ANESTHESIOLOGY

Reconstituted High-density Lipoprotein Therapy Improves Survival in Mouse Models of Sepsis

Sébastien Tanaka, M.D., Ph.D., Claire Genève, M.D., M.Sc., Nathalie Zappella, M.D., M.Sc., Jennyfer Yong-Sang, Ph.D., Cynthia Planesse, M.Sc., Liliane Louedec, Bsc.Tech., Wildriss Viranaïcken, Ph.D., Matthieu Bringart, Bsc.Tech., Philippe Montravers, M.D., Ph.D., Erick Denamur, M.D., Ph.D., Jacques Duranteau, M.D., Ph.D., David Couret, M.D., Ph.D., Olivier Meilhac, Ph.D.

ANESTHESIOLOGY 2020; 132:825–38

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- High-density lipoproteins have multiple positive end-organ effects, and attenuate organ injury and improve survival in mouse models of sepsis

What This Article Tells Us That Is New

- A human injectable formulation of reconstituted high-density lipoprotein, CSL-111, composed of apolipoprotein A1 and phosphatidylcholines, was tested in three mouse models of sepsis
- When administered soon after the insult causing sepsis, CSL-111 improved survival and reduced lung injury, apparent neutrophil activation, and plasma markers of inflammation, but not cytokine concentrations

ABSTRACT

Background: High-density lipoproteins exert pleiotropic effects including antiinflammatory, antiapoptotic, and lipopolysaccharide-neutralizing properties. The authors assessed the effects of reconstituted high-density lipoproteins (CSL-111) intravenous injection in different models of sepsis.

Methods: Ten-week-old C57BL/6 mice were subjected to sepsis by cecal ligation and puncture or intraperitoneal injection of *Escherichia coli* or *Pseudomonas aeruginosa* pneumonia. CSL-111 or saline solution was administrated 2 h after the sepsis. Primary outcome was survival. Secondary outcomes were plasma cell-free DNA and cytokine concentrations, histology, bacterial count, and biodistribution.

Results: Compared with saline, CSL-111 improved survival in cecal ligation and puncture and intraperitoneal models (13 of 16 [81%] survival rate *vs.* 6 of 16 [38%] in the cecal ligation and puncture model; $P = 0.011$; 4 of 10 [40%] *vs.* 0 of 10 [0%] in the intraperitoneal model; $P = 0.011$). Cell-free DNA concentration was lower in CSL-111 relative to saline groups (68 [24 to 123] pg/ml *vs.* 351 [333 to 683] pg/ml; $P < 0.001$). Mice injected with CSL-111 presented a decreased bacterial count at 24 h after the cecal ligation and puncture model both in plasma (200 [28 to 2,302] *vs.* 2,500 [953 to 3,636] colony-forming unit/ml; $P = 0.021$) and in the liver (1,359 [360 to 1,648] *vs.* 1,808 [1,464 to 2,720] colony-forming unit/ml; $P = 0.031$). In the pneumonia model, fewer bacteria accumulated in liver and lung of the CSL-111 group. CSL-111–injected mice had also less lung inflammation *versus* saline mice (CD68+ to total cells ratio: saline, 0.24 [0.22 to 0.27]; CSL-111, 0.07 [0.01 to 0.09]; $P < 0.01$). In all models, no difference was found for cytokine concentration. ^{111}Indium bacterial labeling underlined a potential hepatic bacterial clearance possibly promoted by high-density lipoprotein uptake.

Conclusions: CSL-111 infusion improved survival in different experimental mouse models of sepsis. It reduced inflammation in both plasma and organs and decreased bacterial count. These results emphasized the key role for high-density lipoproteins in endothelial and organ protection, but also in lipopolysaccharide/bacteria clearance. This suggests an opportunity to explore the therapeutic potential of high-density lipoproteins in septic conditions.

(ANESTHESIOLOGY 2020; 132:825–38)

Sepsis remains a major cause of mortality in intensive care units, despite better understanding of the pathophysiology and the development of improved supportive treatments, antibiotic therapies, and surgical techniques.[1–4]

High-density lipoproteins are characterized by the association of apolipoproteins such as apolipoprotein A1, phospholipids, and cholesterol in its free or esterified form. Their most described function is to mediate the reverse transport of cholesterol, from peripheral tissues back to

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

Submitted for publication January 6, 2019. Accepted for publication December 20, 2019. Published online first on February 20, 2020. From Réunion Island University, French Institute of Health and Medical Research (INSERM) U1188, Diabetes atherothrombosis Réunion Indian Ocean (DéTROI), CYROI Plateform, Saint-Denis de La Réunion, France (S.T., J.Y-S., C.P., M.B., D.C., O.M.); Assistance Publique - Hôpitaux de Paris (AP-HP), Department of Anesthesiology and Critical Care Medicine, Bichat-Claude Bernard Hospital, Paris, France (S.T., C.G., N.Z., P.M.); INSERM U1148, Laboratory for Vascular Translational Science, Paris France (C.G., N.Z., L.L.); Réunion Island University, INSERM U1187, CNRS (National Center for Scientific Research) 9192, IRD (Institute for Research and Development) 249, PIMIT Laboratory, Infectious Processes in Tropical Island Environment, CYROI Plateform 2, Saint-Denis de La Réunion, France (W.V.); INSERM U1152, Physiopathology and Epidemiology of Respiratory Diseases, Paris, France (P.M.); INSERM U1137, Infection, Antimicrobials, Modelling, Evolution, Paris, France (E.D.); AP-HP, Department of Anesthesiology and Critical Care Medicine, Paris-Sud Hospitals, Paris-Sud University, Bicêtre Hospital, Le Kremlin-Bicêtre, France (J.D.); Clinical Research Unit (Bio-CANVAS: biomarkers in CardioNeuroVascular DISEASES) U942, Paris, France (J.D.); Réunion Island University-affiliated Hospital, France (D.C., O.M.).

 Anesthesiology 2020; 132:825–38. DOI: 10.1097/ALN.0000000000003155

<zdoi;. DOI: 10.1097/ALN.0000000000003155>

the liver.[5] Many studies have described an inverse association between high-density lipoprotein concentration and cardiovascular events.[6–8] In addition to reverse transport cholesterol, high-density lipoproteins display pleiotropic properties such as antiinflammatory, antioxidant, antiapoptotic, and antithrombotic effects.[9–14] Furthermore, high-density lipoproteins are reported to protect the endothelium *via* limiting the expression of adhesion molecules under inflammatory conditions[15] and are able to neutralize lipopolysaccharides.[16,17]

Previous studies have shown that high-density lipoprotein infusion attenuates organ injury in mouse models of sepsis, potentially *via* lipopolysaccharide neutralization.[18–20] Recently, Zhang *et al.* have reported that the use of apolipoprotein A1 mimetic peptide improved the survival of septic rats.[20]

Several clinical studies have been conducted to assess apolipoprotein A1 concentration in septic conditions.[21–25] Van Leeuwen *et al.* underlined that in septic patients, high-density lipoprotein concentration rapidly fall and can be reduced to 50%.[21] In a previous study, we demonstrated that compared to trauma patients, high-density lipoprotein levels were lower in case of sepsis.[23] Chien *et al.* have shown that low high-density lipoprotein levels at day 1 of severe sepsis were significantly associated with an increased mortality.[24] Barlage *et al.* have also found that in intensive care unit, nonsurvivor septic patients had a statistically significant decreased apolipoprotein A1 concentration.[25] In a recent work comparing septic *versus* nonseptic patients, we reported a marked decrease in high-density lipoprotein concentration and a shift toward large nonfunctional high-density lipoprotein particles.[26]

Based on high-density lipoprotein pleiotropic effects previously demonstrated, we hypothesized that supplementation with functional high-density lipoproteins in a murine model of sepsis could improve the survival. Several studies have tested apolipoprotein A1 mimetic peptides or reconstituted high-density lipoproteins administered before lipopolysaccharide injection or the onset of sepsis. Here we have used reconstituted high-density lipoproteins made of apolipoprotein A1 and phosphatidylcholines (CSL-111; Behring, Switzerland), tested previously in a randomized clinical trial involving post–myocardial infarction patients.[27] The goal of the current study was then to test the potential therapeutic effects of high-density lipoproteins in three different models of sepsis, using reconstituted high-density lipoproteins potentially injectable in clinical settings.

Materials and Methods

Reconstituted HDLs (CSL-111) were provided by CSL Behring AG (Bern, Switzerland). They consist of apolipoprotein AI purified from human plasma combined with soybean phosphatidylcholines to form discoidal particles similar to native reconstituted high-density lipoproteins.[28]

Animals

Ten-week-old C57BL/6 female and male mice weighing 20 to 25g were used in our study (sex ratio, 1:1). All experimentations were performed in accordance with the legislation on the protection of animals and were approved by the ethical committees for animal experimentation, Bichat Hospital, INSERM 1148, Paris (authorization 2012-15/698-097) and CYROI n°114 (authorization *14827-2018012212274175 V3* and *20938-2019031114427274 V7*). Experiments were carried out in animal facility in microsurgery room.

