Bacterial Peptidoglycan-associated Lipoprotein Is Released into the Bloodstream in Gram-negative Sepsis and Causes Inflammation and Death in Mice

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Gram-negative bacterial sepsis commonly causes organ dysfunction and death in humans. Although circu-

lated bacterial toxins trigger inflammation in sepsis, little is known about the composition of bacterial products released into the blood during sepsis or the contribution of various bacterial components to the pathogenesis of sepsis. We have shown that diverse Gram-negative bacteria release bacterial peptidoglyc-

an-associated lipoprotein (PAL) into serum. The present studies explored release of PAL into the blood dur-

ing sepsis and tested the hypothesis that PAL contributes to bacterial virulence and inflammation in Gram-negative sepsis. Released PAL was detected in the blood of 94% of mice following cecal ligation and puncture. Picomolar to nanomolar levels of PAL stimulated macrophages and splenocytes from lipopolysaccharide-

hyporesponsive (C3H/HeJ) mice. Injection of PAL into C3H/HeJ mice stimulated production of serum cyto-

kines and increased pulmonary and myocardial expression of inflammatory markers. PAL caused death in sen-

sitized C3H/HeJ mice. Mutant Escherichia coli bacteria with reduced levels of PAL or truncated PAL were less virulent than wild-type bacteria, as indicated by higher survival rates and lower circulating levels of interleukin-6 and bacteria in a model of peritonitis in lipopolysaccharide-responsive mice. The studies suggest that PAL may be an important bacterial mediator of Gram-nega-

tive sepsis.

Gram-negative sepsis (GNS) is a devastating consequence of Gram-negative bacterial infection that frequently causes severe respiratory failure and cardiovascular dysfunction and is a common cause of death in hospitalized patients (1–3). In sepsis, interactions between microorganisms and host cells trigger inflammatory responses that include release of soluble mediators such as cytokines and nitric oxide, expression of cell surface receptors and adhesion molecules, and recruitment of inflammatory cells into organs (4–9). The similarity in these responses caused by microorganisms as diverse as Gram-nega-

tive and Gram-positive bacteria, fungi, and viruses suggests that multiple microbial components may stimulate common inflammatory signaling pathways and contribute to the pathogene-

sis of sepsis. The concept that multiple bacterial factors are active in sepsis is supported by studies indicating that multiple bacterial components, including lipopolysaccharide (LPS) (10, 11), lipoteichoic acid and peptidoglycan (12, 13), several outer membrane proteins (14–18), flagellin (19), and several lipoproteins (20–22), activate common inflammatory responses. These bacterial products signal through different Toll-like receptors (TLRs) (21–29) and nuclear factor-κB (30).

Although LPS from Gram-negative bacteria has been shown to circulate in GNS and to stimulate inflammation, little is known about the composition of bacterial products that are released into the blood during GNS or the contribution of different Gram-negative bacterial products to the pathogenesis of GNS. Previous studies indicated that antiserum raised to the rough mutant strain Escherichia coli J5 (J5 antiserum) improved survival in experimental GNS and in humans with GNS (31–33). Although J5 antiserum was believed to protect through anti-LPS antibodies, our prior studies indicate that J5 antiserum contains high titers of IgGs that bind bacterial peptido
glycan-associated lipoprotein (PAL), suggesting that anti-

PAL IgG may also have contributed to the protection (34–37). We have also found that PAL is released into human serum by heterologous Gram-negative bacteria in vitro and into the blood of burned rats with E. coli sepsis and that a proportion of PAL is released in fragments that also contain LPS and additional outer membrane proteins (36–39).

PAL is the product of the ExC gene located at 17 min on the E. coli map and is part of the system of cytoplasmic membrane, periplasmic and outer membrane proteins involved in maintaining cell wall integrity (40–44). The precursor protein is composed of 173 amino acids. During posttranslational processing, a 21-amino acid N-terminal signal sequence is removed, and glyc eride and fatty acid groups are added to the N-terminal cysteine (45–47). PAL mutants that either lack PAL or have abnormal PAL are hypersensitive to detergents (42). There is a high degree of homology in PAL among enteric and nonenteric Gram-negative bacteria (44, 48–51).

