyceride levels in the SIRS/MOF group showed a 30% increase in plasma and a 47% decrease in lymph compared to the non-SIRS/MOF patients. In SIRS/MOF a 2.8 fold increase in plasma and a 1.8 fold increase in lymph of the LPS LDL/HDL ratio was observed indicating that the relative LPS binding capacity of the lipoproteins in the SIRS/MOF patient group showed a trend to be shifted mainly towards LDL. Further, in plasma and lymph of four SIRS/MOF patients a novel cholesterol containing HDL-like particle was found which had barely LPS binding capacity (< 5%).

In the SIRS/MOF patients the changes in lipoprotein composition in lymph are a reflection of those in plasma except for the triglyceride levels. In comparison with the non-SIRS/MOF patients, the SIRS/MOF patients show a shifted LPS binding capacity of HDL towards LDL in plasma as well in lymph. Moreover, in plasma and lymph novel cholesterol containing particles, resembling HDL, were identified in the SIRS/MOF patient group.
Chapter V

Introduction

Lipoprotein homeostasis is profoundly affected by LPS exposure, malignancy, surgery and injury. In general, these stimuli cause a decrease of total cholesterol and especially high-density lipoprotein (HDL) cholesterol concentrations (1), (2). Depending on the species involved, total triglyceride concentrations increase or decrease in the early phase of infection (3), (4). All lipoproteins including very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and HDL play a substantial role in the innate immunity by attenuating the host response (5). Further, changes in lipid homeostasis are associated with mortality in animal models as well as in critically ill patients. We have previously reported that all lipoproteins are able to bind bacterial lipopolysaccharides (LPS) (6), (7) and lipoteichoic acid (LTA) (unpublished observations), at concentrations that far exceed the circulating levels in severe sepsis. Moreover it was observed that HDL has the highest LPS binding capacity of all lipoproteins (6), (8), (9).

Transfer of bacterial lipopolysaccharides into lipoproteins is facilitated by specific lipid transfer proteins, that include LPS binding protein (LBP), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) and bactericidal permeability increasing protein (BPI) (10). Of these proteins, LBP is of critical importance for the interaction of endotoxin with several host immune systems. LBP monomerizes LPS from micelles that are shedded from bacteria (11), (12) and presents LPS to membrane CD14 and soluble CD14 (13), (14). LBP also transfers LPS to HDL, which is considered to be a scavenging pathway (15), (16). Indeed, lipoprotein infusions in (hypolipemic) animals or endotoxin-challenged volunteers (17), (18) have provided evidence for the importance of lipoproteins in endotoxin scavenging (19).

Lymph transports endotoxin from the gut and peripheral tissues to the systemic circulation, and LPS is present in thoracic duct lymph from patients with sepsis (20), (21). Moreover, thoracic duct lymph represents the extravascular, interstitial body compartment and therefore may reflect processes at tissue level.

In this study we describe the differences in lipid homeostasis in plasma and thoracic duct lymph from patients with and without the systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF). We also report the LPS neutralizing capability of plasma and lymph of both patient groups after rechallenge with LPS ex vivo.
Patients and Methods

Patients

This study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam. Peripheral blood plasma and thoracic duct lymph were obtained from nine patients with SIRS and multiple organ failure (MOF) (seven men, two women, age (mean ± SE) 60 ± 15 years) and from five patients without SIRS/MOF (3 men, 2 women, aged 65 ± 6 years). Patients with SIRS/MOF, admitted to the intensive care unit of the Academic Medical Center, fulfilled the SIRS-criteria (22) and had organ failure of at least two organ systems. For each parameter, the worst value over the preceding 24 hours was used. The criteria for organ failure were defined by: 1) Respiratory failure (Lung injury score (adapted from reference 23) > 2.5); 2) Cardiovascular dysfunction (Systolic Arterial Pressure < 100 mmHg, or Dopamine and/or Dobutamine and/or Dopamine > 10 μg/kg/min, and/or Noradrenaline > 0.1 μg/kg/min, and/or any concentration Noradrenaline or phosphodiesterase-inhibitor (Perfan) when used in combination with dopamine or dobutamine or dopamine); 3) Disseminated intravascular coagulation (thrombocytes < 50 10/L, or thrombocyte-decrease > 25% or prothrombin time or partial thromboplastin time > 1.2 times the upper limit of normal); 4) Acute renal failure (Urinary output < 0.5 mL/kg body weight/hr for at least two hours after the onset of maximum therapy (volume loading/diuretics), or serum creatinine-increase of 100 μmol/L or acute renal replacement therapy (dialysis/ultrafiltration)); 5) Hepatobiliary dysfunction (total serum-bilirubin > 34 μmol/L or in patients with hepatobiliary disease > 100 μmol/L) and 6) Gastrointestinal dysfunction (endoscopically confirmed stress ulcer). Characteristics of the patients are shown in Table 1.