Models of Sepsis

Animals were anesthetized by 2% isoflurane gas anesthesia and 0.05 mg/kg buprenorphine was injected as analgesia. Cecal ligation and puncture was performed as previously described by Rittirsch *et al.*[29,30] Two hours after the cecal ligation and puncture, each animal was blindly administered either CSL-111 (40 mg/kg) or saline (Supplemental Digital Content, http://links.lww.com/ALN/C220). Computer-based randomization was used to allocate drug regimens to each group, performed by a blinded observer from our laboratory.

Intraperitoneal Injection of Bacteria (Intraperitoneal Model)

In the second model of sepsis, 4×10^7 colony-forming unit/ml of *Escherichia coli* (IAI76 strain) were injected into the intraperitoneal cavity in a volume of 400 μl of saline (Supplemental Digital Content, http://links.lww.com/ALN/C220). This model induced a peritonitis characterized by a rapid intravascular transfer of bacteria.[31] Two hours after the intraperitoneal bacterial injection, each animal was intravenously administered either CSL-111 (40 mg apolipoprotein A1/kg) or saline.

Pseudomonas Aeruginosa Pneumonia Model (Pneumonia Model)

For this third model, a concentration of 4×10^8 colony-forming unit/ml *Pseudomonas aeruginosa* ATCC 27853 strain were instilled in the mouse trachea (Supplemental Digital Content, http://links.lww.com/ALN/C220).[32] Two hours after the bacterial tracheal instillation, each animal was administered intravenously either CSL-111 (40 mg apolipoprotein A1/kg) or saline.

After the surgery, postoperative care consisted in mouse hydration by saline subcutaneous infusion (1.2 ml per 100 g body weight), and analgesia by subcutaneous buprenorphine (5 ng per 100 g body weight). Mice were placed in the facedown position in a bedding-free, prewarmed fresh cage placed over a heating pad. All mice had free access to water and food.

Survival Rate

In each group in the two sepsis procedures (cecal ligation and puncture and intraperitoneal), mice were observed every 2 h for 150 h.

Plasma and Histological Analyses

Twenty-four hours after surgical procedure, the animals were sacrificed, blood, bronchoalveolar lavage fluid and tissues were collected for analysis (Supplemental Digital Content, http://links.lww.com/ALN/C220). An observer blinded to the experimental conditions analyzed the anonymized histological sections.

Bacteria ^{111}Indium Labeling

^{111}Indium was incubated with bacteria for 45 min. Bacteria were then washed with saline by centrifugation in order to discard free ^{111}Indium. Labeled bacteria were injected to the mouse *via* the intraperitoneal route. The same protocol was performed in CSL-111 and saline groups. The distribution of radioactive bacteria was achieved by scintigraphy at different times.

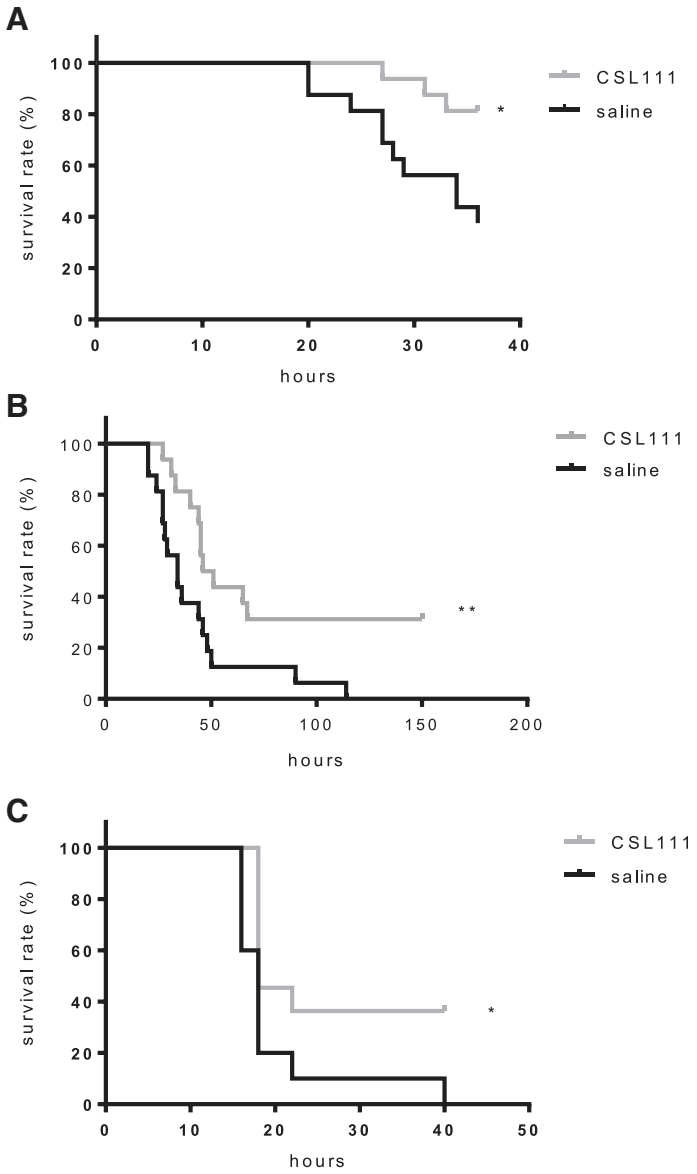

Fig. 1. Survival rate of mice that underwent cecal ligation and puncture at 36 and 150 h after surgery (*A, B*) or injected with 4.10^7 IAI76 *Escherichia coli* at 48 h (*C*). n = 16 mice per group, **P* = 0.01, ***P* < 0.01 comparing CSL-111 *versus* saline-injected using a log-rank (Mantel–Cox) test.

Statistical Analysis

The primary outcome of our study was mortality rate and we defined our secondary outcomes as all plasma and histological analyses as well as bacteria ^{111}Indium labeling. Survival curves according to treatment groups were estimated using the Kaplan–Meier method and compared using a log rank test. No statistical power calculation was conducted before the study. The sample size for survival study was based on previous studies in the field.[20,33,34] Quantitative variables are expressed as median [interquartile range]. Because of a non-Gaussian distribution, univariate comparisons were made using Mann–Whitney U test in case of bivariate comparisons. In case of comparisons between three groups (saline, CSL-111 and sham groups), we used a Kruskal–Wallis test followed by a Dunn multiple comparison test. All analyses were performed at the two-tailed threshold of $P < 0.05$ and data were analyzed using Graph Pad Prism software.

Results

Effect of Intravenous CSL-111 Injections on Survival in the Cecal Ligation and Puncture Model

In saline-injected mice, cecal ligation and puncture resulted in an overall survival of 38% at 36 h *versus* 81% survival rate for CSL-111-treated mice (fig. 1A; $P = 0.011$; n = 16 mice per group). At 110 h, all saline-injected mice were dead whereas CSL-111 treatment significantly improved survival to 31% (fig. 1B; $P = 0.008$). Sham-operated mice (n = 16 mice) all survived (not shown). There were no censured data during the observation period.

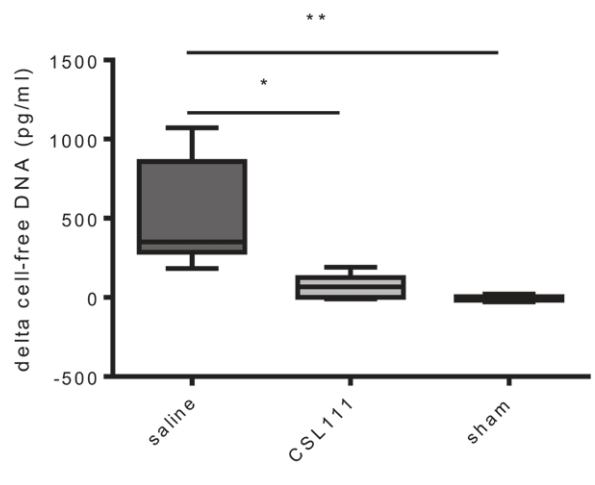

Fig. 2. Cell-free DNA concentration (pg/ml) in plasma 4 h before and 24 h after surgery. Saline group, n = 10; CSL-111 group, n = 8; sham group, n = 9. The data are represented as a box plot.

Effect of Intravenous CSL-111 Supplementation on Survival in the Intraperitoneal Bacterial Injection Model

In order to validate the protective effects of HDLs, we tested CSL-111 in another model of sepsis induced by intraperitoneal injection of *E. coli*. All saline-injected mice were dead at 40h whereas CSL-111 treatment significantly improved survival to 40% at this time point (fig. 1C; $P = 0.011$; n = 10 mice per group). Sham-operated mice (n = 10 mice) all survived (not shown). There were no censured data during the observational period.

Plasma Cell-free DNA

We measured cell-free DNA as a general marker of apoptosis and necrosis, potentially reflecting neutrophil activation *via* production of neutrophil extracellular traps, at baseline and after 24h. This variation of cell-free DNA concentration (delta DNA, pg/ml) was statistically significantly lower in CSL-111 groups relative to saline-injected mice (fig. 2; [68 (24 to 123) pg/ml *vs.* 351 (333 to 683) pg/ml; $P < 0.001$] CSL-111 group, n = 8 mice; saline group, n = 10 mice; sham group, n = 9 mice).