The present studies were performed to explore the release of PAL into the blood in a cecal ligation and puncture (CLP) model of polymicrobial sepsis in mice and to test the hypotheses that...
PAL has inflammatory effects and may contribute to bacterial virulence during GNS. Released PAL was present in the blood of 94% of mice following CLP. Low concentrations of PAL stimulated splenocytes and macrophages in vitro and stimulated multiple responses in vivo, including induction of cytokines in the blood, transcription of proinflammatory pulmonary and myocardial genes, and death in sensitized mice. Survival was higher, and circulating levels of IL-6 and bacteria were lower in mice infected with PAL mutant versus wild-type bacteria in a peritonitis model of GNS. These data indicate that PAL is released by Gram-negative bacteria into the bloodstream during polymicrobial sepsis, that PAL induces inflammation, and that PAL may be an important factor in the development and severity of GNS induced by Gram-negative bacterial peritonitis.

**EXPERIMENTAL PROCEDURES**

**Bacteria**— *E. coli* K12 strains p400, CH202 (PAL-deficient mutant of p400), and CH202/pRc2 (PAL-restored derivative of CH202) were provided by U. Henning (Max-Planck-Institut für Biologie, Tübingen, Germany) (46). *E. coli* K12 strains JC1192 (excC−, PAL wild-type) and JC2721 (1129 excC892 [nonviable PAL-null phenotype] nadhA−:Tn10) were provided by J.-C. Lazzaroni (Université Claude Bernard, Lyon, France) (40, 42). Bacteria were cultured in L-broth (Difco, Detroit, MI). Kanamycin was added to media during growth of CH202/pRc2 for maintenance of the plasmid.

**Animals**—The Institutional Animal Care and Use Committee at the Massachusetts General Hospital approved the animal studies. Release of PAL was studied in 20–25-g C57/HeJmice (Jackson Laboratories, Bar Harbor, ME) and C57/HeN mice (Charles River Laboratories, Wilmington, MA). Inflammatory and lethal effects of purified PAL were studied in 20–25-g C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) and C3H/HeN mice (Charles River Laboratories, Wilmington, MA). Inflammatory and lethal effects of purified PAL were studied in 20–25-g CSH/HeJ mice (Jackson Laboratories, Bar Harbor, ME) and C3H/HeN mice (Charles River Laboratories, Wilmington, MA). Inflammatory and lethal effects of purified PAL were studied in 20–25-g CSH/HeJ mice (Jackson Laboratories, Bar Harbor, ME) and C3H/HeN mice (Charles River Laboratories, Wilmington, MA).

**Antibodies**—Mouse monoclonal anti-PAL IgG (mouse anti-PAL IgG) was produced as described previously (37, 38). Rabbit polyclonal anti-PAL IgG (rabbit anti-PAL IgG) was prepared by immunizing rabbits with purified PAL in incomplete Freund’s adjuvant. Immunoblot analysis revealed that anti-PAL-specific IgGs do not react with components of mouse serum.

**PAL Release in the CLP Model of Sepsis**—PAL release was studied in a CLP model of sepsis as described by Wichterman and others (52, 53). Mice were anesthetized and given a 1-ml subcutaneous normal saline injection, and serum was prepared and analyzed for TNF-α, IL-1β, and IL-6 by enzyme-linked immunosorbent assay (ELISA) (54). BALB/c mice were sensitized with a 10 mg/kg intrasplenic injection of PAL or an equivalent volume of carrier (50 mg sodium phosphate, pH 7.4). At specified time points, mice were euthanized, and blood and organs were collected. Blood was obtained by cardiac puncture up to 16 h after injection, and serum was prepared and analyzed for TNF-α, IL-1β, and IL-6 by enzyme-linked immunosorbent assay (n = 5–8/group).

