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Modulation of Release of Proinflammatory Bacterial Compounds by Antibacterials: Potential Impact on Course of Inflammation and Outcome in Sepsis and Meningitis

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INTRODUCTION

In blood, bacteria or bacterial products elicit a systemic inflammation characterized by massive activation of both macrophages in the reticuloendothelial system and circulating leukocytes, release of cytokines, adhesion molecule expression on endothelial cells, and development of hypotension. The consequences of this systemic inflammation may progress to sepsis, septic shock, and multiple organ failure (70, 73, 85).

Soon after the first adequate dose of an antimicrobial drug to treat a wide spectrum of infectious diseases such as syphilis, relapsing fever, Lyme disease, leptospirosis, brucellosis, Vincent's angina, or African trypanosomiasis, a complex clinical reaction may occur, often characterized by sudden fever, myocloni, and hypotension. This phenomenon was first described by Jarisch (82) and Herxheimer (71) during therapy of secondary syphilis using mercury and is well known as the Jarisch-Herxheimer reaction (JHR). Typically, this systemic reaction occurs 1 to 2 h after the initial treatment of syphilis with effective antibiotics, especially penicillin. It consists of the abrupt onset of fever, chills, myalgias, headache, tachycardia, hyperventilation, vasodilation with flushing, and mild hypotension. It is particularly common (70 to 90%) when secondary syphilis is treated but can occur at any stage. It lasts from 12 to 24 h in various degrees of severity and is self-limiting (170, 187).

Although the molecular mechanisms of the JHR and inflammatory host responses to other bacteria following antibiotic treatment are not yet well understood, the JHR is predictable and of relatively uniform intensity (52). A more detailed analysis of antimicrobial drug-induced inflammatory host responses showed differences between spirochetes, other microbes, and endotoxin injections (23). Furthermore, spirochetes like *Treponema pallidum, Borrelia recurrentis*, and *Borrelia burgdorferi* sensu lato have never been shown to contain classical endotoxins (49, 66, 136). The bacterial compounds involved in JHR remain to be identified.

For the bacteria containing endotoxin, a JHR-like reaction was first described for typhoid fever. In the early days of antibiotic therapy, lethal vasomotor collapse after the first dose of chloramphenicol was noted. A rapid and strong release of endotoxin was suspected as the cause of adverse effects associated with the initiation of treatment. This led to the advice to start with a low dose of the antibiotic (75). Worsening of clinical symptoms after initiation of antibiotic treatment during sepsis or meningitis has also frequently been observed, leading to the concept of immunosuppression by corticosteroids and other agents concomitant or prior to the first antibiotic dose.

A therapeutic approach in meningitis and sepsis, which avoids immunosuppression, is to influence the liberation of bacterial proinflammatory cell wall products. Compared to continuing replication and autolysis, all bactericidal antibiotic regimens eventually reduce the release of bacterial components. Whereas cell wall-active antibacterials can temporarily enhance the liberation of toxic or proinflammatory bacterial compounds, bactericidal antibiotics acting by the inhibition of RNA or protein synthesis or DNA replication (rifamycins, macrolides, clindamycin, ketolides, and quinolones) delay or even circumvent bacterial lysis. They are promising with respect to reducing toxic or proinflammatory bacterial compounds when they possess bactericidal in vivo activity against gram-positive and gram-negative bacteria causing sepsis and meningitis.

The present review aims at a critical reevaluation of in vitro, animal, and clinical data and of whether the use of antibacterials which reduce the release of proinflammatory and toxic compounds from bacteria may be advantageous in comparison to β-lactam antibiotics for initial therapy of severe infections.

PROINFLAMMATORY AND TOXIC EFFECTS OF BACTERIAL COMPONENTS

LPSs

Structure. The cell walls of gram-negative bacteria are composed of three layers, which include outer and inner membranes that enclose a peptidoglycan backbone. The outer membrane is a trilamellar structure which has a different chemical composition from the cytoplasmic membrane and contains the lipopolysaccharide (LPS) moiety. The negatively charged LPS molecules (molecular weight, about 3,000 to

18,000) are noncovalently cross-bridged by divalent cations. This stabilizes the membrane and provides a barrier to hydrophobic molecules. Displacement of LPS by polycationic antibiotics such as polymyxins, aminoglycoside, or chelating agents renders the outer membrane permeable to large hydrophobic molecules. LPS is found only in gram-negative bacteria.

The classic molecule consists of three regions: polysaccharide side chains (O-antigen), core saccharides, and lipid A. The sequence of sugars of the O-antigen is highly variable. The number of repeating oligosaccharide subunits, consisting usually of linear trisaccharides or branched tetra- or pentasaccharides, may be more than 20.

The core saccharides are similar in all gram-negative bacteria that have LPS and consist mainly of *N*-acetylglucosamine, glucose, galactose, heptose, phosphate, and ethanolamine. This unit is linked through its 2-keto-3-deoxyoctonic acid via a glycoside bond to the glucosamine of the lipid A.

The lipid A component contains a dimer of phosphorylated glucosamine bound to O-and N-linked saturated long-chain fatty acids or hydroxy fatty acids and accounts for about half the lipids in the outer bilayer (117, 138, 149).

Proinflammatory activity. The lipid A is responsible for the inflammatory activity of LPS. LPS induces the release of a broad spectrum of inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) from human peripheral mononuclear blood cells and expression of VCAM or ICAM on endothelial cells (21, 104, 105, 174, 175). It elicits many features of septic shock (38, 161).

The mechanisms by which LPS activates cells and initiates inflammatory responses have been investigated extensively. CD14 and the LPS binding protein (LBP) are key mediators in the activation pathway of inflammatory cells (164, 168, 169, 184). CD14 is not a transmembrane protein, i.e., a further transmembrane molecule is needed to explain the cell stimulation by the suggested complex containing LPS, LBP, and membrane-bound CD14. The Toll-like receptor 4 (Tlr4) has recently been found to function as the transmembrane component of the LPS-receptor complex and as the missed transducer of the LPS signal (13, 14). After activation of this signaling cascade, genes encoding inflammatory cytokines are transcribed.