Bronchoalveolar Lavage and Plasma Cytokine Levels

Cecal ligation and puncture markedly induced the production of plasma interleukin-1ß, interleukin-10, and tumor necrosis factor α relative to sham production. There was no statistically significant difference between CSL-111 and saline groups for interleukin-1ß, interleukin-10, and tumor necrosis factor α at 24 h after cecal ligation and puncture (fig. 3; interleukin-1ß, 0 [0 to 133] pg/ml *vs.* 90 [6 to 825] pg/ml; $P = 0.271$; interleukin-10, 3,784 [1,078 to 51,292] ng/ml *vs.* 27,388 [12,817 to 52,383] ng/ml; $P > 0.999$; tumor necrosis factor α, 85 [44 to 266] pg/ml *vs.* 171 [102 to 346] pg/ml; $P > 0.999$; CSL-111 group, n = 8 mice; saline group, n = 10 mice; sham group, n = 8 mice).

In the pneumonia model, there was no statistically significant difference between CSL-111 and saline groups for interleukin-6 (plasma: CSL-111, 108 [19 to 205] pg/mb; saline, 119 [61 to 5,807] pg/ml; $P = 0.431$; bronchoalveolar lavage: CSL-111, 31 [20 to 58] pg/mb; saline, 37 [24 to 240] pg/ml; $P = 0.506$; CSL-111 group, n = 6 mice; saline group, n = 7 mice).

Quantification of Plasma Markers of Inflammation and Endothelial Activation

All plasma markers tested, except vascular cell adhesion molecule-1, were increased under septic conditions relative to sham-operated mice. There was no statistically significant difference between CSL-111– and saline-injected groups for intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin, matrix metallopeptidase-9, and plasminogen activator inhibitor-1 at 24 h after CLP ([fig. S1, Supplemental Digital Content, http://links.lww.com/ALN/C221] intercellular adhesion molecule-1: 23[14 to

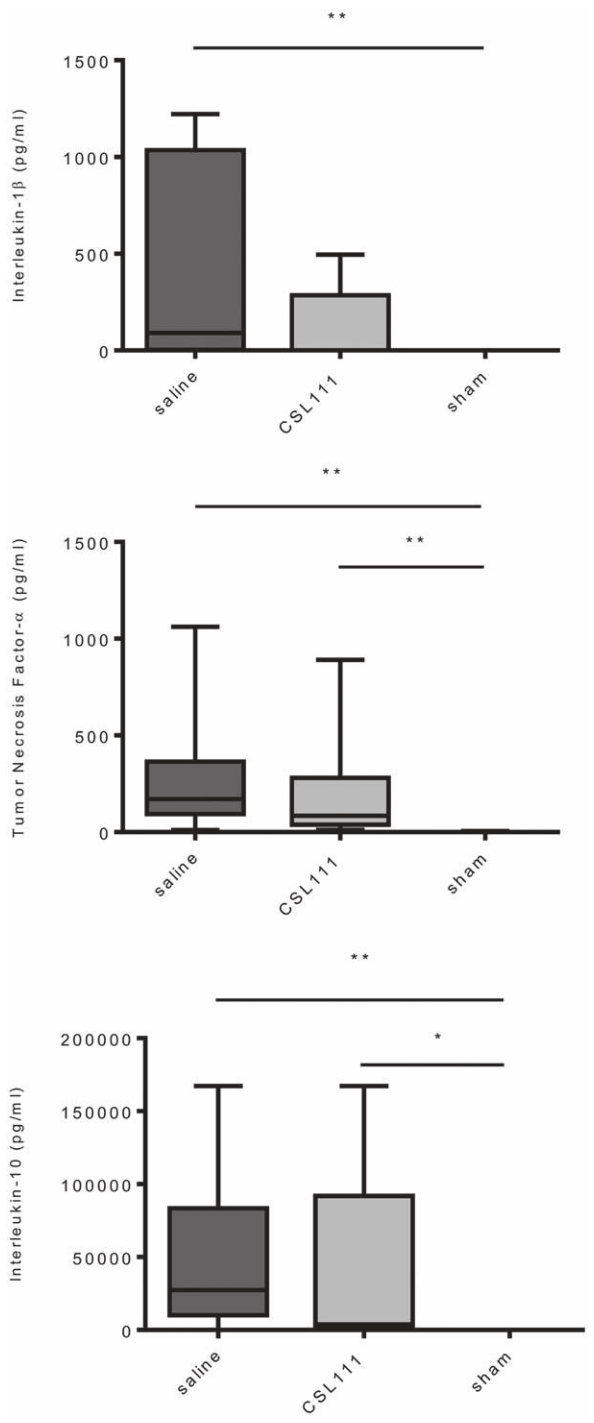

Fig. 3. Interleukin-1ß, interleukin-10, and tumor necrosis factor α concentration in plasma (pg/ml) at 24 h after cecal ligation and puncture. Control group, n = 10; CSL-111 and sham groups, n = 8 mice. The data are represented as a box plot.

37] ng/ml *vs.* 28 [18 to 43] ng/ml, $P > 0.999$; vascular cell adhesion molecule-1: 2,275 [1,390 to 2,823] pg/mL *vs.* 2,203 [1,890 to 3,170] pg/ml, $P > 0.999$; E-selectin: 130 [56 to 252] pg/ml *vs.* 212 [134 to 262] pg/ml, $P = 0.503$; matrix metallopeptidase-9: 28 [19 to 54] pg/ml *vs.* 99 [80 to 177] pg/ml, $P = 0.453$; plasminogen activator inhibitor-1: 149 [92 to 212] ng/ml *vs.* 132 [76 to 232] ng/ml, $P > 0.999$); CSL-111 group, n = 8 mice, saline group, n = 10 mice, sham group, n = 8 mice.

Lung mRNA Expression of Interleukin-6, Tumor Necrosis Factor α, Intercellular Adhesion Molecule-1, Vascular Cell Adhesion Molecule-1 and E-selectin

Cecal ligation and puncture induced lung expression of interleukin-6, tumor necrosis factor α, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, as assessed by reverse transcription polymerase chain reaction. Results are expressed in ratio of messenger RNA (mRNA) to glyceraldehyde 3-phosphate dehydrogenase. There was no difference between CSL-111 group and saline group in lung RNA expression of interleukin-6, tumor necrosis factor α, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin ([fig. S2, Supplemental Digital Content, http://links.lww.com/ALN/C222] interleukin-6, 2.6 [0.7 to 2.8] *vs.* 1.0 [0.8 to 5.1]; $P > 0.999$; tumor necrosis factor α: 1.0 [0.9 to 1.0] *vs.* 1.1 [1.0 to 1.1]; $P > 0.999$; intercellular adhesion molecule-1, 3.0 [2.4 to 3.8] *vs.* 6.3 [4.4 to 8.2]; $P = 0.957$; vascular cell adhesion molecule-1, 1.0 [0.9 to 1.0] *vs.* 1.0 [0.9 to 1.0]; $P > 0.999$; E-selectin, 0.6 [0.0 to 1.0] *vs.* 1.1 [1.0 to 2.6]; $P = 0.151$). Results are expressed in ratio of mRNA to glyceraldehyde 3-phosphate dehydrogenase; CSL-111 group, n = 5 mice; saline group, n = 5 mice; sham group, n = 5 mice).

Bacteremia

Mice treated with CSL-111 have a statistically significant decrease of bacteremia at 24 h from the cecal ligation and puncture compared to saline-injected mice (200 [28 to 2,302] colony-forming unit/ml of blood in CSL-111 group; 2,500 [953 to 3,636] colony-forming unit/ml of blood in saline group; $P = 0.021$; n = 18 mice/group). Sham-operated mice were exempt of bloodborne bacteria.

Bacterial Count in Liver, Lung, Spleen, and Kidney

CSL-111 injection led a statistically significant decrease in bacterial rate in the liver at 24 h from the cecal ligation and puncture relative to the saline group (1,359 [360 to 1,648] colony-forming unit/ml in CSL-111 group; 1,808 [1,464 to 2,720] colony-forming unit/ml in saline group; $P = 0.031$). No difference between the two groups was observed in the lung, spleen, and/or kidney (lung, 844 [104 to 1,489] colony-forming unit/ml *vs.* 807 [492 to 3,762] colony-forming unit/ml; $P = 0.489$; spleen, 320 [114 to 568] colony-forming unit/ml *vs.* 247 [53 to 503] colony-forming unit/ml; $P = 0.757$; kidney, 308 [137 to 336] colony-forming unit/ml *vs.* 122 [52 to 904] colony-forming unit/ml; $P = 0.436$; n = 9 mice/group) (fig. 4).

