Apolipoprotein E Protects Against Bacterial Lipopolysaccharide-induced Lethality

A NEW THERAPEUTIC APPROACH TO TREAT GRAM-NEGATIVE SEPSIS*

Received for publication, October 31, 2000, and in revised form, January 2, 2001
Published, JBC Papers in Press, January 2, 2001, DOI 10.1074/jbc.M009915200

Marijke Van Oosten‡§, Patrick C. N. Rensen‡§, Edwin S. Van Amersfoort‡, Miranda Van Eek‡, Anne-Marie Van Dam†, John J. P. Brevé, Tikva Vogel**, Amos Panet**, Theo J. C. van Berkel‡, and Johan Kuiper‡‡

From the †Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, University of Leiden, Sylvis Laboratories, P. O. Box 9503, 2300 RA Leiden, The Netherlands; the |Research Institute Neurosciences Vrije Universiteit, Faculty of Medicine, Department of Pharmacology, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands; and **Bio-Technology General Ltd., Kiryat Weizmann, Rehovot 76326, Israel

This paper is available on line at http://www.jbc.org

‡ This work was supported by Medical Sciences Grant 902-23-139 from The Netherlands Organization for Scientific Research, Council for Medical Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
§ These authors contributed equally to this work.
¶ Present address: Radiation Genetics and Chemical Mutagenesis, University of Leiden, Sylvis Laboratories, P. O. Box 9503, 2300 RA Leiden, The Netherlands.
†‡ To whom correspondence should be addressed. Tel.: 31 71 5276040; Fax: 31 71 5276032; E-mail: j.kuiper@lacdr.leidenuniv.nl.
† The abbreviations used are: LPS, lipopolysaccharide; apoE, apolipoprotein E; TNF, tumor necrosis factor; i.v., intravenous; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; IL, interleukin.

Septic shock is the most common cause of death in intensive care units and no effective treatment is available at present. Lipopolysaccharide (LPS) is the primary mediator of Gram-negative sepsis by inducing the production of macrophage-derived cytokines. Previously, we showed that apolipoprotein E (apoE), an established modulator of lipid metabolism, can bind LPS, thereby redirecting LPS from macrophages to hepatocytes in vivo. We now report that intravenously administered LPS strongly increases the serum levels of apoE. In addition, apoE can prevent the LPS-induced production of cytokines and subsequent death in rodents. Finally, apoE-deficient mice show a significantly higher sensitivity toward LPS than control wild-type mice. These findings indicate that apoE may have a physiological role in the protection against sepsis, and recombinant apoE may be used therapeutically to protect against LPS-induced endotoxemia.

Sepsis is a syndrome referring to an exaggerated systemic response to infections, which can ultimately lead to death from septic shock. In fact, in the United States the incidence of sepsis has increased during the last decade (1) and sepsis has become the most common cause of death in intensive care units, with 150,000 deaths annually (2, 3). Many cases of sepsis are caused by Gram-negative bacteria (1). Lipopolysaccharide (LPS), a component of the outer membrane of these bacteria, is the primary cause of Gram-negative sepsis and gives rise to the same clinical features as are observed in patients with sepsis (3–6). Within the blood, the lipid A-moiety of LPS binds to both splenic and hepatic macrophages. The effect is caused by a lipoprotein-mediated redirection of LPS to liver parenchymal cells, which results in a reduced production of proinflammatory mediators.

Lipopolysaccharide-induced Lethality

Apolipoprotein E Protects Against Bacterial Lipopolysaccharide-induced Lethality

A NEW THERAPEUTIC APPROACH TO TREAT GRAM-NEGATIVE SEPSIS*

Received for publication, October 31, 2000, and in revised form, January 2, 2001
Published, JBC Papers in Press, January 2, 2001, DOI 10.1074/jbc.M009915200

Marijke Van Oosten‡§, Patrick C. N. Rensen‡§, Edwin S. Van Amersfoort‡, Miranda Van Eek‡, Anne-Marie Van Dam†, John J. P. Brevé, Tikva Vogel**, Amos Panet**, Theo J. C. van Berkel‡, and Johan Kuiper‡‡