Teichoic and Lipoteichoic Acids

Structure. Most gram-positive bacteria contain considerable amounts of teichoic acid (TA) and lipoteichoic acid (LTA) in the cell wall, accounting for up to 50% of its dry weight. The molecules are water-soluble polymers, containing ribitol or glycerol residues joined through phosphodiester linkages and carrying one or more amino acid or sugar substituents. The repeat units may be glycerol, joined by 1,3- or 1,2-linkages, or ribitol, joined by 1,5-linkages. More complex units in which glycerol or ribitol is joined to a sugar residue such as glucose, galactose, or *N*-acetylglucosamine also occur. Although 10 or fewer of the repeat units are common, 30 or more may be possible. D-Alanine is often part of TA, usually linked to position 2 or 3 of glycerol or position 3 or 4 of ribitol or attached to one of the sugar residues.

The composition of the TA molecule can vary with the composition of the growth medium. A defined species may have more than one type of sugar substituent in addition to D-alanine. Two types of TA can be differentiated. The wall TA is covalently linked to peptidoglycan, and the LTA is covalently linked to membrane glycolipid (9, 107, 130).

The function of TA and LTA is still a matter of speculation. LTAs may serve to anchor the wall to the underlying cell membrane. TAs may participate in adhesion and magnesium ion supply to the bacterium. In pneumococci, TAs contribute to resistance, autolysis, and DNA transformation (54, 55, 118). LTAs are heterogeneous in molecular details, but their principal structure is similar in gram-positive bacteria. With respect to the induction of inflammation, they are thought to be the counterpart of the LPS of gram-negative bacteria (67, 68).

Proinflammatory activity. Gram-positive as well as gram-negative microorganisms can cause septic shock. The cell walls of gram-positive organisms contain various components, mainly LTA and peptidoglycan, which elicit the host's inflammatory response (15, 29, 167, 180). LTAs exhibit many biological activities mediated by the induction of cytokines like IL-1, IL-6, IL-8, IL-12, and other molecules activating inflammation (15, 24, 34, 111). In anesthetized rats, administration of LTA caused a release of TNF-α, induction of inducible nitric oxide synthase, and circulatory failure (39). The pathogenetic consequences of LTA can vary among bacterial species (68, 99, 100).

CD14, the major receptor of LPS, also recognizes bacterial components like LTA (34). LTA can act synergistically or as an antagonist of LPS depending on the origin of the LTA (162).

Peptidoglycans

Structure. Due to the structure of the peptidoglycan, cell walls of gram-positive and gram-negative bacteria are rigid, thereby protecting bacteria against osmotic rupture. In Gram's stain, the gram-positive cell wall retains crystal violet because of its thick, multilayered peptidoglycan. The peptidoglycan layer of gram-positive bacteria is 50 to 100 molecules thick, whereas the cell wall of gram-negative bacteria is only one or two molecules thick. Peptidoglycans are heteropolymers of long polysaccharide chains in which *N*-acetylglucosamine and *N*-acetylmuramic acid alternate in a linear form. Peptides are attached to each *N*-acetylmuramic acid, and the strands are cross-linked by peptide linkages. Typical linkage exists between the carboxyl group of a D-alanine residue that forms the terminal peptide, linked either directly or through a short peptide cross bridge to lysine or diaminopimelic acid (33, 41, 65, 141, 152).

Inhibition of peptidoglycan synthesis is the aim of several important classes of antibiotics, including the penicillins, cephalosporins, and glycopeptides.

Proinflammatory activity. A number of biological activities in the host have been attributed to peptidoglycan, but its role in pathogenesis remains unclear. Gram-positive cell wall fragments as well as purified peptidoglycan induce the release of TNF-α, IL-1β, and IL-6 from cultured macrophages/monocytes (15, 72, 111, 167). The molecular mechanism of this induction is still a matter of discussion.

Peptidoglycan from gram-positive bacteria also binds CD14, suggesting that this receptor is involved in cytokine induction in gram-positive infection (47, 48, 182). Peptidoglycan from *Staphylococcus aureus* induced IL-6 and IL-10 mRNA in both

T cells and monocytes. LTA induced IL-6 and IL-10 production in monocytes. The results in T cells were inconclusive. Only peptidoglycan was a potent inducer of TNF-α, whereas peptidoglycan and LTA induced the release of similar amounts of IL-6 and IL-10 (182).

In cultured macrophages, LTA but not peptidoglycan from *S. aureus* induced the release of nitric oxide. Coadministration of LTA and peptidoglycan resulted in a fourfold increase in the production of nitric oxide elicted by LTA alone. For this reason, LTA and peptidoglycan probably work synergistically in systemic inflammation induced by gram-positive bacteria (39). A specific part of the peptidoglycan molecule (*N*-acetylglucosamine-β-[1→4]-*N*-acetylmuramyl-L-alanine-D-isoglutamine) was found to be responsible for the synergism of peptidoglycan and LTA in NO formation, septic shock, and organ injury (85).

In anesthetized rats, administration of LTA caused a release of TNF-α, induction of inducible nitric oxide synthase, and circulatory failure. In cultured macophages, LTA but not peptidoglycan from *S. aureus* induced the release of nitric oxide. Coadministration of LTA and peptidoglycan resulted in a fourfold increase in the production of nitric oxide elicited by LTA alone. For this reason, LTA and peptidoglycan probably work synergistically in systemic inflammation induced by gram-positive bacteria (39).

Bacterial DNA

Bacterial DNA can induces B-cell proliferation. In contrast, mammalian DNA does not show this biological activity (97, 185). Similarly, CpG motifs in fragments of bacterial DNA trigger direct B-cell activation. Methylation of bacterial DNA with CpG methylase abolishes mitogenicity, demonstrating that this difference in CpG status is the cause of the immune-stimulatory effects of bacterial DNA (97).

Unmethylated CpG motifs are about 20-fold more common in bacterial DNA than in vertebrate genomes. When CpG motifs are present in vertebrates, they are more likely to be methylated. It has been suggested that the vertebrate immune system uses these unique characteristics of bacterial DNA to launch innate immune defenses against bacteria.