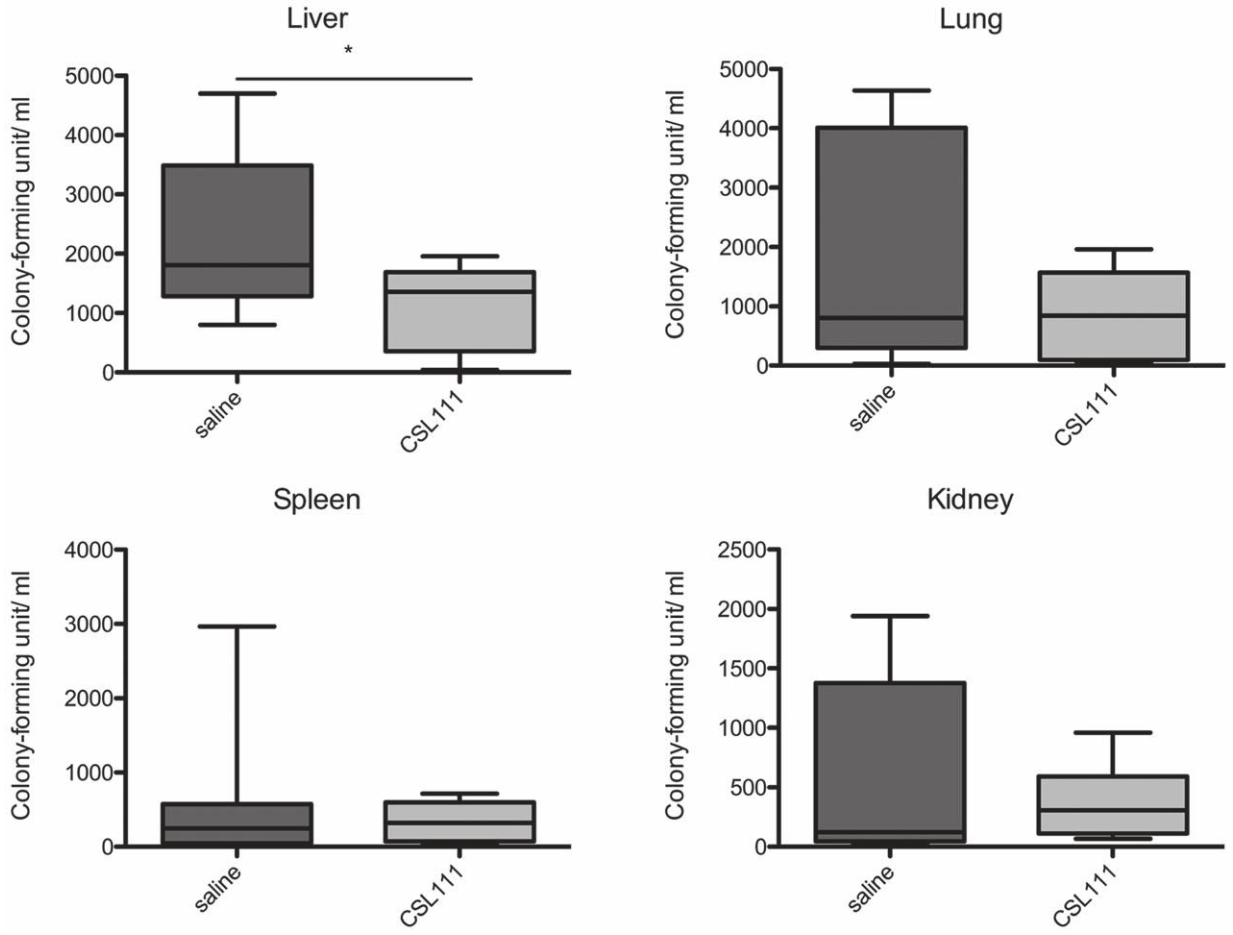

Fig. 4. Bacterial count (colony-forming unit/ml). n = 9 mice/group. Serial dilutions of tissue homogenates (liver, lung, spleen, kidney) were performed in saline and then seeded in Luria-Bertani Agar culture medium. After a 24-h incubation at 37°C, the colony-forming units were counted in order to evaluate the bacterial concentration. The data are represented as a box plot.

In the pneumonia model, CSL-111 injection led to a decreased bacterial concentration in the liver at 24 h comparing sepsis to saline group, 8.0 × 10^7 (6.9 × 10^7 to 8.4 × 10^7) in CSL-111 group; 1.1 × 10^8 (9.9 × 10^7 to 1.3 × 10^8) in saline group; $P < 0.001$. In the pneumonia model, CSL-111 injection also led a decreased bacterial concentration in the lung at 24 h comparing sepsis to saline group, 4.9 × 10^7 (4.6 × 10^7 to 5.6 × 10^7) in CSL-111 group; 3.2 × 10^8 (5.8 × 10^7 to 4.2 × 10^8) in saline group; $P = 0.004$; n = 7 mice in saline group, n = 5 mice in CSL-111 group (fig. 5).

Lung Histological Evaluation and Immunodetection of Apolipoprotein A1 and CD68

Hematoxylin and eosin staining showed an increase of alveolar destruction and infiltration by inflammatory cells in the saline mice group compared to mice treated with CSL-111 (cells to total area ratio, 0.19 [0.13 to 0.20] in the saline group; 0.03 [0.03 to 0.04] in the CSL-111 group; 0.05 [0.04 to 0.06] in the sham group; $P = 0.01$; n = 3 mice/group; fig. 6). In the cecal ligation and puncture model, immunohistological analysis of septic lungs 24 h after intravenous injection of CSL-111 or saline demonstrates that apolipoprotein A1 reached and accumulated in pulmonary tissue in high-density lipoprotein-injected mice, suggesting that high-density lipoprotein particles can locally exert their protective effects (apolipoprotein A1 immunopositive area to cells ratio, 0.5 [0.4 to 1.1] in high-density lipoprotein-injected mice *vs.* 0.02 [0.01 to 0.03] in the saline group mice; $P = 0.016$; fig. 7 and fig. S3, Supplemental Digital Content, http://links.lww.com/ALN/C223). Immunodetection of CD68+ macrophage shows a statistically significant increased accumulation of these inflammatory cells in septic conditions relative to controls (sham-operated mice) (CD68-immunopositive area to cells ratio: saline, 0.24 [0.22 to 0.27]; CSL-111 group, 0.07 [0.01 to 0.09]; sham group,

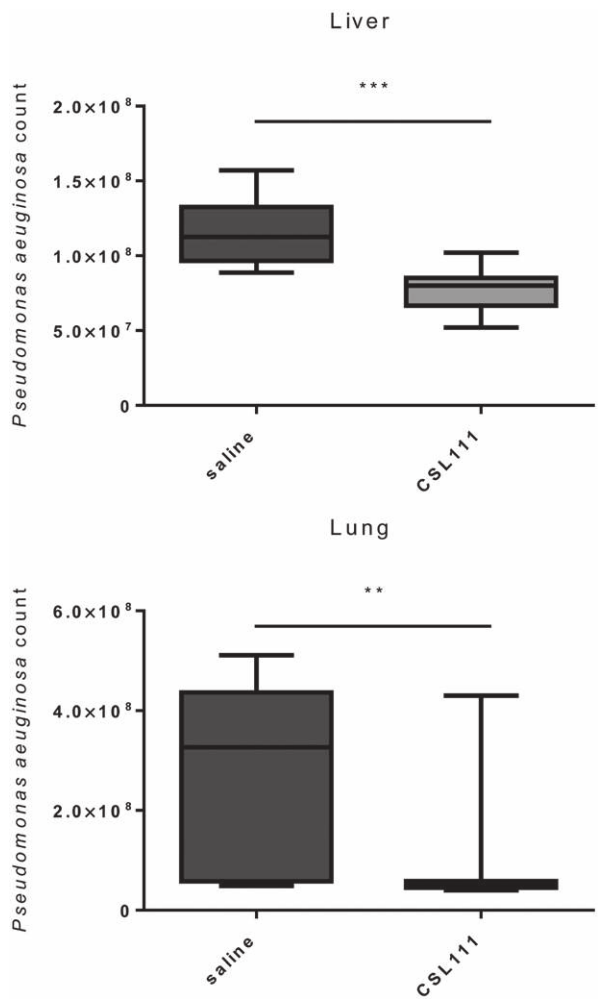

Fig. 5. *Pseudomonas aeruginosa* bacterial count in liver and lung, 24 h after pneumonia model. n = 7 mice in saline group; n = 5 in CSL-111 group. $^{**}P < 0.01$, $^{***}P < 0.001$. The data are represented as a box plot.

0.02 [0.0 to 0.04]; $P < 0.0001$). High-density lipoprotein injection markedly limited the recruitment of macrophages in septic conditions (fig. 8 and fig. S4, Supplemental Digital Content, http://links.lww.com/ALN/C224).

In the pneumonia model, hematoxylin and eosin staining showed an increase of alveolar destruction and infiltration by inflammatory cells in the saline mice group compared to mice treated with CSL-111 and the sham group (cells to total area ratio: 0.04 [0.03 to 0.05] in the saline group; 0.01 [0.01 to 0.02] in the CSL-111 group; 0.01 [0.01 to 0.02] in the sham group; $P < 0.001$; n = 3 mice/group).