Table 1. Characteristics of patients with SIRS and organ failure (study group) (SIRS/MOF).

<table>
<thead>
<tr>
<th>sex/age (years)</th>
<th>Diagnosis</th>
<th>Failing organ systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/67</td>
<td>Generalized fecal peritonitis</td>
<td>Respiratory, cardiovascular, DIC, hepatobiliary</td>
</tr>
<tr>
<td>M/75</td>
<td>Generalized fecal peritonitis</td>
<td>Cardiovascular, DIC, acute renal failure</td>
</tr>
<tr>
<td>M/66</td>
<td>Generalized fecal peritonitis</td>
<td>Cardiovascular, DIC</td>
</tr>
<tr>
<td>M/39</td>
<td>Generalized fecal peritonitis</td>
<td>Respiratory, cardiovascular, DIC, acute renal failure</td>
</tr>
<tr>
<td>M/46</td>
<td>Obstructed ileostoma</td>
<td>Cardiovascular, DIC (abdominal fluid culture positive)</td>
</tr>
<tr>
<td>M/40</td>
<td>Acute sterile pancreatitis</td>
<td>Cardiovascular, DIC, acute renal failure</td>
</tr>
<tr>
<td>F/79</td>
<td>Acute sterile pancreatitis</td>
<td>Respiratory, cardiovascular, DIC</td>
</tr>
<tr>
<td>M/56</td>
<td>Hemorrhagic shock</td>
<td>Cardiovascular, acute renal failure</td>
</tr>
<tr>
<td>M/69</td>
<td>Abdominal aortic aneurysm</td>
<td>Cardiovascular, DIC, acute renal failure</td>
</tr>
</tbody>
</table>

F: female; M: Male; DIC: disseminated intravascular coagulation.

Patients without SIRS/MOF, serving as controls, underwent a transthoracic resection for a carcinoma of the esophagus or gastro-oesophageal junction. Thoracic duct ligation and resection was carried out as part of the oncologically demanded wide resection. These patients did not have
Chapter V

SIRS and multiple organ failure at the time of operation, and therefore were suitable as control group to address the aim of the study.

Previously, we have shown that there were no significant differences between endotoxin concentrations in lymph and blood of SIRS/MOF-patients; nor between blood of patients with and without SIRS/MOF, or lymph of patients with and without SIRS/MOF (20). Moreover, we have shown that LBP concentrations in plasma of patients with SIRS/MOF were significantly higher as compared to LBP concentrations in plasma of patients without SIRS/MOF. This was also found for LBP concentrations in lymph of patients with SIRS/MOF as compared to lymph of patients without SIRS/MOF (24).

Plasma and thoracic duct lymph collection

In patients with SIRS/MOF, the thoracic duct was cannulated with a 14-gauge single lumen catheter (Ohmeda, Swindon, UK) in the neck. Lymph was obtained immediately after the catheter was inserted in the thoracic duct (25). In the control group, the thoracic duct was cannulated with a 14-Gauge single lumen catheter (Ohmeda, Swindon, UK) approximately at the level of the fifth thoracic vertebra. Cannulation was performed immediately prior to resection. From both patient groups an arterial blood sample was drawn at the time of lymph sampling. Blood was collected in sterile, pyrogen-free 4.5 ml tubes containing 0.048 ml EDTA-K3 (Vacutainer Systems, Becton-Dickinson, Rutherford, NJ, USA). Lymph was collected in pyrogen-free plastic tubes (Sarstedt, Nümbrecht, Germany), containing pyrogen-free heparin (Tromboliquin®, Organon, Oss, The Netherlands, final concentration 50 IU/mL). Following centrifugation (1600 g, 20 min), lymph-supernatant and plasma were aliquoted and stored at -80°C until further processing.