**Lungs and hearts (myocardia) were collected up to 4 h after injection and homogenized in guanidine thiocyanate and centrifugation on a CsCl cushion (57). The murine TNF-α probe was generated by Bruce Beutler (University of Texas Southwestern Medical Center, Dallas, TX). The IL-1β probe was made using polymerase chain reaction, sense (5′-ataagacgccagccacca-3′) and antisense primers (5′-ccacgcagttgacagc-3′), and cDNA generated from reverse transcription using 2 μg of RNA isolated from PAL-treated mouse lungs, Moloney murine leukemia virus reverse transcriptase, RNasin, and the Moloney murine leukemia virus reverse transcriptase primer, as described previously (58). The ICAM probe was made using H1M1 and a murine expressed sequence tag fragment cloned into pBluescript SK (American Type Culture Collection). To confirm that equal quantities of RNA were analyzed, the blots were stripped of the labeled cDNA probes and then hybridized with a 15 nt excess of an oligonucleotide corresponding to rat 18 S ribosomal RNA (5′-agctatgttggctgac-3′) that had been end-labeled with [γ-32P]ATP and T4 polynucleotide kinase (59).

**Lung MPO levels** were measured 4 h after injection of PAL (n = 6/group), essentially as described previously (60, 61). The right lung was weighed, homogenized in 50 mM sodium phosphate (pH 7.4), and centrifuged (12,000 × g). Lung homogenates were resuspended in 0.5 ml of hexadecyl-trimethyl-ammonium bromide (Sigma) in 50 mM sodium phosphate (pH 6.2), homogenized, sonicated, freeze-thawed three times, and sonicated again. The solution was centrifuged, and MPO levels were measured in the supernatants (62). Twenty μl of the supernatant was mixed with 200 μl of the extraction buffer.
Wild-type control (H11005) blood cultures from the PAL mutant peritonitis experiments were antagonized with purified PAL or carrier. Lung MPO data and quantitative test was used to assess serum cytokine levels over time in mice statistical analysis because mortality, IL-6 levels, and degree of bacteremia were not considered statistically significant. In the figures, * and ** signify p values of <0.05, <0.01, and <0.001, respectively.

RESULTS

PAL Circulates in Mice after CLP—The CLP model approximates human sepsis resulting from intestinal disruption (52, 53). PAL was affinity-purified from sterile-filtered plasma collected from CLP and sham mice using mouse anti-PAL IgG and detected by immunoblotting with rabbit anti-PAL IgG. A band with a molecular mass of 19–19 kDa, which is identical to results obtained by others (13, 19).

Inflammatory and Lethal Effects of PAL Mutants in E. coli Peritonitis and Sepsis—E. coli K12 wild-type (p400 and JC1129) and PAL mutant (CH202 and JC7271) strains were grown to mid-log phase and washed in sterile normal saline, and the optical density at 550 nm was adjusted to 0.8 using normal saline. Bacteria were then pelleted and resuspended in normal saline at one-twentieth the original volume, and quantitative cultures were performed to ensure that mice received comparable amounts of wild-type and PAL mutant bacteria. Live bacteria were administered to C3H/HeN mice by intraperitoneal injection. Bacterial doses were 3.7 × 10^6 (± 1.9 × 10^6) and 3.4 × 10^6 (± 7.3 × 10^6) CFU for the PAL-deficient strain and its progenitor wild-type strain, respectively, and 1.7 × 10^6 (± 5.9 × 10^6) and 1.7 × 10^6 (± 7.4 × 10^6) CFU for the PAL nonsense strain and its progenitor wild-type strain, respectively. Blood was collected via the tail artery at 20 h, and IL-6 levels were measured, and quantitative blood cultures were performed. Survival was followed for 72 h. Data were compiled from three separate experiments for each PAL mutant strain (n = 18/group) and paired wild-type control (n = 12–18/group). The data from the two wild-type E. coli K12 strains were combined into one group for the figures and for statistical analysis because mortality, IL-6 levels, and degree of bacteremia were not statistically different between these groups (final wild-type n = 30).