In the pneumonia model, CSL-111–injected mice presented less CD68+ cells than in saline group mice. There was no difference between CSL-111 mice and sham (CD68-immunopositive area to cells ratio: saline, 0.07 [0.05 to 0.13]; CSL-111 group, 0.03 [0.02 to 0.04]; sham group, 0.0 [0.0 to 0.0]; $P < 0.0001$; n = 3 mice/group; fig. 9).

^{111}Indium Bacterial Labeling

Scintigraphy has shown that bacteria have mainly an intraperitoneal localization at 1h after intraperitoneal injection. At 2.5 h, in the group of mice treated with CSL-111, bacteria have an epigastria localization that could be gall bladder. In the saline-injected group, labeled bacteria were essentially localized in intraperitoneal position and in testis. There was no epigastria accumulation in this group of mice (n = 2 mice/group) (fig. S5, Supplemental Digital Content, http://links.lww.com/ALN/C225).

Discussion

Our main finding is that injection of reconstituted high-density lipoproteins (CSL-111) markedly improved survival in three different mouse models of sepsis. At different experimental time points after cecal ligation and puncture, (36, 40, or 110 h), mortality was reduced by more than 30% in the CSL-111–injected group *versus* the saline-injected group. These results are in the line with several studies showing a reduced mortality after injection of either reconstituted high-density lipoproteins or apolipoprotein A1 mimetic peptides in endotoxemic models.[15,19,35] It was shown that apolipoprotein A1 knockout mice are more susceptible to cecal ligation and puncture-induced death.[34] Apolipoprotein A1 knockout mice exhibited a decreased plasma lipopolysaccharide neutralization capacity relative to control mice. In this context, our results and the conclusions of these studies emphasized a major protective role of high-density lipoproteins during experimental sepsis. We performed these two models of sepsis (cecal ligation and puncture/intraperitoneal bacterial injection models) because they are more similar to human sepsis as compared to lipopolysaccharide infusion, which appears to be simple and reproducible, but probably does not reflect the complex physiologic human response to the bacterial insult. In the intraperitoneal bacterial injection model, the presence of bacteria allows insights into mechanisms of host response to pathogens. Moreover, cecal ligation and puncture model represents a polymicrobial sepsis model and may be similar to human sepsis progression with similar hemodynamic and metabolic phases.[36] We also performed a sepsis induced by an intratracheal injection of *Pseudomonas aeruginosa*, in order to test the potential of high-density lipoproteins therapy in a pneumonia, nonintraabdominal sepsis model.

In both peritonitis and pneumonia models, histological analysis showed that mice treated with CSL-111 had less lung inflammatory cell infiltration and less alveolar septal destruction. Our results are in line with McDonald *et al.* study who found that pretreatment of lipopolysaccharide-rats with reconstituted high-density lipoproteins attenuated intestinal injury by reducing edema, cell infiltration or destruction of the normal architecture.[15] We also

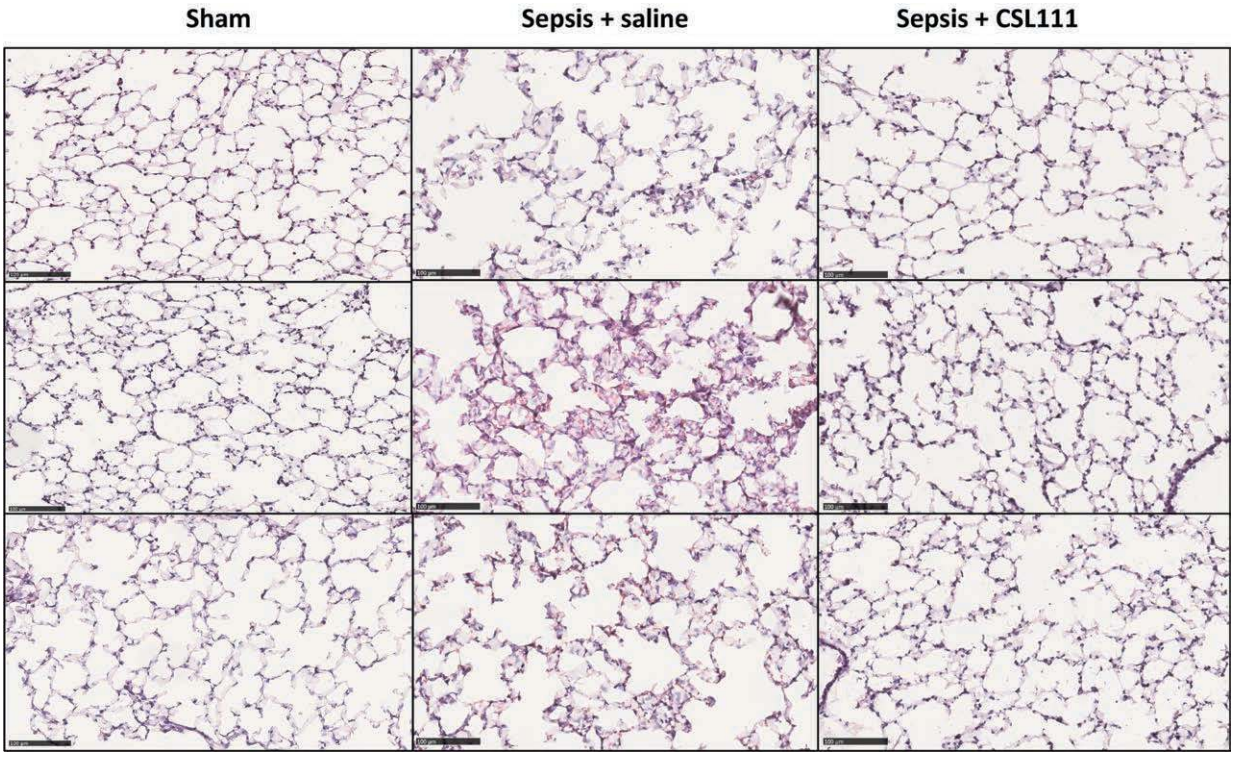

Fig. 6. Histological sections of lung tissue stained with hematoxylin and eosin, the *black scale bar* represents 100 mm. n = 3/group.

underline an interesting anti-inflammatory property of high-density lipoproteins particles. Macrophage recruitment is associated with inflammation in acute lung injury and acute respiratory distress syndrome.[37] In our study, in both peritonitis and pneumonia models, mice treated with CSL-111 had less macrophage infiltration *versus* saline-injected mice conferring to CSL-111 a protective antiinflammatory effect. For histological analysis, only three mice per group could be included due to technical problems.

Inflammatory states are also characterized by increased levels of plasma markers, such as cell-free DNA. Neutrophil activation by pathogens leads to the liberation of DNA associated with antimicrobial proteins contained in granules called neutrophil extracellular traps.[38] Neutrophil extracellular trap production has been highlighted in different pathologies other than sepsis such as cancer, trauma, or myocardial infarction.[39,40] DNA may be released into the circulation from apoptotic and necrotic cells. Apoptosis plays a major role in sepsis and in particular NETosis, consisting in the release of neutrophil extracellular traps by activated neutrophils, leading to production of cell-free DNA. Circulating DNA concentration has been reported to be increased in the plasma of septic patients.[41] Cell-free DNA also appears to be a predictor of outcome in septic shock patients.[42–44] Because of the increase of cell death and NETosis in septic conditions, cell-free DNA concentration was higher at admission in ICU nonsurvivor than in survivors according to Saukkonen *et al.* In our study, the reduced cell-free DNA concentration in high-density lipoprotein *versus* saline-injected mice is probably due to the antiapoptotic protective effect of CSL-111, as well as their capacity to limit neutrophil activation.

As expected, tumor necrosis factor α, interleukin-1ß, and interleukin-10 levels were increased by the cecal ligation and puncture procedure relative to sham-operated mice. However, in our different models of sepsis, we failed to show a reduction in cytokine production in high-density lipoprotein-treated mice. Compared with recent literature in the field, cytokine expression is time- and model-dependent: reconstituted high-density lipoproteins treatment in rats subjected to endotoxemia did not reduce the serum level increase of tumor necrosis factor α after lipopolysaccharide administration.[15] Zhang *et al.* reported a decrease in interleukin-6 plasma levels 12 h after cecal ligation and puncture in apolipoprotein A1 mimetic peptide–treated mice, but not at 24 h.[20] Dai *et al.* also found reduced tumor necrosis factor α levels in apolipoprotein A1 mimetic peptide–treated mice at 2 h, but not at 6 h.[19] In our study, cytokine measurement was only performed at 24 h after the cecal ligation and puncture which may be inappropriate to show differences in high-density lipoprotein-treated mice.