In addition to B-cell proliferation, the immunological activity of DNA containing unmethylated CpG motifs includes stimulation of the release of IL-6 and Il-10, natural killer cell activation, secretion of gamma interferon and activation of monocytes, with increases in the levels of TNF-α and IL-12. Furthermore, in a murine model, CpG-containing oligonucleotides modified Th2 inflammatory response, making it more Th1-like (7, 30, 32, 37, 63, 92, 93, 94, 97, 185).

On the one hand, bacterial DNA has been found to cause septic shock; on the other, it can reduce LPS-induced inflammation through an IL-12-dependent pathway (148, 156). In inducing septic shock, on a weight basis bacterial DNA is approximately 10-fold less potent than LPS (156).

Bacterial DNA alone induced little nitric oxide production in cultured macrophages, yet DNA from both gram-negative and gram-positive bacteria synergized with subthreshold concentrations of endotoxin (59). Although the exact role of bacterial DNA in the signal pathway and its biological significance during severe infections remain unclear, it probably contributes to the proinflammatory activity of lysed bacteria.

Bacterial Proteins

A variety of bacterial proteins can participate in the pathogenesis of inflammation and injury to the host during infection. Since the influence of antibiotic treatment on the expression and release of bacterial proteins has been less thoroughly investigated than its influence on the release of the compounds mentioned above, this review focuses on exotoxins and hemolysins.

Exotoxins. Only a minority of gram-positive organisms produce entero- or exotoxins, e.g., toxic shock syndrome toxin and staphylococcal enterotoxins A to E, which are able to induce shock in humans (17, 96). These toxins are called superantigens because of their ability to activate T cells by binding to major histocompatibility complex class II molecules and to the T-cell receptor at picomolar concentrations, leading to excessive production of proinflammatory cytokines (96, 166).

Hemolysins. Several genera, particularly of gram-positive bacteria, produce hemolysins. Pneumolysin has been studied extensively. It consists of a single 53-kDa polypeptide chain, is localized in the cytoplasm, and is produced by virtually all clinical isolates of *Streptococcus pneumoniae*. It can activate the classical complement pathway by binding to the Fc portion of immunoglobulin G and forms transmembrane pores by oligomerization (129).

Modes of Action on the Host

Proinflammatory and toxic bacterial products may affect the host on several levels.

Leukocytes. Many of the harmful effects of bacterial compounds on the host organism are mediated by mechanisms of the immune response. Stimulation of circulating cells of the immune system leading to cytokine and chemokine secretion, degranulation of granulocytes, and production of free radicals by granulocytes and monocytes has been firmly established (72) (see above also).

Resident immune cells. The action of bacterial compounds on immune cells does not necessarily involve the circulating pool of monocytes and granulocytes. In rats, LPS induces caspase-3-mediated apoptosis of hepatocytes, which depends on the presence of intact Kupffer cells (64). Stimulation of cultured microglia with LPS (1 ng/ml) resulted in a strong release of TNF-α. Higher concentrations of LPS caused time- and dose-dependent apoptotic death of microglia. In contrast, astrocytes were insensitive to LPS-induced cytotoxicity (102).

Heat-inactivated pneumococci and pneumococcal cell wall preparations damage astrocytes and microglia in vitro. Neuronal injury, however, only occurs during coculture with glial cells (88). The production of nitric oxide by glial cells plays an important role in neuronal cell death (89). In organotypic hippocampal slices in vitro, i.e., in the absence of leukocytes, necrotic and apoptotic cell damage occurs after exposure to heat-inactivated *S. pneumoniae* R6, lipoteichoic acid, peptidoglycans, and pneumococcal DNA (144). This indicates that several bacterial components may stimulate local immunocompetent cells.

Endothelia. High-dose endotoxin produces oligonucleosomal and random DNA fragmentation in liver, lung, kidney, intestine, and vascular endothelial cells in vivo (18, 84). Although this may be, in part, mediated by circulating immune

cells, a direct effect of bacterial components on endothelial cells has been demonstrated. In vitro, endotoxin stimulated endothelial cells to release TNF-α, ICAM-1, and inducible nitric oxide synthetase (57) and disrupted the endothelial cell barrier function by a mechanism which does not involve protein synthesis (8). Upon stimulation with supernatants of antibiotically treated *S. aureus*, human umbilical cord vein endothelial cells released IL-8 and MCP-1 and showed increased adhesiveness for granulocytes (178). Heat-inactivated unencapsulated pneumococci induced the release of matrix metalloproteinase-2 from aortic and cerebral endothelial cells (114). Pneumolysin damaged brain microvascular endothelial cells and decreased transendothelial electrical resistance (191) and caused a dose-dependent inhibition of ciliar beat frequency in ependymal cells (74).

Neurons. Pneumolysin is capable of directly damaging hair cells of the cochlea (35). It may induce apoptosis in neurons of the hippocampal formation, possibly by direct action on the cell membrane (22; A. Stringaris and R. Nau, unpublished data).

RELEASE OF BACTERIAL COMPOUNDS DURING TREATMENT

Gram-Negative Bacteria

During normal growth, endotoxin is shed continuously by gram-negative organisms (3, 50). Furthermore, various gram-negative pathogens liberate bilayered membrane vesicles containing endotoxin, outer membrane proteins, and several hydrolytic enzymes, including proteases, alkaline phosphatase, phospholipase C, and peptidoglycan hydrolase (83). Antibiotic treatment causes up to 100-fold rises in the endotoxin levels in the supernatants of bacterial cultures (44). The release of endotoxin by gram-negative bacteria during antibiotic therapy is drug and dose dependent. In general, high concentrations of antibacterials release smaller quantities of endotoxin than concentrations close to the MIC (44, 78, 133, 171).

β-Lactam antibiotics. Meningococci exposed to penicillin in vitro for 1 to 2 h released more endotoxin than untreated meningococci (2, 3, 113). After an observation period of 4 h, however, multiplying bacteria not treated with antibacterials released more endotoxin than bacteria treated with penicillin or ceftriaxone (132).