Reagents and materials

Lipopolysaccharides of the highest purity available were obtained from commercial sources. *Escherichia coli* O111:B4 LPS was obtained from Sigma Chemical Co. (St Louis, MS, USA). Pyrogen-free distilled water used throughout the experiments was from Ecotainer (Braun Medical AG, Melsungen, Germany). The fluorescent label BODIPY was obtained from Molecular Probes Inc. (Eugene, OR, USA). Phenol Amino anti-Pyrene (PAP) reagent for post-column cholesterol and triglyceride detection was from Bio-Merieux (Marcy l'Etoile, France). Tris-hydroxymethylaminomethane, from Boehringer Mannheim (Mannheim, Germany). Di-methyl sulphoxide (DMSO). NaCl and Tween-20 of the highest purity were purchased from Merck (Darmstadt, Germany).

BODIPY labeling of LPS

Purified *E. coli* O111:B4 LPS was labeled using the BODIPY-R6G oligonucleotide amine labeling kit (Molecular Probes, Eugene, OR, USA) after modifications of the manufacturer's protocol for oligosaccharide labeling as described previously (6). The fluorescent labeled LPS was depleted of free fluorescent label by binding of the excess label to glycine, which was subsequently separated from the labeled LPS liposomes using a sephadex-15 column. The labeling efficiency was determined by measurement of the optical density at 528 nm using the
LPS binding capacity in lymph and plasma during SIRS

Quoted extinction coefficient of 70,000 cm⁻¹ M⁻¹ and the stoichiometry of labeling was found to be approximately 1 BODIPY per 1 LPS and the final 0111:B4–BODIPY-R6G concentration was determined by a modified 2-Keto-3-deoxy Octonate (KDO) assay (Purpald assay) (26).

Separation of lipoprotein classes by High Performance Gel permeation Chromatography (HPGC)

The system contained a PU-980 ternary pump with an LG-980-02 linear degasser, a FP-920 fluorescence and UV-975 UV/VIS detector (Jasco, Tokyo, Japan). An extra P-50 pump (Pharmacia Biotech, Uppsała, Sweden) was used for in-line cholesterol PAP reagent addition at 0.1 ml/min. Plasma lipoprotein separations were performed with a Superose 6 HR 10/30 column (Pharmacia Biotech, Sweden) with TBS containing 0.005 % (v/v) tween-20, pH 7.4 as eluent at a flow rate of 0.31 ml/min. Computer analysis of the chromatograms was carried out using the Borwin Chromatographic software, version 1.23 (JMBS Developments, Le Fontanil, France).

Distribution, lipoprotein-binding capacity and kinetics of different LPS chemotypes

For the LPS distribution experiments, 3 μl of labeled LPS preparations in saline were added to 100 μl plasma (ex vivo) in polypropylene tubes, achieving a final concentration of 23 μg/ml plasma, and subsequently incubated for 1 hour at 37 °C. Profiles of the association of fluorescent LPS with lipoproteins in plasma and lymph were analyzed by HPGC with fluorescence and post-column cholesterol detection. BODIPY-LPS fluorescence was monitored by an excitation wavelength at 530 nm and an emission wavelength at 550 nm. The chromatograms used for analysis were the result of the subtraction of a plasma or lymph chromatogram with spiked fluorescent-labeled LPS and a blank of the same plasma or lymph sample of each individual. This resulted in a fluorescent profile, originating only from lipoprotein associated fluorescent LPS. Cholesterol was continuously monitored at 505 nm by an enzymatic total cholesterol detection method using PAP reagent.

Total Cholesterol, triglycerides, apo A and apo B analysis in plasma and lymph.

Total cholesterol and triglyceride concentrations were determined enzymatically resulting in a substrate conversion measured by spectrophotometry using PAP reagent. Apo A-I and Apo B levels were measured by an automated nephelometric assay (APA and APB) using an Array Protein System Nephelometer (Beckman, Mijdrecht, The Netherlands).