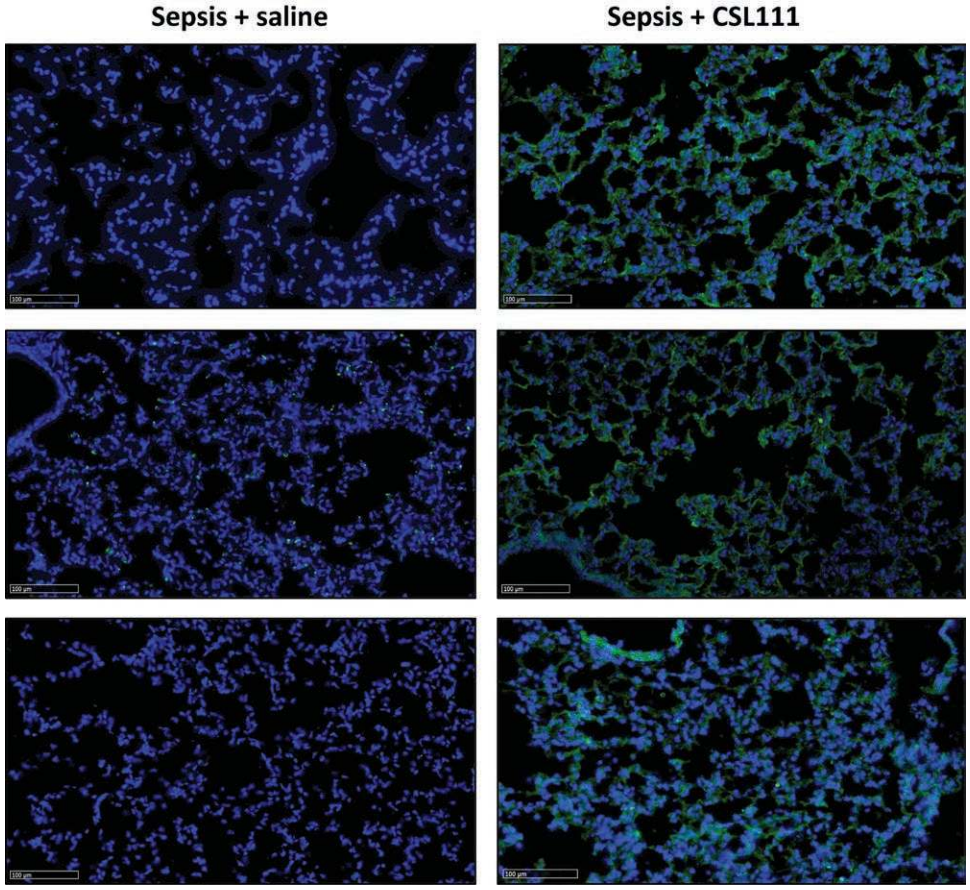

Fig. 7. Immunodetection of apolipoprotein A1 in the lung. Nuclei are stained with 4′,6-diamidino-2-phenylindole (*blue*), apolipoprotein A1 fluorescence appears in *green* (Alexa 488 secondary antibody). The *black scale bar* represents 100 mm. n = 3/group.

High-density lipoproteins display a variety of endothelial protective effects.[14] In human umbilical vein endothelial cells stimulated with tumor necrosis factor α for 4 h, Cockerill *et al.* have demonstrated that preincubation with physiologic concentration of mature high-density lipoproteins attenuated the expression of adhesion molecules.[45] McDonald *et al.* reported that reconstituted high-density lipoproteins attenuated the upregulation of intercellular adhesion molecule-1 and *P*-selectin observed by immunohistochemistry in the kidneys of rats subjected to a 6-h endotoxemia.[15] In our work, interleukin-6, tumor necrosis factor α and vascular cell adhesion molecule-1 mRNA lung levels were unchanged at 24 h by the CLP procedure, whereas intercellular adhesion molecule-1 and E-selectin expression was increased in the lungs of septic mice. Interaction between sepsis and acute respiratory distress syndrome (ARDS) are complex, involving complement system activation, neutrophil infiltration, vascular endothelial system damage, and activation of coagulation cascades.[46,47] The lung damage occurring during sepsis increases the morbimortality; patients with sepsis-related ARDS have a higher 60-day mortality rate than patients with nonsepsis-related ARDS.[48]

In addition to their antiinflammatory potential, high-density lipoproteins can bind and neutralize lipopolysaccharides.[49,50] We show that mice treated by CSL-111 presented a statistically significant decrease in bacteremia at 24 h from the cecal ligation and puncture *versus* saline-injected mice. Whereas in the pneumonia model we found a decreased of bacterial count in both liver and lung of high-density lipoproteins-treated mice, in the cecal ligation and puncture model, this decreased bacterial tissue contamination was only statistically significant in the liver. It could be hypothesized that high-density lipoproteins may bind and improve lipopolysaccharides and/or bacteria clearance *via* the liver and subsequent bile excretion. Increased mortality was observed in rats subjected to bile duct ligation in a model of endotoxemia induced by lipopolysaccharide and prevented by reconstituted high-density lipoprotein treatment.[51] After labeling bacteria with ^{111}Indium, we show that they were directed to the bile vesicle in reconstituted high-density lipoprotein-treated mice.

We also have demonstrated that apolipoprotein A1 is able, after intravenous infusion, to reach and accumulate in the

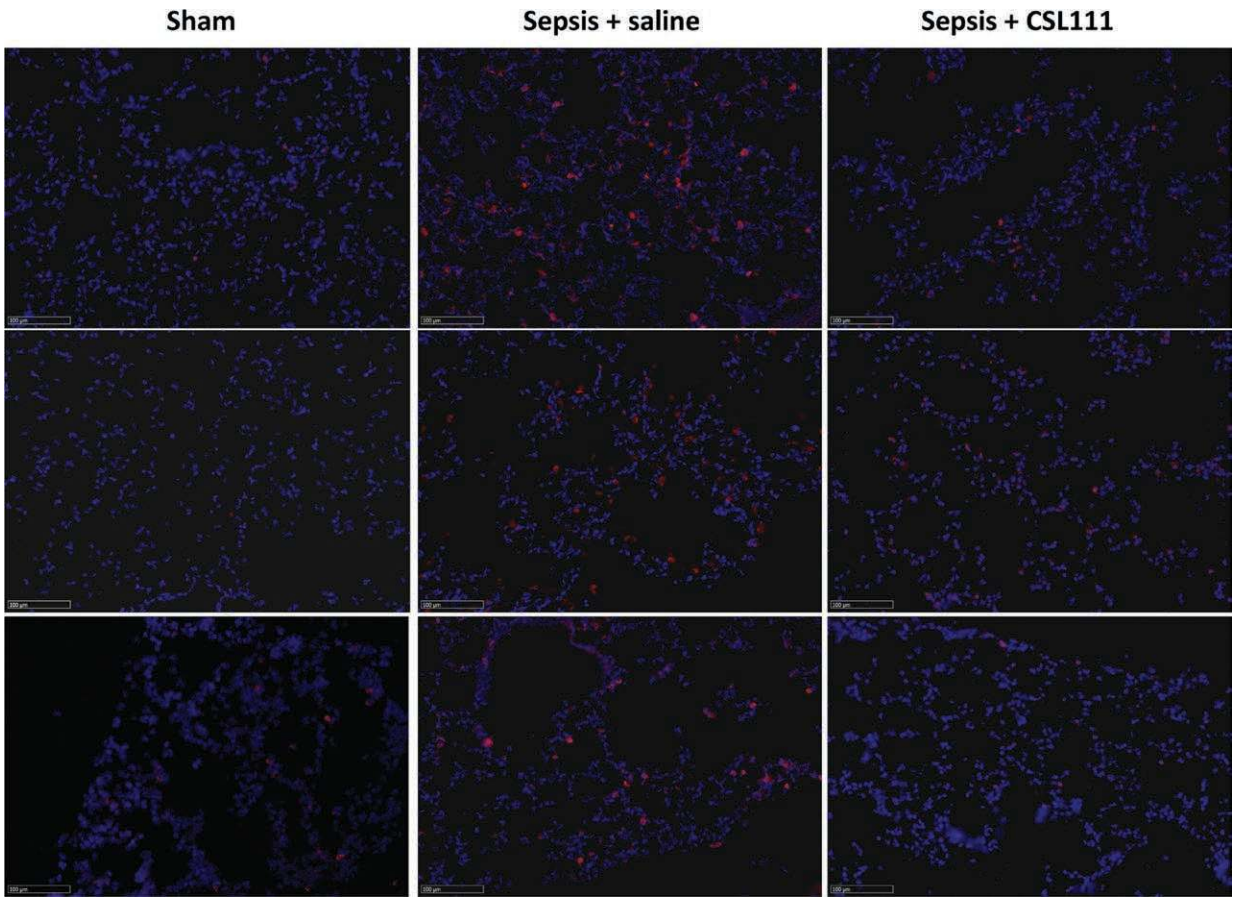

Fig. 8. Immunodetection of CD68 in the lung. Nuclei are stained with 4′,6-diamidino-2-phenylindole (*blue*), CD68 fluorescence appears in *red* (Alexa 594 secondary antibody). The *black scale bar* represents 100 mm. n = 3/group.

lung. Immunohistological analysis of septic lungs showed an intense staining for apolipoprotein A1 pulmonary tissue in high-density lipoprotein-injected mice, suggesting that high-density lipoproteins may exert their pleiotropic effects (in particular antioxidant and antiinflammatory). We have previously reported that high-density lipoproteins could accumulate in the lung under inflammatory conditions after intravenous injection (in pulmonary emphysema).[52]

Our work has limitations. First, we did not monitor hemodynamic parameters. Monitoring blood gases to collect lactate levels could have been interesting. Therefore, the mechanism for death is not provided (shock/hypotension or respiratory failure, or both). Second, tolerance parameters such as renal or hepatic function were not measured. Third, CSL-111 was injected only 2 h after the cecal ligation and puncture. A later infusion might be of interest in order to better fit to a potential clinical situation. Finally, the lung wet-to-dry weight ratio was not performed but could have been informative in the assessment of edema across different groups.