Three main responses to β-lactam antibiotics by gram-negative bacteria have been observed: rapid cell lysis due to the inhibition of penicillin-binding protein 1A (PBP-1A) and PBP-1B; production of osmotically sensitive, cell wall-deficient round cells by the inhibition of PBP-2; and filament formation by inhibition of PBP-3. Filament formation leads to strong endotoxin production and, ultimately, endotoxin release when such cells lyse (61). The amount of endotoxin released correlates with cell wall morphology and bacterial shape before lysis (76). Antibacterials inhibiting PBP-3 at low concentrations (e.g., ceftazidime) also bind to PBP-2 at higher concentrations, which causes round cells not containing large amounts of endotoxin (61).

At two times the MIC, PBP-3-specific ceftazidime liberated 10 to 40 times more endotoxin from *Pseudomonas aeruginosa* than the PBP-2-specific imipenem (78). In contrast to the difference between ceftazidime and imipenem with respect to *P. aeruginosa*, the endotoxin release by ceftazidime and imipenem from *Escherichia coli* in one study was almost identical at concentrations between 0.06 and 256 mg/liter (Fig. 1) (51). In two other studies (134, 171), however, the amount of endotoxin was lower after exposure of *E. coli* to imipenem and meropenem than to ceftazidime. At two times the MIC, the release of endotoxin from *E. coli* was higher than at 50 times the MIC for all antibacterials tested (meropenem, imipenem, ceftazidime, and ciprofloxacin) (171).

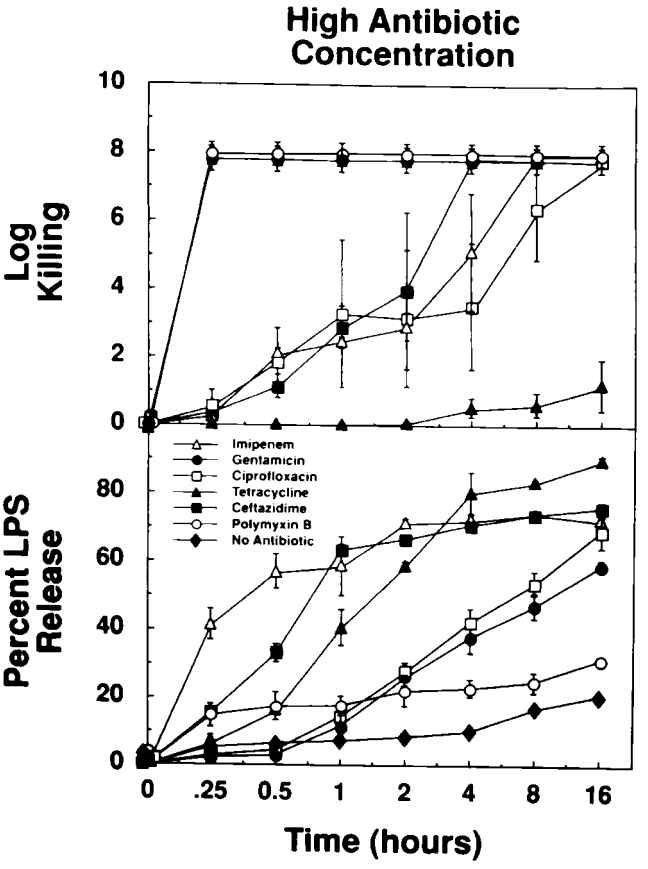

FIG. 1. Release of LPS from *E. coli* following exposure to various antibacterials (tetracycline, 16 mg/liter; all other agents, 256 mg/liter) (means ± standard deviations). Protein synthesis inhibitor antibiotics released smaller LPS quantities than β-lactam antibiotics. Significant differences (analysis of variance) were detected between tetracycline versus polymyxin B and no antibiotic; imipenem and ceftazidime versus polymyxin B; imipenem versus gentamicin, ciprofloxacin, and no antibiotic; and ceftazidime versus gentamicin and no antibiotic. Reprinted from reference 51 with permission of the publisher.

Incubation of *Salmonella enterica* serovar Typhi with ceftazidime and imipenem led to a maximum release of 33 and 27% of the radiolabeled endotoxin, respectively. The delay between bacterial killing and LPS release was approximately 1 h for both antibiotics (177).

Quinolones. *E. coli* exposed for 60 min to ciprofloxacin showed a 1,000-fold decrease in CFU but no equivalent decrease in bacterial numbers, as determined by light microscopy and flow cytometry, suggesting that the bacteria did not lyse (108). In another study investigating *E. coli*, at two and 50

times the MIC, after 8 h of exposure ciprofloxacin had induced the release of approximately two- and one-fifth, respectively, of the endotoxin released by ceftazidime (171). The release of endotoxin was slower during ciprofloxacin than during ceftazidime treatment (171). Although in two other studies the absolute amount of endotoxin released from *E. coli* by ciprofloxacin was similar to that liberated by ceftazidime (51, 133), in timed experiments ciprofloxacin, despite rapid bactericidal action, induced the release of only 12.7% of the total LPS within the first hour of exposure, whereas during ceftazidime treatment 61.9% was released (Fig. 1) (51).

Polymyxin. The hydrophobic fatty acid chain of polymyxin is able to bind the LPS core glycolipid, inactivating its biological function (139). The amounts of intact drug or the reactive side chain necessary to achieve anti-LPS activity, however, are toxic and preclude systemic use of polymyxin in humans (140, 183). Nevertheless, in several animal models of gram-negative sepsis, subinhibitory polymyxin doses bound endotoxin and increased or prolonged survival (36, 181). As for polymyxin and aminoglycosides (see below), the glycopeptide teicoplanin was able to bind and neutralize endotoxin. After incubation of teicoplanin with LPS for 3 h, it reduced in vitro reactivity and lethality of D-galactosamine-sensitized mice challenged intraperitoneally with *Salmonella enterica* serovar Minnesota R595 LPS (56).