From the †Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, University of Leiden, Sylvis Laboratories, P. O. Box 9503, 2300 RA Leiden, The Netherlands; the |Research Institute Neurosciences Vrije Universiteit, Faculty of Medicine, Department of Pharmacology, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands; and **Bio-Technology General Ltd., Kiryat Weizmann, Rehovot 76326, Israel

This paper is available on line at http://www.jbc.org

‡ This work was supported by Medical Sciences Grant 902-23-139 from The Netherlands Organization for Scientific Research, Council for Medical Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
§ These authors contributed equally to this work.
¶ Present address: Radiation Genetics and Chemical Mutagenesis, University of Leiden, Sylvis Laboratories, P. O. Box 9503, 2300 RA Leiden, The Netherlands.
†‡ To whom correspondence should be addressed. Tel.: 31 71 5276040; Fax: 31 71 5276032; E-mail: j.kuiper@lacdr.leidenuniv.nl.
† The abbreviations used are: LPS, lipopolysaccharide; apoE, apolipoprotein E; TNF, tumor necrosis factor; i.v., intravenous; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; IL, interleukin.

Septic shock is the most common cause of death in intensive care units and no effective treatment is available at present. Lipopolysaccharide (LPS) is the primary mediator of Gram-negative sepsis by inducing the production of macrophage-derived cytokines. Previously, we showed that apolipoprotein E (apoE), an established modulator of lipid metabolism, can bind LPS, thereby redirecting LPS from macrophages to hepatocytes in vivo. We now report that intravenously administered LPS strongly increases the serum levels of apoE. In addition, apoE can prevent the LPS-induced production of cytokines and subsequent death in rodents. Finally, apoE-deficient mice show a significantly higher sensitivity toward LPS than control wild-type mice. These findings indicate that apoE may have a physiological role in the protection against sepsis, and recombinant apoE may be used therapeutically to protect against LPS-induced endotoxemia.

Sepsis is a syndrome referring to an exaggerated systemic response to infections, which can ultimately lead to death from septic shock. In fact, in the United States the incidence of sepsis has increased during the last decade (1) and sepsis has become the most common cause of death in intensive care units, with 150,000 deaths annually (2, 3). Many cases of sepsis are caused by Gram-negative bacteria (1). Lipopolysaccharide (LPS), a component of the outer membrane of these bacteria, is the primary cause of Gram-negative sepsis and gives rise to the same clinical features as are observed in patients with sepsis (3–6). Within the blood, the lipid A-moiety of LPS binds to the LPS-binding protein (7, 8), and the resulting complex displays a high affinity for CD14-toll-like receptor 4 (TLR4) complex on mononuclear phagocytes (9, 10). Activation of these cells induces the release of inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukins (IL-1α, IL-1β, and IL-6). These cytokines are responsible for the metabolic and physiologic changes that ultimately lead to pathologic conditions (11–13). The importance of these cytokines in LPS-induced death arises from observations that administration of TNFα or IL-1 to animals provokes a similar reaction as detected after injection of LPS (14–17). In addition, antibodies against TNFα protect monkeys (18–20), rabbits (21), and mice (17) against LPS-induced death. Also, blockade of IL-1 production prevents LPS-induced death of mice (22). Current therapeutic strategies are, therefore, directed against LPS (bacterial/permeability-increasing protein (BPI), antibodies against LPS (23, 24)), cytokines (soluble TNF receptor, anti-TNF antibodies (25)), and receptors (soluble CD14, IL-1 receptor antagonist (26), antibodies against LBP), but the initial clinical data are merely disappointing.

Lipoproteins are suggested to play an important role in the protection against infection and inflammation. All lipoproteins (high density lipoproteins (HDL), low-density lipoproteins (LDL), lipoprotein(a), very-low-density lipoproteins (VLDL), and chylomicrons) can bind endotoxin (27–32) and thereby reduce the toxic properties of LPS. In particular, incubation of VLDL or chylomicrons with LPS before administration to rodents significantly reduces the serum levels of TNFα and protects against endotoxin-induced death (28, 29). The protective effect is caused by a lipoprotein-mediated redirection of LPS from Kupffer cells to parenchymal liver cells (29, 33) and a subsequent secretion of LPS into the bile, where it is inactivated (34). Consequently, macrophages become less activated, which results in a reduced production of proinflammatory mediators.