In clinical practice, reconstituted high-density lipoprotein injection has been tested in several clinical settings, including atherosclerosis and type 2 diabetes.[28,53–55] The Effect of rHDL on Atherosclerosis-Safety and Efficacy (ERASE) study consisting of short-term infusions of CSL-111 resulted in no statistically significant reductions in percentage change in atheroma volume compared with placebo, but did allow a statistically significant improvement in the plaque characterization index and coronary score.[27]

In clinical situation, only two studies have tested the protective effects of reconstituted high-density lipoproteins in human endotoxemia.[56,57] In one of them, reconstituted high-density lipoprotein infusion dramatically reduced the endotoxin-induced inflammatory response.[56] Moreover, several observational studies, including ours, conducted in septic patients have shown that high-density lipoprotein concentration is low and that these particles are potentially dysfunctional.[21,23–25]

In this context, in acute conditions such as sepsis, high-density lipoprotein supplementation may represent a new therapeutic approach due to its potential to limit inflammation, protect the endothelial barrier, and improve lipopolysaccharide neutralization.

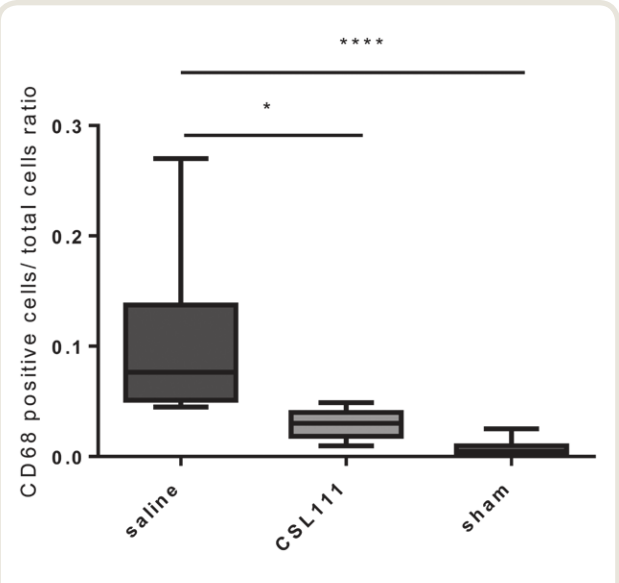

Fig. 9. CD68+ cells to total cells ratio in the lung 24 h after the *Pseudomonas aeruginosa* pneumonia model. n = 3 mice/group. *$P < 0.05$, ****$P < 0.0001$. The data are represented as a box plot.

In conclusion, CSL-111 infusion improved survival in different mouse models of sepsis. In peritonitis and pneumonia models, CSL-111 injection reduced inflammation in both plasma and lung. Mice treated with CSL-111 presented a statistically significant decrease in bacterial count at 24 h after the sepsis in plasma, liver, and lung, and also had less macrophage infiltration in the lung *versus* the saline group conferring to CSL-111 a protective antiinflammatory effect. These results emphasized the key role for high-density lipoproteins in lipopolysaccharide/bacteria neutralization and clearance. Further mechanistic insights are needed before performing a clinical trial using apolipoprotein A1–containing reconstituted high-density lipoproteins in human sepsis.

Acknowledgments

The authors thank Devy Diallo, Ph.D., and Sandrine Delbosc, Ph.D. (French Institute of Health and Medical Research [INSERM] U1148, Paris, France), for their contributions: performing plasma cell-free DNA measurement and RNA isolation and real-time quantitative polymerase chain reaction; Dan Longrois, M.D., Ph.D. (INSERM U1148, Paris, France, and Assistance Publique - Hôpitaux de Paris [AP-HP], Department of Anesthesiology and Critical Care Medicine, Bichat-Claude Bernard Hospital, Paris, France), and Jean-Baptiste Michel, M.D., Ph.D. (INSERM U1148, Paris, France), for their contribution in study conception and design; and Giuseppina Caligiuri, M.D., Ph.D. (INSERM U1148, Paris, France), for performing cytokine and endothelial marker measurements.

Competing Interests

The authors declare no competing interests.

Research Support

This work was supported by Fondation de France, National Agency for Research Grant for Young Researchers (ANR JCJC) 1105, and the Biosecurity in Tropical Environment (BIOST) Federation from the University of Reunion Island. Dr. Tanaka was recipient of a research grant from the French Society of Anesthesia-Resuscitation – Resuscitation Society of French language – National Institute of Health and Medical Research (Société française d'Anesthésie-Réanimation–Société de Réanimation de Langue Française–Institut national de la Santé et de la Recherche Médicale). Dr. Yong-Sang received a research grant from Medical Research Foundation (Fondation pour la Recherche Médicale). Dr. Genève received a research grant from Public Assistance Paris Hospitals (Assistance Publique Hôpitaux de Paris).

Correspondence

Address correspondence to Dr. Meilhac: Université de La Réunion, INSERM, UMR 1188 Diabète athérothombose Réunion Océan Indien, Saint-Denis de La Réunion, France. olivier.meilhac@inserm.fr. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Balk RA: Severe sepsis and septic shock. Definitions, epidemiology, and clinical manifestations. Crit Care Clin 2000; 16:179–92
2. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP: Surviving sepsis campaign: International guidelines for management of sepsis and septic shock: 2016. Crit Care Med 2017; 45:486–552
3. Polderman KH, Girbes AR: Drug intervention trials in sepsis: Divergent results. Lancet 2004; 363:1721–3

4. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC: The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315:801–10
5. Fielding CJ, Fielding PE: Molecular physiology of reverse cholesterol transport. J Lipid Res 1995; 36:211–28
6. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S, Tyroler HA: High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989; 79:8–15
7. Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J: Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009 Jul 22;302(4):412–23
8. Jacobs DR Jr, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA: High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: The follow-up study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol 1990; 131:32–47
9. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM: Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 1991; 353:265–7
10. Miller GJ, Miller NE: Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet 1975; 1:16–9
11. Kitamura A, Iso H, Naito Y, Iida M, Konishi M, Folsom AR, Sato S, Kiyama M, Nakamura M, Sankai T: High-density lipoprotein cholesterol and premature coronary heart disease in urban Japanese men. Circulation 1994; 89:2533–9
12. Wu A, Hinds CJ, Thiemermann C: High-density lipoproteins in sepsis and septic shock: Metabolism, actions, and therapeutic applications. Shock 2004; 21:210–21
13. Theilmeier G, Schmidt C, Herrmann J, Keul P, Schäfers M, Herrgott I, Mersmann J, Larmann J, Hermann S, Stypmann J, Schober O, Hildebrand R, Schulz R, Heusch G, Haude M, von Wnuck Lipinski K, Herzog C, Schmitz M, Erbel R, Chun J, Levkau B: High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury *in vivo* via the S1P3 lysophospholipid receptor. Circulation 2006; 114:1403–9
14. Tran-Dinh A, Diallo D, Delbosc S, Varela-Perez LM, Dang QB, Lapergue B, Burillo E, Michel JB, Levoye A, Martin-Ventura JL, Meilhac O: HDL and endothelial protection. Br J Pharmacol 2013; 169:493–511
15. McDonald MC, Dhadly P, Cockerill GW, Cuzzocrea S, Mota-Filipe H, Hinds CJ, Miller NE, Thiemermann C: Reconstituted high-density lipoprotein attenuates organ injury and adhesion molecule expression in a rodent model of endotoxic shock. Shock 2003; 20:551–7
16. Vesy CJ, Kitchens RL, Wolfbauer G, Albers JJ, Munford RS: Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. Infect Immun 2000; 68:2410–7
17. Wurfel MM, Kunitake ST, Lichenstein H, Kane JP, Wright SD: Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. J Exp Med 1994; 180:1025–35
18. Levine DM, Parker TS, Donnelly TM, Walsh A, Rubin AL: *In vivo* protection against endotoxin by plasma high density lipoprotein. Proc Natl Acad Sci USA 1993; 90:12040–4
19. Dai L, Datta G, Zhang Z, Gupta H, Patel R, Honavar J, Modi S, Wyss JM, Palgunachari M, Anantharamaiah GM, White CR: The apolipoprotein A-I mimetic peptide 4F prevents defects in vascular function in endotoxemic rats. J Lipid Res 2010; 51:2695–705
20. Zhang Z, Datta G, Zhang Y, Miller AP, Mochon P, Chen YF, Chatham J, Anantharamaiah GM, White CR: Apolipoprotein A-I mimetic peptide treatment inhibits inflammatory responses and improves survival in septic rats. Am J Physiol Heart Circ Physiol 2009; 297:H866–73
21. van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP: Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003; 31:1359–66
22. Trinder M, Genga KR, Kong HJ, Blauw LL, Lo C, Li X, Cirstea M, Wang Y, Rensen PCN, Russell JA, Walley KR, Boyd JH, Brunham LR: Cholesteryl ester transfer protein influences high-density lipoprotein levels and survival in sepsis. Am J Respir Crit Care Med 2019; 199:854–62
23. Tanaka S, Labreuche J, Drumez E, Harrois A, Hamada S, Vigué B, Couret D, Duranteau J, Meilhac O: Low HDL levels in sepsis *versus* trauma patients in intensive care unit. Ann Intensive Care 2017; 7:60
24. Chien JY, Jerng JS, Yu CJ, Yang PC: Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. Crit Care Med 2005; 33:1688–93
25. Barlage S, Gnewuch C, Liebisch G, Wolf Z, Audebert FX, Glück T, Fröhlich D, Krämer BK, Rothe G, Schmitz G: Changes in HDL-associated apolipoproteins relate to mortality in human sepsis and correlate to monocyte and platelet activation. Intensive Care Med 2009; 35:1877–85
26. Tanaka S, Diallo D, Delbosc S, Genève C, Zappella N, Yong-Sang J, Patche J, Harrois A, Hamada S, Denamur E,