Statistical analysis

Values are presented as median [range]. Results were processed with Prism version 3.0 (Graph Path Software Inc., San Diego, CA, USA) and SPSS version 10.1.0 (Chicago, IL, USA) using the Wilcoxon signed ranks test for parametric comparison of the data of lymph and plasma in the non-SIRS/MOF or in the SIRS/MOF group, and the Mann Whitney test for non-parametric comparison between the non-SIRS/MOF and SIRS/MOF data of total cholesterol, total triglycerides, apo A-I, apo B, LPS distribution in plasma and lymph. A probability of less than 0.05 (two-sided) was considered to represent a statistically significant difference.
Chapter V

Results

Lipid specific parameters in SIRS/MOF and non-SIRS/MOF patients

In both patient groups lipid profiles in plasma compared to lymph showed a similar distribution, but the absolute concentrations of apo B, apo A-I and total cholesterol were lower in lymph. In addition, plasma triglycerides concentrations were higher. In table 2 the comparison of the lipid parameters is shown between the non-SIRS/MOF patients, representing the control group, and the SIRS/MOF patients. As compared to the control group, in the SIRS/MOF group a decrease of apo A-I (-55% in plasma and lymph), a decrease of apo B (-43% in plasma and -38% in lymph) and a decrease in total cholesterol levels (-54% in plasma and -37% lymph) was demonstrated.

Table 2. The median [range] of the total cholesterol, triglycerides, apo A-I and apo B in plasma (P) and lymph (L) in the non-SIRS/MOF and SIRS/MOF patient group.

<table>
<thead>
<tr>
<th></th>
<th>Non-SIRS/MOF</th>
<th>SIRS/MOF</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol (mM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>2.88</td>
<td>1.31*</td>
<td>-54</td>
</tr>
<tr>
<td>L</td>
<td>1.05</td>
<td>0.66**</td>
<td>-37</td>
</tr>
<tr>
<td><strong>Triglycerides (mM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.67</td>
<td>0.87</td>
<td>+30</td>
</tr>
<tr>
<td>L</td>
<td>0.97</td>
<td>0.51</td>
<td>-47</td>
</tr>
<tr>
<td><strong>Apo A-I (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>600</td>
<td>270**</td>
<td>-55</td>
</tr>
<tr>
<td>L</td>
<td>450</td>
<td>202**</td>
<td>-55</td>
</tr>
<tr>
<td><strong>Apo B (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>440</td>
<td>250</td>
<td>-43</td>
</tr>
<tr>
<td>L</td>
<td>280</td>
<td>175</td>
<td>-38</td>
</tr>
</tbody>
</table>

In the last column the percent change of the median in the SIRS/MOF group compared to the non-SIRS/MOF group is noted. Bold = p < 0.05 (non SIRS/MOF vs. SIRS/MOF) and * = p < 0.05 (plasma vs. Lymph).

The triglyceride levels in the SIRS/MOF group showed an increase of 30% in plasma but a 47% decrease in lymph compared to the non-SIRS/MOF patients.
The cholesterol distribution among the lipoprotein classes

In figure 1a an example of the cholesterol distribution is depicted in a non-SIRS/MOF patient over the different lipoprotein classes in plasma and in lymph. Figure 1b represents the cholesterol distribution among the lipoproteins in plasma and lymph of a patient with SIRS/MOF. In the non-SIRS/MOF control group median cholesterol levels in plasma of VLDL (0.27 mM), LDL (1.69 mM) and HDL (0.58 mM) were observed (figure 2a), whereas in lymph median cholesterol levels of VLDL (0.22 mM), LDL (0.62 mM) and HDL (0.28 mM) were found (figure 2c). Compared to the non-SIRS/MOF control group, the SIRS/MOF patient group, showed lower median plasma cholesterol levels
Chapter V

Fig. 2. The absolute cholesterol distribution (mM) among the main lipoprotein classes of non-SIRS/MOF patients in plasma (A) and lymph (C) and SIRS/MOF patients in plasma (B) and in lymph (D). All individual cholesterol values are plotted. The horizontal bars indicate the median.

of VLDL (0.18 mM), LDL (0.60 mM) and HDL (0.24 mM) (figure 2b) and showed lower lymph cholesterol levels of VLDL (0.16 mM), LDL (0.32 mM) and HDL (0.12 mM) (figure 2d). All absolute cholesterol levels in lymph were lower compared to plasma levels in both patient groups. In the plasma compartment of 4 SIRS/MOF patients we observed a novel cholesterol-containing particle (Figure 1b and 2b). The molecular weight was below that of the normal HDL particles of the non-SIRS/MOF patient group. These novel cholesterol-containing particles were also observed in the lymph compartment of 5 SIRS/MOF patients (figure 1b and 2d). Isolation of the HDL subtypes as seen after HPGC analysis revealed that indeed both particles were of HDL origin (figure 3a). $K_{av}$ analysis showed a mean size of the novel HDL particle of 150 kDa (fraction 2) compared to a mean size of 300 kDa of the normal HDL (fraction 1). Immuno electrophoresis (fig. 3b) showed low apo A-I and apo A-II
Composition of HDL subtypes in SIRS/MOF plasma