Triglyceride-rich lipoproteins may be used therapeutically to protect against Gram-negative sepsis or septic shock, but the need for isolation from human lymph or blood impedes their possible application. Within our laboratory, we have developed an emulsion model for chylomicrons from commercially available lipids and human recombinant apoE (35), which is selectively taken up via apoE-specific receptors on liver parenchymal cells. In a rat model, we demonstrated that recombinant chylomicrons can target LPS to liver parenchymal cells, which prevents its binding to both splenic and hepatic macrophages. Furthermore, it was shown that apoE binds LPS directly and alters its metabolic fate, suggesting that apoE in triglyceride-
Cultured in IMDM containing 5% fetal calf serum, 3.02 g/liter NaHCO₃, in short, the IL-6-dependent B9 cells (mouse hybridoma cell line) were injected of LPS, which was preincubated with PBS ( ), apoE-free emulsion ( ), apoE ( ), or apoE-enriched emulsion ( ). Blood samples were taken at the indicated times from the tail vein and allowed to clot for 30 min at room temperature. Serum was screened for its TNFα (A), IL-1β (B), and IL-6 (C) content. Values are the mean ± S.E. of three experiments.

**Materials and Methods**

**Rats and Mice**—Nine to ten-week-old male Wistar rats of mass 260–310 g and 10–12-week old C57Bl/6 mice of mass 21–27 g and apoE-deficient mice (crossed back on a C57Bl/6 background; Refs. 37, 38) were obtained from Broekman Institut BV, Someren, The Netherlands and were fed ad libitum with regular chow (unless otherwise stated).

**Cytokine Determination**—Emulsion was prepared as described earlier (36). Rats were given an intravenous (i.v.) injection of LPS (10 μg/kg) derived from Salmonella minnesota R595 (Re) (List Biological Laboratories Inc., Campbell, CA), which was preincubated with PBS, apoE-free emulsion (20 mg of triglycerides per kg), apoE (800 μg/kg) or apoE-enriched emulsion for 30 min at 37°C. Blood samples were taken up to 150 min after injection from the tail vein and allowed to clot for 30 min at room temperature. Serum samples, obtained after centrifugation at 16,000 × g for 5 min, were screened for their IL-1β, IL-6, and TNFα content.

ApoE-deficient mice (22–27 g) and control mice (22–27 g) were given an intravenous injection of LPS (25 μg/kg body weight). At the indicated time points (up to 180 min), blood samples were taken, and serum was obtained as described above. In the serum samples TNFα levels were determined.

**IL-1β Assay**—Rat IL-1β was detected using a rat IL-1β-specific ELISA. 96-well plates (NUNC MAXISORP) were coated overnight at 4°C with sheep anti-rat IL-1β antibodies in coating buffer (PBS containing 0.14 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, pH 7.2–7.4). Plates were washed three times with wash/dilution buffer (0.5 mM NaCl, 2.5 mM NaH₂PO₄, 1.4 mM Na₂HPO₄, 0.1% Tween 20, pH 7.2). The plates were incubated overnight at 4°C with serum samples and recombinant rat IL-1β (diluted in normal rat serum) as a standard. The plates were washed and subsequently incubated with biotinylated sheep anti-rat IL-1β for 1 h at room temperature. Plates were washed again and streptavidin/polyhorseradish peroxidase was added. After 30 min at room temperature, plates were washed and 1,2-β-phenylenediamine dihydrochloride was added, and plates were left for 15 min at room temperature. The reaction was stopped by addition of 1 M H₂SO₄, and the absorbance was measured at 495 nm.

**IL-6 Bioassay**—IL-6 was detected as described by Aarden et al. (39). In short, the IL-6-dependent B9 cells (mouse hybridoma cell line) were cultured in IMDM containing 5% fetal calf serum, 3.02 μl/g NaHCO₃, 0.1% β-mercaptoethanol, penicillin/streptavidin, and 30 units/ml human recombinant IL-6 (Central Laboratory for Blood research (CLB), Amsterdam, The Netherlands). For detection of IL-6, B9 cells (5 × 10⁵ cells/well) and dilutions of serum samples were added to 96-well plates in a total volume of 200 μl/well. Human IL-6 was used as a standard. Cells were incubated for 72 h at 37°C, 5% CO₂. After this period [³H]thymidine (125 nCi/well) was added, and the cells were incubated for another 4 h at 37°C, 5% CO₂. Cells were harvested on a glass filter, and [³H]radioactivity was counted using a Wallac Microbeta plus LCC. 