Statistical Analysis—Representative data from at least three experiments are presented in the figures. Error bars in the figures represent one-half of the material that was affinity-purified from 250 ml of 0.1% hydrogen peroxide in 50 mM sodium phosphate (pH 6.2), and absorption was measured at a wavelength of 450 nm (reference, 620 nm) after 15 min. MPO concentrations (units/g lung) were determined using human MPO standards (Sigma).

PAL-induced Mortality in D-Galactosamine-sensitized Mice—Lethal effects of PAL were studied in D-galactosamine-sensitized mice (n = 7–8 mice/group) (63). D-Galactosamine (800 mg/kg) was administered to mice by intraperitoneal injection 5 min before intravenous injection of 1, 10, and 100 μg of PAL or an equivalent volume of carrier. Survival was assessed up to 96 h after treatment.

PAL Circulates in Sepsis and Causes Inflammation and Death (Fig. 2), and co-incubation of PAL with polymyxin B (5 μg/ml) did not attenuate the proliferative response to PAL (data not shown).

PAL Increases the Production of TNF-α and IL-6 but not IL-1β by Cultured Peritoneal Macrophages—PAL caused a dose-dependent increase in production of TNF-α and IL-6 by macrophages at PAL concentrations ≥ 5 ng/ml (280 pm) (p < 0.001, Fig. 3, A and B). IL-1β was not detected when macrophages were treated with up to 5 μg/ml PAL (data not shown). TNF-α and IL-6 were not detected when macrophages were treated with LPS at concentrations up to 100 ng/ml (≥ 50 -fold in excess of the LPS in the purified PAL) and polymyxin B (5 μg/ml) did not inhibit macrophage responses to PAL (data not shown).

PAL Induces Nitrite Production by Cultured Peritoneal Macrophages in the Presence of IFN-γ—Expression of inducible nitric oxide synthase is up-regulated during sepsis. This may increase nitric oxide production and contribute to vasodilatation and shock (65, 66). Accumulation of nitrite, a marker of nitric oxide production, was measured in macrophage supernatants. Although neither PAL (up to 5 μg/ml) nor IFN-γ (10 units/ml) alone increased nitrite levels, nitrite levels were increased by the combination of ≥500 pg/ml PAL and IFN-γ (p < 0.001; Fig. 3C). The requirement for IFN-γ for PAL-induced nitrite production observed in these studies is consistent with results obtained by others (13, 19).
were analyzed by RNA blot hybridization 1, 2, and 4 h after administration of PAL. TNF-α (A) and IL-6 (B) levels were measured in culture supernatants at 18 h. C, macrophages were incubated with dilutions of PAL plus IFN-γ (10 units/ml). Nitrite levels were measured in supernatants at 18 h. Nitrite was not detected in supernatants of macrophages incubated with PAL alone or IFN-γ alone.

**PAL Increases MPO Levels in Lungs**—Levels of MPO, a neutrophil granule protein, were quantitated to assess infiltration and/or activation of neutrophils in the lung. MPO levels were nearly 6-fold higher in PAL-treated mice (mean, 39.9 units/g; range, 7.4–111.7 units/g) than in carrier-treated mice (mean, 7.4 units/g; range, 2.1–11.4 units/g) (p < 0.007).

**PAL Causes Death in D-Galactosamine-sensitized Mice**—The number of mice surviving was determined up to 96 h after intravenous injection with either carrier or PAL (10 μg), fractionated using formaldehyde-agarose gel electrophoresis, and transferred to charged membranes. After the membranes were exposed to 32P-labeled restriction fragments of specific cDNAs that encode murine TNF-α, IL-1β, and ICAM-1, they were washed at high stringency and subjected to autoradiography. After stripping the membranes with formamide, rehybridization with an excess of 32P-end-labeled 18 S oligonucleotide and autoradiography confirmed equal RNA loading. A representative RNA blot from one of three experiments is shown.