![Graph showing HDL subtypes](image)

**Tab. 3.** A representative isolation of the HDL subtypes as found with size exclusion chromatography in patients with SIRS/MOF (A). Fraction 1 has a molecular size of approximately 300 kDa, whereas fraction 2 an average molecular size of 150 kDa revealed. Rocked immunoelectrophoresis shows the Lp A-I and Lp A-I/A-II composition of fraction 1 and 2 (B). The absolute concentrations of Lp A-I, Lp A-I/A-II apo A-I and apo B are presented in panel C.

levels in both fractions. However, fraction 2 had a higher LP A-I / LP A-I/A-II ratio. In both fractions no apo B could be detected (fig. 3c).

To obtain more insight in the alterations of the relative cholesterol distribution between HDL and LDL or VLDL in both patient groups LDL/HDL and VLDL/HDL ratios were calculated (Table 3). The median plasma LDL/HDL cholesterol ratio in the SIRS/MOF versus the non-SIRS/MOF patients was increased (1.7 times) indicating a cholesterol distribution shift towards LDL, whereas the median lymph cholesterol ratio was virtually unchanged (Table 3). The median plasma VLDL/HDL cholesterol ratio in the SIRS/MOF patients versus the non-SIRS/MOF group was slightly increased (1.4 times), whereas the median VLDL/HDL lymph ratio in the SIRS/MOF versus the non-SIRS/MOF showed a decrease (2.4 times) indicating that no relative cholesterol shift towards VLDL had occurred. Further, we observed a striking increase of the
ratio ranges (VLDL/HDL and LDL/HDL) in the SIRS/MOF group as compared to the non-SIRS/MOF patient group.

Table 3: The relative total cholesterol ratio among the lipoproteins in patients in the non-SIRS/MOF and the SIRS/MOF group in plasma (P) and lymph (L).

<table>
<thead>
<tr>
<th>Chol. RATIO</th>
<th>non-SIRS/MOF</th>
<th>Median</th>
<th>Range</th>
<th>SIRS/MOF</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL/HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>202</td>
<td>[181 - 343]</td>
<td>348</td>
<td>[52.3 - 4111]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>165</td>
<td>[146 - 219]</td>
<td>155</td>
<td>[36.6 - 961]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL/HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>29.1</td>
<td>[19.4 - 166.7]</td>
<td>40.4</td>
<td>[0 - 5368]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>107</td>
<td>[46.9 - 201]</td>
<td>44.1</td>
<td>[0 - 1709]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cholesterol ratios, LDL/HDL and VLDL/HDL, are obtained using the equation LDLc x 100 /HDLc and VLDLc x 100 /HDLc respectively.

The LPS distribution among the lipoprotein classes

Figure 1c depicts an example of the LPS distribution among the lipoproteins of a control patient, whereas figure 1d represents the LPS distribution of a typical SIRS/MOF patient. In figure 4 the percentile LPS distribution over the different lipoprotein classes in plasma (figure 4a and 4b) and lymph (figure 4c and 4d) is shown. In the non-SIRS/MOF control group the relative median plasma LPS distribution among the lipoproteins was: VLDL (5%), LDL (10%) and HDL (73%) (Figure 4a) respectively. A 12% non-lipoprotein bound LPS residual was observed (not shown). The relative median lymph LPS distribution in the control group was: VLDL (13%), LDL (16%) and HDL (62%) respectively (figure 4c). A residual non-lipoprotein bound LPS of 9% was observed (not shown).

The SIRS/MOF patient group had a relative median plasma LPS distribution of VLDL (5%), LDL (17%) and HDL (70%) (figure 4b). A novel HDL-like particle accounts for 4% of LPS binding and additionally a non-LP bound residual of 4% was observed. In two subjects of the SIRS/MOF patient-
Table 4: The relative LPS distribution ratio among lipoproteins in patients with or without SIRS/MOF in plasma (P) and lymph (L).