**TNFα Assay**—TNFα content of the serum samples was determined using a commercially available ELISA kit for rat or mouse TNFα (Immunosource, Zoersel-Halle, Belgium). The assay was performed according to the manufacturer’s instructions.

**Mortality**—Mice received an intraperitoneal injection of 20 mg of β-galactosamine. Subsequently, they were injected i.v. with LPS (150 ng/kg), which was preincubated for 30 min at 37°C with PBS or apoE (25 μg/kg). During the 72-h period after injection, the survival was determined, after which no further loss of animals occurred.

ApoE ELISA and Cholesterol Determination—Mice were injected with PBS or LPS (100 μg/kg). At different time points after injection, blood samples were taken from the tail vein and allowed to clot for 30 min. Serum was obtained by centrifugation for 5 min at 16,000 × g and screened for its apoE content, using a mouse apoE-specific ELISA as described (36), and total cholesterol using a commercial enzymatic kit from Roche Molecular Biochemicals (Mannheim, Germany).

**Western Blot**—Sera were obtained as described above. Proteins within the sera (1-μl aliquots) were separated by 10% SDS-polyacrylamide gel electrophoresis under reducing conditions and blotted onto nitrocellulose membrane in a buffer containing 25 mM Tris, 20% methanol, 192 mM glycine, and 0.02% SDS. Blots were immunoblotted with rabbit anti-mouse apoE antibody and visualized by enhanced chemiluminescence, essentially as described (40).

**Statistical Analysis**—The n value is indicated for each experiment. Statistical differences in cytokine and apoE production were determined using a two-tailed Student’s t test. Statistical differences in survival curves among the groups of mice were analyzed by log rank test. For both analyses, Graphpad software (Prism and Instat) was used.

**Results**

**Effect of apoE on LPS-induced Cytokine Levels**—To determine the effect of apoE on LPS-induced proinflammatory cytokine levels, rats were injected with LPS in the absence or presence of apoE and/or emulsion, and the cytokine levels were determined up to 4 h after injection. Injection of LPS resulted in a strong induction of TNFα, IL-1β, and IL-6 in serum reaching peak levels at 60, 90, and 120 min after LPS administration, respectively (Fig. 1). Injection with PBS alone had no effect on cytokine levels (not shown). Administration of the apoE-enriched emulsion together with LPS largely inhibited the LPS-induced maximum levels of TNFα, IL-1β, and IL-6.
mice and control mice were injected with a sublethal dose of LPS, which was preincubated with PBS (○) or apoE (●). Survival was determined during the 72-h period after injection, after which no further loss of animals occurred (n = 7). A Kaplan-Meier survival curve is shown. Asterisk indicates a significant difference (p = 0.022) between the LPS-treated animals and the LPS-apoE-treated animals.

80%, 91%, and 98%, respectively (p < 0.05, Fig. 1). In fact, apoE alone inhibited the LPS-induced maximum serum levels of these cytokines (p < 0.05) to a similar extent (68%, 99%, and 99%) as the apoE-enriched emulsion. The effects observed with free and emulsion-associated apoE were not significantly different, suggesting that the apoE moiety of the recombinant chylomicrons is responsible for the detoxification of LPS. In agreement with this assumption, the apoE-free emulsion did not significantly reduce the LPS-induced cytokine levels (Fig. 1).

Effect of apoE on LPS-induced Lethality—The proinflammatory cytokines TNFα and IL-1 may both play an important role in the fatal outcome of Gram-negative sepsis. Inhibition of the LPS-induced serum levels of these cytokines (p < 0.05) to a similar extent (68%, 99%, and 99%) as the apoE-enriched emulsion. The effects observed with free and emulsion-associated apoE were not significantly different, suggesting that the apoE moiety of the recombinant chylomicrons is responsible for the detoxification of LPS. In agreement with this assumption, the apoE-free emulsion did not significantly reduce the LPS-induced cytokine levels (Fig. 1).