Aminoglycosides. Gentamicin and other aminoglycosides, in addition to inhibiting protein synthesis, also perturb the packing order of lipids by interaction with anionic lipopolysaccharides on the surface of gram-negative bacteria and thereby may induce cell lysis. By destabilizing the membranes of *P. aeruginosa*, gentamicin increased the release of membrane vesicles three- to fivefold (83). Similarly, in several studies the release of endotoxin by protein synthesis-inhibiting aminoglycosides was comparable to the release observed during treatment with β-lactam antibiotics (176). For this reason, aminoglycosides may not be ideal candidates for the reduction of proinflammatory bacterial compounds. In two studies, however, tobramycin induced the release of less LPS from *E. coli* than ceftazidime and cefuroxime (155, 171).

Aminoglycosides, although less potent than polymyxin B, can bind and neutralize endotoxin (116, 150). Binding of endotoxin to the aminoglycoside is one explanation for the observation that the combination of tobramycin and cefuroxime caused less endotoxin liberation from *E. coli* than treatment with cefuroxime alone (43, 44).

Chloramphenicol. In meningococci treated with penicillin, ceftriaxone, and chloramphenicol, the release of endotoxin was lowest during chloramphenicol treatment. The difference between chloramphenicol versus the β-lactams reached statistical significance with only one of the four strains investigated, whereas with the other three strains a trend towards differences between chloramphenicol and β-lactams was observed (132).

Clindamycin. Antibacterials usually not employed for treatment of infections by gram-negative bacteria have also been shown to influence the release of proinflammatory compounds and virulence factors. Clindamycin, although not effective against *E. coli*, suppressed the production of hemolysin (16). Treatment with clindamycin at 25 μg/ml for 4 h strongly reduced and treatment for 18 h almost completely abolished endotoxin release during treatment with ceftazidime (91).

As mentioned above, endotoxin is not the only proinflammatory compound of gram-negative bacteria. Other bacterial products probably contribute to the pathogenesis of septic shock, meningeal inflammation, and tissue damage (134). The influence of antibiotic treatment on the release of peptidoglycans and bacterial DNA by gram-negative bacteria, however, has not been studied, to our knowledge.

Gram-Positive Bacteria

Until recently, the lack of convenient analytical methods impeded the comparative investigation of the liberation of LTA and TA from gram-positive bacteria by various antibacterials.

β-Lactam antibiotics. The release of peptidoglycans and TA in pneumococci treated with penicillin and ampicillin has been demonstrated after incorporation of [^{3}H]lysine and [^{3}H]choline (53) in vitro. Benzylpenicillin enhanced the release of radiolabeled LTA from *Lactobacillus casei*, whereas chloramphenicol inhibited its release (131).

The release of LTA and TA from an *S. pneumoniae* type 3 strain grown in tryptic soy broth was monitored by enzyme immunoassay (EIA) (159). At a concentration of 10 μg/ml, a rapid and intense release of LTA/TA occurred during exposure to ceftriaxone and meropenem (160).

Protein synthesis inhibitors. The LTA/TA concentrations during treatment of *S. pneumoniae* with rifampin, rifabutin, and quinupristin/dalfopristin were substantially lower than during treatment with ceftriaxone and meropenem (Fig. 2). The release of LTA/TA during exposure to trovafloxacin was slower than during ceftriaxone; the difference after 12 h, however, failed to reach statistical significance. At low antibiotic concentrations (MIC and MBC), larger quantities of LTA/TA were released than in cultures treated with 10 to 300 times the MIC of the respective antibacterial (160). Rifampin treatment resulted in less bacterial DNA release than treatment with ceftriaxone and spontaneous growth (60).

Similarly, LTA in the supernatant of *S. aureus* cultured in vitro was quantified by EIA, and peptidoglycan was measured by means of a silkworm larva plasma test 4 h after the addition of antibiotics at concentrations of 20 times the MIC. Erythromycin, clindamycin, and gentamicin acting as inhibitors of protein synthesis released smaller quantities of LTA and peptidoglycan from *S. aureus* than the β-lactam antibiotics cefamandole, flucloxacillin, and imipenem (177, 179) (Fig. 3). The different antibiotic supernatants were then tested for TNF-α production in a whole-blood stimulation assay without and after degradation of peptidoglycan in the bacterial supernatants. The enzymatic treatment reduced the TNF-α-inducing capacity of supernatants exposed to β-lactams by approximately 80%. For protein synthesis inhibitors, no residual TNF-α production was observed. This suggests that the stimulation of TNF-α release in whole blood exerted by supernatants of *S. aureus* cultures was primarily effected by peptidoglycan (179).

Clindamycin substantially inhibited the production of pyrogenic exotoxin C (toxic shock syndrome toxin 1) from *S. aureus*, as measured by double immunodiffusion at concentrations