**PAL Mutants Are Less Virulent than Wild-type Controls during E. coli Peritonitis**—Two different PAL mutants were used for these experiments. The PAL-deficient strain contains markedly reduced levels of PAL by immunoblotting with anti-PAL IgG (37). The PAL nonsense strain contains a mutation in the PAL gene with a stop codon that results in production of a truncated protein (42). Overall survival was markedly increased in the PAL mutant groups versus the wild-type controls.

**fig. 3.** PAL increases cytokine and nitrite production by C3H/HeJ thioglycollate-elicited peritoneal macrophages. A and B, macrophages were incubated with dilutions of PAL. TNF-α (A) and IL-6 (B) levels were measured in culture supernatants at 18 h. C, macrophages were incubated with dilutions of PAL plus IFN-γ (10 units/ml). Nitrite levels were measured in supernatants at 18 h. Nitrite was not detected in supernatants of macrophages incubated with PAL alone or IFN-γ alone.

**fig. 4.** PAL induces serum cytokines in C3H/HeJ mice. Carrier (☐) or PAL (10 μg; □) was injected intravenously at t = 0, and TNF-α (A), IL-6 (B), and IL-1β (C) levels were measured in sera at the specified time points.

**fig. 5.** PAL modulates cytokine and adhesion molecule mRNA levels in lungs and hearts (myocardia) of C3H/HeJ mice. RNA was extracted from the lungs and myocardia 1, 2, and 4 h after intravenous injection with either carrier or PAL (10 μg), fractionated using formaldehyde-agarose gel electrophoresis, and transferred to charged membranes. After the membranes were exposed to 32P-labeled restriction fragments of specific cDNAs that encode murine TNF-α, IL-1β, and ICAM-1, they were washed at high stringency and subjected to autoradiography.
Fig. 6. PAL causes death in N-galactosamine sensitized C3H/HeJ mice. Mice were sensitized by intraperitoneal injection of N-galactosamine 5 min before intravenous injection of carrier or PAL at the doses indicated. Survival was followed over 96 h. (Fig. 7A). Survival was 7% in the wild-type group as compared with 33% and 100% in the PAL-deficient and PAL nonsense groups, respectively (p < 0.001). Plasma IL-6 concentrations (Fig. 7B) and levels of bacteremia (Fig. 7C) were reduced in the PAL mutant groups as compared with the wild-type group (p < 0.001).

**DISCUSSION**

We have demonstrated that PAL is released into the blood by Gram-negative bacteria during experimental GNS and that PAL has potent inflammatory effects and contributes to the virulence of *E. coli* K12 bacteria during peritonitis and sepsis. Previously, we found that PAL is released into the blood in an infected wound model of monomicrobial sepsis in rats (38, 39). The present studies indicate that PAL is also released into the blood in a polymicrobial model of sepsis caused by intestinal disruption. Although these studies were not specifically designed to quantitate PAL release, a rough estimate of the concentration of PAL circulating in the plasma can be made based on the immunoblots for PAL. In some CLP samples, the intensity of staining for PAL was similar to that for a 16-ng standard of purified PAL, as can be seen in Fig. 1 (lanes 1 and 2). Based on the quantity of affinity-purified CLP sample loaded per well, PAL levels in CLP plasma are at least 128 ng/ml. This concentration is well within the range that stimulates splenocyte and macrophage responses in our studies. Although we have been unable to find reports in the literature that other bacterial membrane proteins are released into the bloodstream at concentrations that stimulate inflammatory responses, we have previously detected released murein lipoprotein in the blood of septic rats (39). Prior studies have focused substantially on LPS. It seems likely that multiple other bacterial products, including proteins and lipids, are released into the blood during infection and contribute to the inflammation in sepsis.

Gram-negative bacteria have been reported to contain between 10^9 and 1.2 × 10^9 molecules of PAL/cell, depending on the genus and species of the bacteria (47, 49). The highest level of bacteremia in the present studies was 3.8 × 10^5 CFU/ml, which should contain 3.8 × 10^9 to 4.5 × 10^10 molecules/ml or 0.1–1.3 ng PAL/ml, depending on the bacteria involved in this polymicrobial infection. Based on these rough estimates, released PAL levels were 100–1000-fold higher than predicted by the culture data. This may be due to shedding of PAL by live bacteria during GNS and/or slow clearance of released PAL and raises the possibility that released PAL may remain in the circulation at levels that stimulate inflammatory responses after sterilization of the infection.