Effect of apoE on LPS-induced Lethality—The proinflammatory cytokines TNFα and IL-1 may both play an important role in the fatal outcome of Gram-negative sepsis. Inhibition of the LPS-induced serum levels of these cytokines (p < 0.05) to a similar extent (68%, 99%, and 99%) as the apoE-enriched emulsion. The effects observed with free and emulsion-associated apoE were not significantly different, suggesting that the apoE moiety of the recombinant chylomicrons is responsible for the detoxification of LPS. In agreement with this assumption, the apoE-free emulsion did not significantly reduce the LPS-induced cytokine levels (Fig. 1).

ApoE-deficient (○) and control mice (●) were injected intravenously with LPS (25 μg/kg). At the indicated time points, blood samples were taken and TNFα levels were determined by ELISA. Data indicate the mean ± S.E. (n = 5). Asterisk indicates a significant difference between LPS-treated apoE-deficient mice and LPS-treated control mice (p < 0.05).
with ELISA (Fig. 4C). The intensity of the apoE band was increased at 12 h after the injection of LPS, whereas in the controls a slight reduction of the apoE staining was observed. The antibody reacted solely with a single 34-kDa protein band in the serum of fasted C57Bl/6J mice that were injected with PBS or LPS. This band could not be detected in the serum of apoE-deficient mice, which accounts for the specific staining of apoE.

**DISCUSSION**

The present work demonstrates that LPS strongly increases the endogenous serum level of apoE in a rodent model. The mechanism by which apoE is increased may involve either de novo protein synthesis as induced by LPS and/or cytokines or release from existing intra- and extracellular pools. It is likely that a continuous LPS stimulus may provoke a long-term elevation of serum apoE levels. Indeed, septic patients demonstrated that, although these patients show a severe hypocholesterolemia, both the HDL and LDL fractions were largely enriched in apoE (43), whereas a single LPS-challenge increased the apoE content of HDL in African green monkeys (44).

In addition, we observed that exogenous apoE decreased the LPS-induced production of proinflammatory cytokines, probably by decreasing the release of cytokines by macrophages. These data are in full accordance with our earlier findings that free and emulsion-bound apoE, but not the emulsion alone, altered the in vivo kinetics of radioiodinated LPS and largely decreased the association of LPS with macrophages (36). The interference of apoE with the metabolic fate of LPS is not only accompanied by a strong inhibition of the cytokine levels in serum, but also protects against LPS-induced death, indicating that the reduction in cytokine levels is followed by a reduction in mortality. The absence of apoE from the serum (apoE-deficient mice) led to a 2-fold higher sensitivity of the mice for treatment with LPS than control mice, because in the absence of apoE, a 2-fold increase in TNFα levels was observed. This is surprising because apoE-deficient mice have 8-fold higher cholesterol levels than control mice, but the absence of apoE from the lipoproteins apparently leads to an inability to neutralize LPS. These data are in agreement with very recent data of de Bont et al. (45), who showed that apoE-deficient mice produce significantly more TNFα in response to LPS than control mice, and the mortality after injection of LPS was significantly higher in apoE-deficient mice than in control mice. The levels of IL-1 and IL-6 however were similar in apoE-deficient and control mice in response to LPS.

Until recently, apoE has been assigned a classical antiatherogenic role in lipid metabolism (46). Recent data, however, indicate that only 2–10% of the endogenous apoE serum level in rodents is sufficient for the maintenance of cholesterol homeostasis (38, 47). These data imply that serum apoE may have other functions that are unrelated to lipid metabolism. Indeed, initial data do indicate that apoE may have immunomodulatory functions (48–51) and that apoE may also influence the extension of neurites in the brain (50). The present observations strongly suggest that we have now identified a protective role of apoE in Gram-negative sepsis.

In conclusion, we postulate that in severe Gram-negative bacterial infection, a physiological increase in endogenous apoE forms a defense mechanism against the development of sepsis. If, withstanding this protection mechanism, the infection is not adequately neutralized, administration of exogenous apoE may be of highly therapeutic significance to overcome failure of this endogenous defense mechanism.

**Acknowledgment**—We thank Dr. N. Pearce of SmithKline Beecham Pharmaceuticals, Harlow, UK for kindly providing us with the apoE antibody.