Montravers P, Duranteau J, Meilhac O: High-density lipoprotein (HDL) particle size and concentration changes in septic shock patients. Ann Intensive Care 2019; 9:68
27. Tardif JC, Grégoire J, L'Allier PL, Ibrahim R, Lespérance J, Heinonen TM, Kouz S, Berry C, Basser R, Lavoie MA, Guertin MC, Rodés-Cabau J; Effect of rHDL on Atherosclerosis-Safety and Efficacy (ERASE) Investigators: Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: A randomized controlled trial. JAMA 2007; 297:1675–82
28. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, Thomas WG, Mukhamedova N, de Courten B, Forbes JM, Yap FY, Kaye DM, van Hall G, Febbraio MA, Kemp BE, Sviridov D, Steinberg GR, Kingwell BA: High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009; 119:2103–11
29. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA: Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009; 4:31–6
30. Deitch EA: Animal models of sepsis and shock: A review and lessons learned. Shock 1998; 9:1–11
31. O'Dwyer MJ, Starczewska MH, Schrenzel J, Zacharowski K, Ecker DJ, Sampath R, Brealey D, Singer M, Libert N, Wilks M, Vincent JL: The detection of microbial DNA but not cultured bacteria is associated with increased mortality in patients with suspected sepsis-A prospective multi-centre European observational study. Clin Microbiol Infect 2017; 23:208.e1–6
32. Sun F, Xiao G, Qu Z: Murine bronchoalveolar lavage. Bio-Protoc 2017; 7(10):e2287
33. Moreira RS, Irigoyen M, Sanches TR, Volpini RA, Camara NO, Malheiros DM, Shimizu MH, Seguro AC, Andrade L: Apolipoprotein A-I mimetic peptide 4F attenuates kidney injury, heart injury, and endothelial dysfunction in sepsis. Am J Physiol Regul Integr Comp Physiol 2014; 307:R514–24
34. Guo L, Ai J, Zheng Z, Howatt DA, Daugherty A, Huang B, Li XA: High density lipoprotein protects against polymicrobe-induced sepsis in mice. J Biol Chem 2013; 288:17947–53
35. Zhang X, Wang L, Chen B: Recombinant HDL (Milano) protects endotoxin-challenged rats from multiple organ injury and dysfunction. Biol Chem 2015; 396:53–60
36. Esmon CT: Why do animal models (sometimes) fail to mimic human sepsis? Crit Care Med 2004; 32(5 Suppl):S219–22
37. Aggarwal NR, King LS, D'Alessio FR: Diverse macrophage populations mediate acute lung inflammation and resolution. Am J Physiol Lung Cell Mol Physiol 2014; 306:L709–25
38. Brinkmann V, Zychlinsky A: Beneficial suicide: Why neutrophils die to make NETs. Nat Rev Microbiol 2007; 5:577–82
39. Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA: Plasma DNA as a prognostic marker in trauma patients. Clin Chem 2000; 46:319–23
40. Margraf S, Lögters T, Reipen J, Altrichter J, Scholz M, Windolf J: Neutrophil-derived circulating free DNA (cf-DNA/NETs): A potential prognostic marker for posttraumatic development of inflammatory second hit and sepsis. Shock 2008; 30:352–8
41. Hotchkiss RS, Nicholson DW: Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol 2006; 6:813–22
42. Saukkonen K, Lakkisto P, Pettilä V, Varpula M, Karlsson S, Ruokonen E, Pulkki K; Finnsepsis Study Group: Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. Clin Chem 2008; 54:1000–7
43. Rhodes A, Wort SJ, Thomas H, Collinson P, Bennett ED: Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. Crit Care 2006; 10:R60
44. Zeerleder S, Zwart B, Wuillemin WA, Aarden LA, Groeneveld AB, Caliezi C, van Nieuwenhuijze AE, van Mierlo GJ, Eerenberg AJ, Lämmle B, Hack CE: Elevated nucleosome levels in systemic inflammation and sepsis. Crit Care Med 2003; 31:1947–51
45. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ: High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995; 15:1987–94
46. Angus DC, van der Poll T: Severe sepsis and septic shock. N Engl J Med 2013; 369:2063
47. Matthay MA, Ware LB, Zimmerman GA: The acute respiratory distress syndrome. J Clin Invest 2012; 122:2731–40
48. Sheu CC, Gong MN, Zhai R, Chen F, Bajwa EK, Clardy PF, Gallagher DC, Thompson BT, Christiani DC: Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. Chest 2010; 138:559–67
49. Freudenberg MA, Bøg-Hansen TC, Back U, Galanos C: Interaction of lipopolysaccharides with plasma high-density lipoprotein in rats. Infect Immun 1980; 28:373–80
50. Gupta H, Dai L, Datta G, Garber DW, Grenett H, Li Y, Mishra V, Palgunachari MN, Handattu S, Gianturco SH, Bradley WA, Anantharamaiah GM, White CR: Inhibition of lipopolysaccharide-induced inflammatory responses by an apolipoprotein AI mimetic peptide. Circ Res 2005; 97:236–43
51. Sewnath ME, Levels HH, Oude Elferink R, van Noorden CJ, ten Kate FJ, van Deventer SJ, Gouma DJ: Endotoxin-induced mortality in bile duct-ligated rats after administration of reconstituted high-density lipoprotein. Hepatol Baltim Md 2000 Dec;32(6):1289–99
52. Moreno JA, Ortega-Gomez A, Rubio-Navarro A, Louedec L, Ho-Tin-Noé B, Caligiuri G, Nicoletti A,

Levoye A, Plantier L, Meilhac O: High-density lipoproteins potentiate α1-antitrypsin therapy in elastase-induced pulmonary emphysema. Am J Respir Cell Mol Biol 2014; 51:536–49

53. Michael Gibson C, Korjian S, Tricoci P, Daaboul Y, Yee M, Jain P, Alexander JH, Steg PG, Lincoff AM, Kastelein JJ, Mehran R, D'Andrea DM, Deckelbaum LI, Merkely B, Zarebinski M, Ophuis TO, Harrington RA: Safety and tolerability of CSL112, a reconstituted, infusible, plasma-derived apolipoprotein A-I, after acute myocardial infarction: The AEGIS-I Trial (ApoA-I Event Reducing in Ischemic Syndromes I). Circulation 2016; 134:1918–30
54. Calkin AC, Drew BG, Ono A, Duffy SJ, Gordon MV, Schoenwaelder SM, Sviridov D, Cooper ME, Kingwell BA, Jackson SP: Reconstituted high-density lipoprotein attenuates platelet function in individuals with type 2 diabetes mellitus by promoting cholesterol efflux. Circulation 2009; 120:2095–104
55. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, Halpern S, Crowe T, Blankenship JC, Kerensky R: Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: A randomized controlled trial. JAMA 2003; 290:2292–300
56. Pajkrt D, Doran JE, Koster F, Lerch PG, Arnet B, van der Poll T, ten Cate JW, van Deventer SJ: Antiinflammatory effects of reconstituted high-density lipoprotein during human endotoxemia. J Exp Med 1996; 184:1601–8
57. Pajkrt D, Lerch PG, van der Poll T, Levi M, Illi M, Doran JE, Arnet B, van den Ende A, ten Cate JW, van Deventer SJ: Differential effects of reconstituted high-density lipoprotein on coagulation, fibrinolysis and platelet activation during human endotoxemia. Thromb Haemost 1997; 77:303–7