There are several possible explanations for the observation that PAL was detected in 94% of the CLP mice, whereas Gram-negative bacteria were detected in only 56% of the CLP mice. First, some mice may have been bacteremic, but at a lower level than the ≥100 CFU/ml required for detection. Second, PAL may be released at the site of infection and then diffuse into the blood. Third, PAL may be released into the blood and accumulate during transient bacteremic events that were missed by our single culture. The high rate of detection of released PAL suggests that PAL may be a sensitive marker for GNS.

PAL potently stimulates multiple immune effector cells, including macrophages, lymphocytes, and neutrophils. Picomolar levels of PAL stimulated production of TNF-α, IL-6, and nitrite by peritoneal macrophages. Injection of PAL into mice stimulated responses that are characteristic of early sepsis, including induction of serum cytokines, increased pulmonary and myocardial expression of cytokine and adhesion molecule mRNA, and accumulation and/or activation of neutrophils within the lungs.

Respiratory failure, myocardial dysfunction, and vasodilatation frequently occur during sepsis. Inflammatory responses...
within organs include increased expression of cytokines and vascular adhesion molecules such as ICAM and up-regulation of inducible nitric oxide synthase leading to increased nitric oxide production (2, 6, 9, 67). The increased myocardial and pulmonary expression of these genes, the elevated pulmonary MPO levels, and the increased macrophage production of nitrite induced by PAL suggest that PAL may contribute to the acute respiratory failure, myocardial dysfunction, and shock observed in GNS.

The notion that PAL may be important in GNS is further supported by the reduced mortality, lower plasma IL-6 levels, and lower levels of bacteremia induced by bacterial mutants defective in PAL as compared with wild-type strains of bacteria in LPS-responsive mice. There are several potential mechanisms for the decreased virulence of the PAL mutant bacteria. Abnormalities of this structural cell wall component may render the bacteria more sensitive to host defenses, may influence the quantity of other cell wall constituents that may also be important in the pathogenesis of GNS, and/or may influence the pattern of release of bacterial components such as LPS during infection. In addition, PAL may contribute more directly to the pathogenesis of GNS by triggering inflammation either on its own or in conjunction with other bacterial or host mediators or by facilitating movement of the bacteria from the peritoneum into the bloodstream.

Additional studies will be required to define the cellular mechanisms of PAL-mediated inflammation. The broad inflammatory responses elicited by PAL suggest a nuclear factor κB-dependent pathway (30). TLR2 is a candidate receptor for PAL because it is a receptor for lipoproteins from other bacteria (24, 27, 28). TLR4 is not required for the responses observed in these studies because C3H/HeJ mice lack functional TLR4 (23). Lipoproteins from spirochetes, Mycoplasma, and Mycobacteria and murein lipoprotein from Gram-negative bacteria have been shown to activate nuclear factor κB-mediated inflammatory responses (20–30). However, unlike PAL, most of these lipoproteins are derived from bacteria that do not cause Gram-negative sepsis, and they have not been reported to circulate in the blood separately from bacteria during active infection.

The presence of PAL in the circulation at concentrations that stimulate inflammatory cells, the potent inflammatory and toxic effects of PAL, and the reduced injury induced by PAL mutant bacteria as compared with wild-type control bacteria in a model of infection all support the hypothesis that PAL may play an important role in GNS. If PAL is an important mediator of GNS, the high degree of homology between PAL from diverse Gram-negative bacteria suggests that it may be a suitable bacterial target for future antisepsis therapies.