**REFERENCES**

1. Glaser, M. P., Zanetti, G., Baumgartner, J. D., and Cohen, J. (1991) *Lancet* 338, 732–736.
2. Parillo, J. E. (1990) *Ann. Intern. Med.* 113, 227–242.
3. Parillo, J. E. (1993) *Am. J. Respir. Crit. Care Med.* 147, 1471–1477.
4. Raetz, C. H. R. (1990) *Annu. Rev. Biochem.* 59, 129–170.
5. Raetz, C. H. R., Ulevitch, R. J., Wright, S. D., Sibley, C. H., Ding, A. D., and Nathan, C. F. (1991) *FASEB J.* 5, 2652–2660.
6. Riechel, E. T., Kirikae, T., Schade, F. U., Mamat, U., Schmidt, G., Leppnow, H., Ulmer, A. J., Zahringer, U., Seydel, U., and Di Padova, F. (1994) *FASEB J.* 8, 217–225.
7. Tobias, P. S., Soldau, K., and Ulevitch, R. J. (1989) *J. Biol. Chem.* 264, 10867–10871.
8. Gallay, P., Heumann, D., Le Roy, D., Barrantes, C., and Glaser, M. P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 9935–9938.
9. Beutler, B. (2000) *Curr. Opin. Microbiol.* 3, 23–28.
10. Poltorak, A., He, X. L., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., and Beutler, B. (1998) *Science* 282, 2085–2088.
11. Waage, A., Brandtzaeg, P., Halstensen, A., Kierulf, P., and Espevik, T. (1987) *Lancet* 1, 8823.
12. Chensue, S. W., Terebuh, P. D., Remick, D. G., Scales, W. E., and Kunkel, S. L. (1996) *Annu. Rev. Med.* 47, 2085–2088.
13. Henderson, B., Poole, S., and Wilson, M. (1996) *Microbiol. Rev.* 60, 316–334.
14. Tracey, K. J., and Cerami, A. (1994) *Annu. Rev. Med.* 45, 491–503.
15. Dinarello, C. A. (1994) *Adv. Pharmacol.* 25, 21–51.
16. Dinarello, C. A. (1996) *Blood* 87, 2095–2147.
17. Heumann, D., Le Roy, D., and Glaser, M. P. (1996) *J. Endotoxin Res.* 3, 87–92.
18. Tracey, K. J., Fong, Y., Hesse, D. G., Manogue, K. R., Lee, A. T., Kuo, G. C., Lowry, S. F., and Cerami, A. (1987) *Nature* **330**, 662–664
19. Hinshaw, L. B., Tekamp-Olson, P., Chang, A. C. K., Lee, P. A., Taylor, Jr., F. B., Corder, C. R., Peet, T. C., Emerson, T. E., Passey, R. B., and Kuo, G. C. (1990) *Circ. Shock* **30**, 279–292
20. Fiedler, V. B., Loof, I., Sander, E., Voehringer, V., Galanos, C., and Fournel, M. A. (1991) *J. Lab. Clin. Med.* **120**, 574–588
21. Mathison, J. C., Wolfson, E., and Ulevitch, R. J. (1988) *J. Clin. Invest.* **81**, 1925–1937
22. Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., and Salfeld, J. (1995) *Cell* **80**, 401–411
23. Bone, R. C., Balk, R. A., Fein, A. M., Perl, T. M., Wenzel, R. P., Reines, H. D., Quenzer, R. W.; Iberti, T. J., Macintyre, N., and Schein, R. M. (1995) *Crit. Care Med.* **23**, 994–1005
24. Giroir, B. P., Quint, P. A., Barton, P., Kirsch, E. A., Kitchen, L., Goldstein, B., Nelson, B. J., Wedel, N. J., Carroll, S. F., and Scannon, P. J. (1997) *Lancet* **350**, 1439–1443
25. Abraham, E., Wunderink, R., Silverman, H., Perl, T. M., Nasraway, S., Levy, H., Bone, R., Wenzel, R. P., Balk, R., and Allred, R. (1995) *J. Am. Med. Assoc.* **273**, 934–941
26. Fisher, Jr., C. J., Dhainaut, J. F., and Opal, S. M. (1994) *J. Am. Med. Assoc.* **271**, 1836–1843
27. Van Lenten, B. J., Fogelman, A. M., Haberland, M. E., and Edwards, P. A. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 2704–2708