<table>
<thead>
<tr>
<th>LPS RATIO</th>
<th>Median</th>
<th>Range</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL/HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>8.5</td>
<td>[3.8-16.6]</td>
<td>23.9</td>
<td>[0-9277]</td>
</tr>
<tr>
<td>L</td>
<td>22.9</td>
<td>[19.8-43.2]</td>
<td>42.1</td>
<td>[15.2-1200]</td>
</tr>
<tr>
<td>VLDL/HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>7.0</td>
<td>[3.7-9.6]</td>
<td>9.3</td>
<td>[0-623]</td>
</tr>
<tr>
<td>L</td>
<td>18.2</td>
<td>[12.2-36.4]</td>
<td>23.4</td>
<td>[0-563]</td>
</tr>
</tbody>
</table>

The lipoprotein associated LPS ratios, LDL/HDL and VLDL/HDL, are obtained using division of the corrected LPS fluorescence peak areas of LDL x 100/HDL and VLDL x 100/HDL respectively. * = P < 0.05 (plasma vs. lymph), # = P < 0.05 (non-SIRS/MOF vs. SIRS/MOF).

The decreased LPS association with HDL in plasma was compensated by increased LDL-LPS binding. In lymph of the SIRS/MOF group, an altered lipoprotein LPS distribution was observed of VLDL (11%), LDL (31%), HDL (49%), novel HDL like particle (5%), and a non-lipoprotein bound residual of 4% (not shown) (figure 4d). In addition, in four subjects the LPS-lipoprotein distribution was shifted from HDL towards LDL. Of further notice is that the observed novel cholesterol containing particles were capable to bind LPS in plasma (4 subjects) as well in lymph (5 subjects).

To investigate the overall alterations in lipoprotein bound LPS, ratios between VLDL/HDL and LDL/HDL were determined in plasma and lymph (Table 4). In the non-SIRS/MOF patient group the median LPS LDL/HDL ratio was 2.7 times higher in lymph compared to plasma whereas in the SIRS/MOF patient group only a 1.8 times higher LDL/HDL ratio in lymph was found indicating a lower relative LPS association with HDL in lymph than in plasma. Comparison of the LDL/HDL plasma ratios between the two patient groups revealed a 2.8 times higher median ratio in plasma in the SIRS/MOF group compared to the non-SIRS/MOF group, whereas in lymph only a 1.8 times higher ratio was seen. Also these observations demonstrated a shifted LPS distribution towards LDL in SIRS/MOF.
Fig. 3. A representative isolation of the HDL subtypes as found with size exclusion chromatography in patients with SIRS/MOF (A). Fraction 1 has a molecular size of approximately 300 kDa, whereas fraction 2 an average molecular size of 150 kDa revealed. Rocked immunoelectrophoresis shows the Lp A-I and Lp A-I/A-II composition of fraction 1 and 2 (B). The absolute concentrations of Lp a-I, Lp A-I/A-II apo A-I and apo B are presented in panel C.

The median VLDL/HDL LPS ratio in lymph was higher compared to plasma (2.5 times in non-SIRS/MOF (p<0.05) and 2.6 times in SIRS/MOF patient group (Table 4) indicating that, in lymph, the higher VLDL concentrations result in binding of LPS to this lipoprotein. The VLDL/HDL ratio in plasma was slightly increased in the SIRS/MOF group compared to the non-SIRS/MOF group. In lymph, the same slight increase of the ratio VLDL/HDL in the SIRS/MOF patient group was observed versus the non-SIRS/MOF group. None of the aforementioned differences were statistically significant.
Discussion

We have investigated the plasma and lymph lipid composition of the main lipoprotein classes (VLDL, LDL and HDL) of patients with the systemic inflammatory response syndrome and multiple organ failure and without SIRS and MOF (SIRS/MOF). Moreover, we assessed the LPS distribution among the main lipoprotein classes by rechallenge (ex vivo) plasma and lymph samples with fluorescently labeled LPS (BODIPY-O111:B4). Compared to the non-SIRS/MOF control group, decreased total cholesterol. Apo A-I and Apo B levels were observed in the plasma and lymph of the SIRS/MOF patients. Total plasma triglycerides of the SIRS/MOF patients were increased whereas in lymph a triglyceride decrease was observed. The relative lymph cholesterol distribution among the main lipoprotein classes in the non-SIRS/MOF patients and SIRS/MOF patients paralleled the plasma cholesterol distribution but the absolute concentrations were much lower (30 – 50%) in lymph. The relative LPS distribution among the main lipoprotein classes revealed that, during SIRS/MOF, the LPS distribution in plasma as well as in lymph shifted mainly towards LDL and not towards VLDL.