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REFERENCES
1. Centers for Disease Control. (1990) J. Am. Med. Assoc. 263, 937–938
2. Parrillo, J. E., Parker, M. M., Natanson, C., Suffredini, A. F., Danner, R. L., Cunnion, R. E., and Ognibene, F. P. (1990) Ann. Intern. Med. 113, 227–242
3. Sands, K. E., Bates, D. W., Lanken, P. N., Graman, P. S., Hibberd, P. L., Kahn, K. L., Parsonnet, J., Panzer, R., Orav, E. J., and Szoka, F. A. (1988) J. Infect. Dis. 157, 237–240
4. Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolfe, S., Milsark, T. W., Mares, R. J., Fahey, T. J., Zentella, A., Albert, J. D., Giusti, S., Terek, R., and Cerami, A. (1986) Science 234, 470–474
5. Dijkstra, J., Larrick, J., Brian, J. L., and Szoka, F. C. (1988) J. Leukocyte Biol. 43, 436–444
6. Dastin, M. L., Robien, R., Bhan, A. K., Dinarello, C. A., and Springer, T. A. (1987) J. Immunol. 137, 245–254
7. Glausier, M. P., Zanetti, G., Baumbach, J. D., and Cohen, J. (1991) Lancet 338, 732–736
8. Bone, R. C. (1991) Ann. Intern. Med. 115, 457–469
9. Kamochi, M., Kamochi, F., Kim, Y. B., Sawh, S., Sanders, J. M., Sarembock, I., Green, S., Young, J. S., Leys, K. P., Su, M., and Rose, C. E. Jr. (1991) Am. J. Physiol. 277, L310–L319
10. Beutler, B., Milsark, I. W., and Cerami, A. C. (1985) Science 229, 869–871
11. Kengen, T. K., and Cerami, A. (1993) Immunologic Rev. 147, 246–256
12. De Kimpe, S. J., Kengen, M., Tiemersmann, C., and Vane, J. R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10359–10363
13. Kengen, T. K., Kengen, S. D., Robson, C., Foster, S. J., and Tiemersmann, C. (1998) Science 282, 305–315
14. Melchers, F., Braun, V., and Galanos, C. (1975) J. Exp. Med. 142, 473–482
15. Goodman, G. W., and Sultzman, B. M. (1979) Infect. Immun. 24, 685–696
16. Chedid, L., Parant, M., and Boyer, F. (1968) J. Immunol. 100, 292–301
17. Braude, A. I., Douglas, H., and Davis, C. E. (1973) J. Infect. Dis. 128, 851–864
18. scrapie, C., and Salzman, A. L. (2001) J. Immunol. 166, 1248–1260
19. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Huffel, C. V., 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
20. Poltorak, A., Yuan, R., Mark, M. R., Sultzer, B. M., and Cerami, A. (1993) J. Exp. Med. 177, 1746–1749
21. Means, P. K., Rosen, J. W., Schromm, A. B., Smith, A. J., Keane, J., and Tufano, M. J. (2001) J. Immunol. 166, 4074–4082
22. Nicotera, A., He, X., Smirnova, I., Liu, M. Y., Huffel, C. V., 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
60. Takano, T., Fiore, S., Maddox, J. F., Brady, H. R., Petasis, N. A., and Serhan, C. N. (1997) *J. Exp. Med.* **185**, 1693–1704
61. Salkowski, C. A., Detore, G., Franks, A., Falk, M. C., and Vogel, S. N. (1998) *Infect. Immun.* **66**, 3569–3578
62. Renkema, T. J. E., Postma, D. S., Noorhoeck, J. A., Sluiter, H. J., and Kaufmann, H. P. (1991) *Eur. Respir. J.* **4**, 1237–1244

63. Galanos, C., Freudenberg, M. A., and Reutter, W. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5939–5943
64. Reed, L. J., and Muench, H. (1938) *J. Hyg.* **27**, 493–497
65. Parratt, J. R. (1998) *J. Antimicrob. Chemother.* **41**, Suppl. A, 31–39
66. Kilbourn, R. G., Gross, S. S., Juhran, A., Adams, J., Griffith, O. W., Levi, R., and Lodato, R. F. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 3629–3632
67. Martin, M. A., and Silverman, H. J. (1992) *Clin. Infect. Dis.* **14**, 1213–1228
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