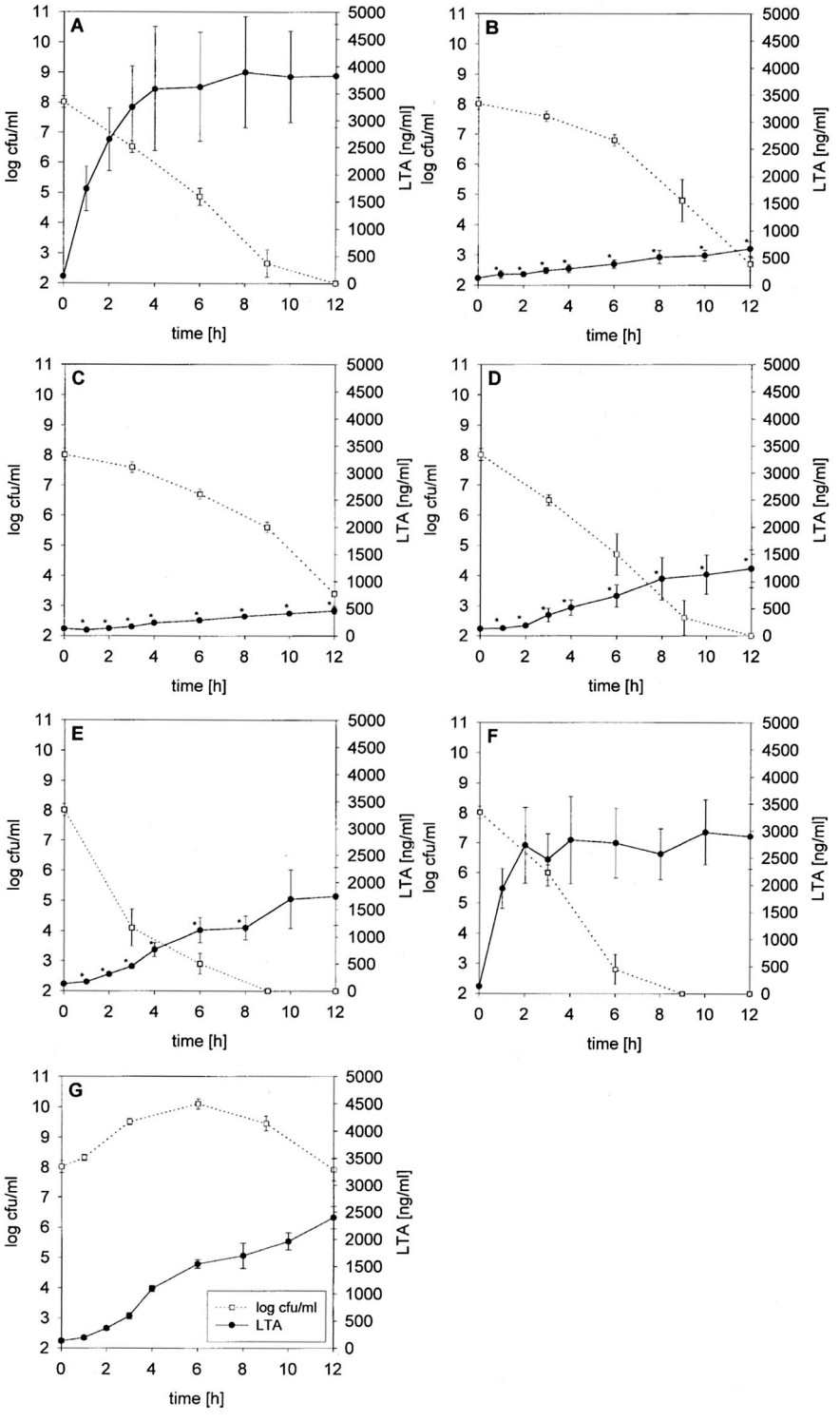

FIG. 2. Release of LTA and TA by *S. pneumoniae* during treatment with 10 μg of ceftriaxone (A), rifampin (B), rifabutin (C), quinupristin/dalfopristin (D), trovafloxacin (E), and meropenem (F) per ml (solid circles). Means ± standard errors of means of five experiments are shown. *, $P \leq 0.05$ versus ceftriaxone-treated bacteria (open squares, bacterial titers, means ± SEM). Reprinted from reference 160 with permission of the American Society for Microbiology. Antibacterials inhibiting bacterial protein synthesis released smaller quantities of LTA and TA than β-lactam antibiotics.

(≥0.01 mg/liter) which did not significantly inhibit bacterial growth (142). At subinhibitory concentrations, β-lactam antibiotics strongly induced and clindamycin almost completely inhibited the expression of the gene encoding the staphylococcal alpha-toxin (126). Tetracycline and kanamycin also inhibited the production of staphylococcal exotoxin at subinhibitory concentrations, but were less potent than clindamycin, erythromycin, and lincomycin (40). Similarly, the ability of *Strepto-*

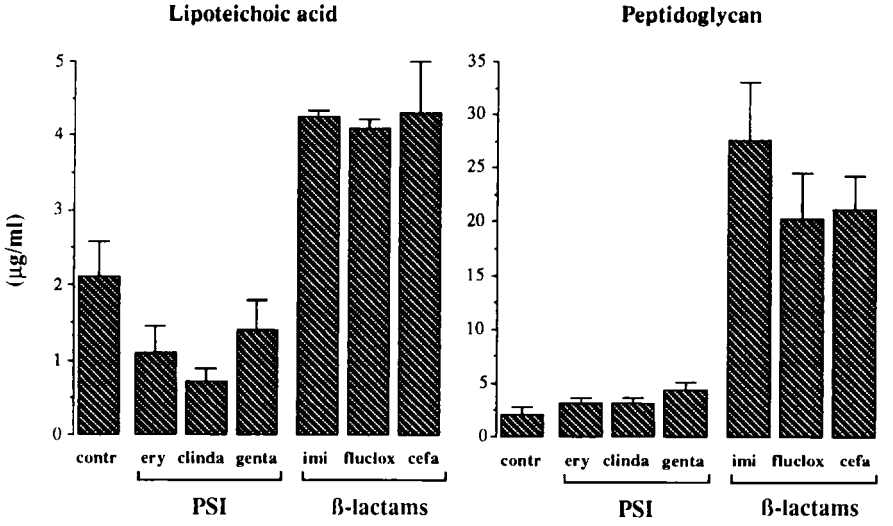

FIG. 3. Release of LTA and peptidoglycans from *Staphylococcus aureus* after 4 h of exposure to various antibacterials (means ± standard errors of the mean for three to four separate experiments). Reprinted from reference 178 with permission of the American Society for Microbiology. The protein synthesis inhibitors (PSI) erythromycin (ery), clindamycin (clinda), and gentamicin (genta) released substantially smaller amounts of LTA and peptidoglycans than imipenem (imi), flucloxacillin (fluclox), and cefamandole (cefa). Contr, control.

coccus pyogenes to produce streptococcal pyrogenic exotoxin A (as estimated by [^{3}H]thymidine uptake of mononuclear cells) was lower in cultures treated with clindamycin than with ampicillin (concentration 0.5 times the MIC) (157). Furthermore, lincomycin and clindamycin at concentrations below those which partially inhibited bacterial growth completely suppressed the production of streptolysin S in streptococci. Chloramphenicol and erythromycin, two other bacterial protein inhibitors, had no effect on hemolysin production (151). Upon exposure to clindamycin, *Clostridium perfringens* released substantially less α-toxin than during penicillin treatment (158). Based on these observations, several authors recommend the use of clindamycin for therapy of staphylococcal or streptoccal toxic shock syndrome and *Clostridium perfringens* gas gangrene.