The total cholesterol, apo A-I and apo B levels in plasma and lymph were all decreased in the SIRS/MOF group compared to the non-SIRS/MOF group. The plasma triglyceride levels of the SIRS/MOF group were increased, which is consistent with previous observations in the early phase of infection (4). However, in contrast to the changes observed early after infection (27), we found a decrease in lymph triglycerides during the development of sepsis. It should be noted that all patients included in this study were surgical patients who had fasted for at least 12 hours. Our data indicate that in critically ill patients the overall measured lipoprotein homeostasis in lymph is a reflection of the plasma homeostasis. However, the absolute apolipoprotein and cholesterol levels are 30 – 50% lower in lymph as compared to plasma which is consistent with earlier findings (21).

In animal models, intravenous administration of reconstituted HDL (rHDL) or triglyceride-rich particles protects against the mortality of lethal endotoxemia (7). Similar effects have been observed in low dose endotoxemia in healthy volunteers who were pretreated with rHDL infusion (28), (29). It has been previously reported that HDL cholesterol concentrations in severe sepsis are decreased, and the mortality of SIRS/MOF patients is correlated with the HDL cholesterol concentration (17). However it remains uncertain to what
Chapter V

extent the alterations in plasma lipoprotein concentrations in critical illness translate into differences in LPS binding capacity. In the SIRS/MOF patients of this study the LPS binding capacity among the main lipoprotein classes was clearly shifted towards LDL and to a lesser extent to VLDL indicating that during SIRS/MOF VLDL is not a potent scavenger to compensate the loss of the LPS binding capacity of HDL as shown in an experimental infection study where hyper-triglyceridemia, mostly located in VLDL and LDL particles, did not inhibit the in vivo responses to endotoxin in humans (30).

The interaction of LPS with HDL is mediated by lipopolysaccharide binding protein (LBP), which has significant sequence homology with the phospholipid transporting proteins CETP and PLTP. A significant fraction of circulating LBP is associated with HDL (14), which implicates that this lipoprotein has a major role as a LPS scavenger. However, others have observed that in plasma of septic patients LBP can also be associated with LDL (31), (32). In this study and in a previous study we report that, when the number of HDL particles decreases, LPS will associate with the other available lipoproteins such as LDL and VLDL (7) possibly enhanced by a shifted LBP association towards LDL.

A novel finding in this study is that 5 SIRS/MOF patients had a new LPS and cholesterol containing particle which appeared to be smaller in size (150 kDa) than the average molecular size of HDL (300 kDa) and contained very small amounts of cholesterol. Furthermore, we observed a different apo A-I/A-II ratio compared to the larger HDL particle indicating a changed apolipoprotein composition. We consider these particles to be lipid poor HDL particles that appear as a result of the inflammation-dependent inhibition of HDL lipid transport proteins that are responsible for normal HDL maturation (33). We hypothesize that these smaller HDL particles are acute phase HDL particles that are known to have a higher affinity for macrophages than for hepatocytes (27). However, acute phase HDL is less capable to protect LDL against oxidation (34), which may lead to an additional increased risk of damage to the arterial wall during SIRS/MOF.

In conclusion, we have found that an altered lipid composition in plasma and lymph during SIRS/MOF results in a marked alteration of LPS distributions among the lipoproteins. SIRS/MOF is associated with low HDL concentrations in plasma and lymph, and in these conditions the other lipoprotein classes (esp. LDL) are capable of binding LPS. In addition, a new endotoxin-binding particle
LPS binding capacity in lymph and plasma during SIRS of HDL origin appears in plasma and lymph of SIRS/MOF patients. The LPS binding shift towards LDL and VLDL may result in less effective endotoxin scavenging.

Acknowledgement:
We thank K. Bakhtiari for performing the nephelometric assays and H.P. van Barreveld for his assistance with the HPGC analysis.

References

Chapter V


LPS binding capacity in lymph and plasma during SIRS


33. Hayat S. Raynes JG. Acute phase serum amyloid A protein increases high density lipoprotein binding to human peripheral blood mononuclear cells and an endothelial cell line. Scan J Immun 2000; 51(2):141-146.