28. Harris, H. W., Grunfeld, C., Feingold, K. R., and Rapp, J. H. (1990) *J. Clin. Invest.* **86**, 696–702
29. Harris, H. W., Grunfeld, C., Feingold, K. R., Read, T. E., Kane, J. P., Jones, A. L., Eichbaum, E. B., Bland, G. F., and Rapp, J. H. (1993) *J. Clin. Invest.* **91**, 1028–1034
30. Fink, W. A., Baumstark, M. W., Weinstock, C., Berg, A., and Northoff, H. (1993) *Infect. Immun.* **61**, 5140–5146
31. Parker, T. S., Levine, D. M., Chang, J. C. C., Laxer, J., Coffin, C. C., and Rubin, A. L. (1995) *Infect. Immun.* **63**, 253–258
32. Netea, M. G., de Bont, N., Demacker, P. N. M., Verschueren, I., Kullberg, B. J., van Dijk, K. W., van der Meer, W. J., Stalenhoef, A. F., and van Berkel, T. J. C. (1998) *J. Clin. Invest.* **101**, 221–225
33. Rensen, P. C. N., van Oosten, M., van der Heuvel, E. C., Hijstuberbensch, M. K., and Van Berkel, T. J. C. (1999) *J. Clin. Invest.* **104**, 221–225
34. Harris, H. W., Rockey, D. C., and Chau, P. (1998) *Hepatology* **27**, 1341–1348
35. Read, T. E., Harris, H. W., Grunfeld, C., Feingold, K. R., Calhoun, M. C., Kane, J. P., and Rapp, J. H. (1993) *Infect. Immun.* **61**, 3496–3502
36. Rensen, P. C. N., Van Dijk, M. C. M., Havenaar, E. C., Bijsterbosch, M. K., and Van Berkel, T. J. C. (1995) *J. Clin. Invest.* **95**, 1028–1034
37. Rensen, P. C. N., van Oosten, M., van de Bilt, E., Havekes, L. M., and Helfker, M. H. (1994) *Atherosclerosis* **111**, 25–37
38. Van Eck, M., Herijgers, N., Yates, J., Pearse, N. J., Hoogerbrugge, P. M., Groot, P. H. E., and Vanberkel, T. J. C. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17**, 3117–3126
39. Aarden, L. A., De Groot, E. R., Schaap, O. L., and Lansdorp, P. M. (1997) *Eur. J. Immunol.* **17**, 1411–1416
40. Van Velzen, A. G., Da Silva, P. P., Gordon, S., and Van Berkel, T. J. C. (1997) *Biochem. J.* **322**, 411–415
41. Galanos, C., Freudenberg, M. A., and Reutter, W. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5939–5943
42. Ulevitch, R. J., Johnston, A. R., and Weinstein, D. B. (1979) *J. Clin. Invest.* **64**, 1516–1524
43. Fraunherger, P., Parhofer, K. G., Cremer, P., Gerling, A., Siegle, B., Walli, A. K., and Seidel, D. (1997) *Atherosclerosis* **134**, 349 (abstr.)
44. Auerbach, B. J., and Parks, J. S. (1989) *J. Biol. Chem.* **264**, 10264–10270
45. De Bont, N., Netea, M. G., Demacker, P. N. M., Verschueren, I., Kullberg, B. J., van Dijk, K. W., van der Meer, W. J., Stalenhoef, A. F. (1999) *J. Lipid Res.* **40**, 660–665
46. Mahley, R. W. (1988) *Science* **240**, 622–630
47. Linton, M. F., Atkinson, J. B., and Fazio, S. (1995) *Science* **267**, 1034–1037
48. Mistry, M. J., Clay, M. A., Kelly, M. E., Steiner, M. A., and Harmony, J. A. (1995) *Cell Immunol.* **160**, 14–23
49. Roselaar, S. E., and Daugherty, A. (1997) *Circulation* **96**, I-347 (abstr.)
50. Laskowitz, D. T., Goel, S., Bennett, E. R., and Matthew, W. D. (1997) *J. Neuroimmunol.* **76**, 70–74
51. Laskowitz, D. T., Matthew, W. D., Bennett, E. R., Schmechel, D., Herbstreith, M. H., Goel, S., and McMillian, M. K. (1998) *Neuroreport* **9**, 615–618