EFFECT OF DIFFERENTIAL RELEASE OF PROINFLAMMATORY COMPOUNDS ON MAMMALIAN CELLS

Gram-Negative Bacteria

Mononuclear cells released less TNF after exposure to filtrates of *E. coli* cultures treated with amikacin or imipenem than when they were exposed to filtrates of ceftazidime-, cefotaxime-, and aztreonam-treated cultures. Exposure to ciprofloxacin gave intermediate levels of TNF release (153). Low-dose treatment with cefuroxime and ceftazidime resulted in higher production of TNF than high-dose therapy, and coadministration of tobramycin reduced the TNF release induced by cefuroxime treatment (43). Exposure of *Haemophilus influenzae* to ceftriaxone resulted in greater release of TNF than exposure to imipenem despite similar degrees of bacterial killing (5).

Upon stimulation by endotoxin, endothelial cells increase their surface expression of adhesion molecules and their secretion of chemokines such as IL-6, IL-8, and MCP-1 (6, 106). Killing of *E. coli* by PBP-2-specific imipenem induced less IL-6 release from human umbilical vein endothelial cells than did PBP-3-specific ceftriaxone and meropenem. When endothelial cells were coincubated with 4% human blood, antibiotic-induced IL-6 release was substantially higher than IL-6 liberation produced by endothelial or blood cells alone, indicating amplification of IL-6 release by coincubation of endothelia and monocytes (6).

After 4 and 18 h of clindamycin incubation, the release of IL-1β and TNF-α from a human acute monocytic leukemia cell line (THP-1) induced by supernatants of ceftazidime-treated *E. coli* was smaller than that without clindamycin treatment (91).

Gram-Positive Bacteria

Supernatants from *Staphylococcus epidermidis* cultures incubated with β-lactam antibibiotics induced a stronger TNF release from plastic-adherent monocytes than those obtained from bacteria incubated with culture medium only, vancomycin, and clindamycin (110).

Peptidoglycan and LTA released from *S. aureus* caused TNF-α secretion in a whole-blood stimulation assay (179) and IL-8 and MCP-1 liberation, ICAM-1 expression, and increased adhesiveness of granulocytes in human umbilical vein endothelial cultures (178). The stimulatory effects of supernatants from cultures treated with β-lactam antibiotics was greater than from cultures treated with protein synthesis inhibitors, indicating a stronger release of proinflammatory compounds by β-lactams (178, 179).

RELATIONSHIP BETWEEN PROINFLAMMATORY COMPOUND CONCENTRATION AND OUTCOME IN HUMANS

Meningitis

Pathology. The release of bacterial cell wall components into the cerebrospinal fluid (CSF) during antibiotic-induced bacte-

rial lysis may cause a burst of meningeal inflammation after initiation of antibiotic therapy (119, 173). Endotoxins and peptidoglycans of gram-negative organisms and fragments of the cell wall of gram-positive bacteria (peptidoglycans, TA, and LTA) can stimulate the release of proinflammatory cytokines, induce profound meningeal inflammation, increase intracranial pressure, and cause brain edema in experimental meningitis (26, 119, 137).

In humans, evidence of brain herniation was present at autopsy in approximately one in three cases who die during the acute phase of meningitis (46, 121). More than 50% of the brain herniations occurred later than 2 h after lumbar puncture (42). At this time, antibiotic therapy had presumably been initiated. Since endotoxin injected intracisternally is able to increase brain water content, it has been suspected that the antibiotic-induced release of proinflammatory cell wall products contributed to brain herniation in some of these cases (163, 165).

Adjunctive corticosteroids. In order to attenuate subarachnoid space inflammation, adjunctive anti-inflammatory drugs have been introduced into the therapy of bacterial meningitis (135). However, although different regimens have been proven effective in animal models, only dexamethasone therapy has been shown to decrease hearing loss and overall neurologic sequelae in children with *H. influenzae* meningitis (125). For all other pathogens, the present data are not strong enough to warrant routine use of dexamethasone (112). In animal experiments, dexamethasone increased neuronal damage in the dentate gyrus of the hippocampal formation (190). It has been shown to decrease the concentrations of hydrophilic antibiotics in the CSF in meningitis and in the pus of brain abscesses and to affect CSF sterilization in the case of pathogens with decreased sensitivity to antibiotics (28, 95, 128).

Protein synthesis inhibitors and quinolones versus β-lactam antibiotics. Bactericidal protein synthesis inhibitors, promising for use in infections of the central nervous system (CNS), include rifampin, clindamycin, and perhaps quinupristin/dalfopristin, linezolid, and ketolides. Older quinolones are inadaequate for the majority of CNS infections because of their low activity against *S. pneumoniae* and *Listeria monocytogenes* (120). Newer quinolones, such as clinafloxacin, trovafloxacin, and moxifloxacin, are highly active against gram-positive and gram-negative pathogens causing meningitis. They also appear to kill bacteria without lysing them immediately (58, 123, 129, 143).

Chloramphenicol. In *E. coli* meningitis, the marked increase in endotoxin in CSF 3 h after initiating treatment with cefotaxime was not observed with chloramphenicol, although the reduction in bacterial titers in CSF was not significantly different. Brain edema was less severe in chloramphenicol- than in cefotaxime-treated rabbits (165). Similarly, bacteriostatic concentrations of chloramphenicol did not cause the initial increase in LPS observed 2 h after initiation of antibiotic therapy which occurs during therapy of meningitis with β-lactam antibiotics (58). After 6 h, however, LPS was higher in the chloramphenicol-treated than in any of the β-lactam antibiotic-treated groups (58). For this reason, the bacteriostatic chloramphenicol appears not to be suitable to decrease the release of proinflammatory or neurotoxic bacterial compounds in meningitis.

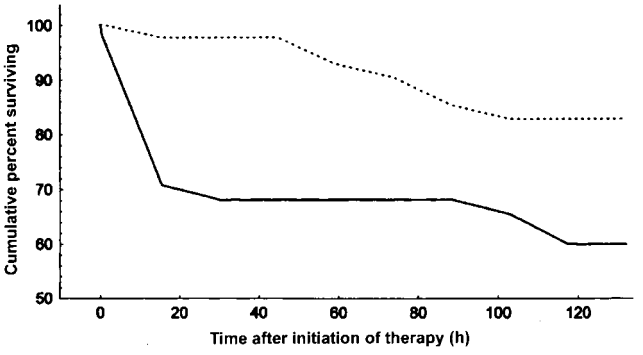

FIG. 4. Kaplan-Meier analysis of mice treated with rifampin (dashed line) and ceftriaxone (solid line) for experimental *S. pneumoniae* meningitis. Reprinted from reference 122 with permission of the publisher. Note the high early mortality in animals treated with ceftriaxone. The survival-versus-time curves for the two antibiotics were significantly different ($P = 0.007$; Cox proportional hazard model). Rifampin reduced overall mortality from 49 to 26% ($P = 0.04$, Fisher's exact test).

Rifampin. In the CSF of experimental rabbits, the release of proinflammatory cell wall products (LTA and TA) of *S. pneumoniae* killed by rifampin, rifabutin, quinupristin/dalfopristin, moxifloxacin, and trovafloxacin was slower and/or smaller than that of pneumococci killed by ceftriaxone (20, 160). *S. pneumoniae* type 3 killed in vitro by trovafloxacin and injected intracisternally caused less CSF pleocytosis in rabbits than bacteria killed in vitro by ceftriaxone (123).

In the rabbit model of *S. pneumoniae* meningitis, trovafloxacin delayed (123) and rifabutin and quinupristin/dalfopristin decreased the activity of TNF in the CSF (145, 172). Trovafloxacin and rifabutin, however, failed to reduce parameters of neuronal damage in this model. Treatment with quinupristin/dalfopristin decreased the CSF concentration of neuron-specific enolase, an indicator of neuronal damage in meningitis (77), in comparison to ceftriaxone, yet this compound only slowly reduced pneumococcal titers in CSF (172).

Rifampin, compared to ceftriaxone, reduced early mortality in a mouse model of *S. pneumoniae* meningitis (Fig. 4) (122). In a rabbit model of *S. pneumoniae* meningitis, CSF leukocytes from rifampin-treated rabbits produced less reactive oxygen intermediates than leukocytes from animals receiving ceftriaxone. The CSF malondialdehyde concentrations and the density of apoptotic neurons in the dentate gyrus were lower in rifampin- than in ceftriaxone-treated animals (20).

The rapid induction of resistance to rifampin during monotherapy precludes its use as a single agent for sepsis and meningitis, yet no substantial release of LTA/TA was observed in vitro when ceftriaxone was added 6 h after initiation of antibiotic therapy with rifampin (J. Gerber, P. Yamini, and R. Nau, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1788, 1999). Therefore, after initial rifampin therapy, β-lactam antibiotics may be added when the majority of the bacteria have already been killed by rifampin (i.e., 12 to 24 h later).

The available data do not permit the introduction of this concept into clinical routine. They strongly suggest a randomized clinical trial comparing standard therapy (β-lactams) ver-

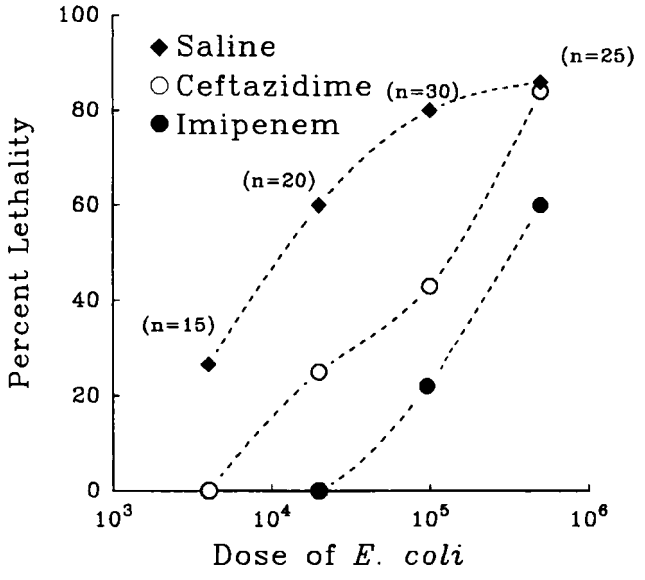

FIG. 5. Protection of mice from *E. coli* induced death by ceftazidime and imipenem. Redrawn from reference 25 with permission of the publisher. Mice were administered 5×10^3 to 5×10^5 CFU of *E. coli* along with a single treatment of saline or antibiotic (20 mg/kg) and D-galactosamine (20 mg/mouse). Bacteria and antibiotic treatments were given intraperitoneally at the same time. At equal bacterial inocula, the percentage of mice surviving was substantially greater with imipenem than with ceftazidime.

sus a protein synthesis-inhibiting antibiotic (rifampin) followed by the combination of rifampin plus β-lactam 12 h later.

Sepsis

Adjunctive corticosteroids. Clinical trials in humans with several immunomodulatory agents, including high-dose methylprednisolone, have been unsuccessful (19). This fact encourages the study of the influence of antibacterials on the release of proinflammatory and toxic bacterial compounds in vivo and on survival.

β-Lactam antibiotics. The potential of antibiotics to cause endotoxin release influenced the outcome of gram-negative sepsis in animal models (25, 79, 127). In mouse models of *E. coli* and *P. aeruginosa* peritonitis/sepsis, where bacteria, D-galactosamine (to sensitize mice for endotoxin), and antibiotics were given at the same time, 20 mg of the PBP-2 inhibitor imipenem per kg resulted in an approximately eightfold increase in the 50% lethal dose (LD_{50}) of bacteria. Conversely, PBP-3-specific ceftazidime led to only a threefold increase in the LD_{50} (Fig. 5) (25). When antibiotic treatment was started 2 h after intraperitoneal inoculation of 4×10^3 *E. coli*, only imipenem provided significant protection (25). In D-galactosamine-sensitized mice infected with an LD_{50} dose of *P. aeruginosa*, treatment with imipenem decreased mortality to 0%, whereas an equivalent dose of ceftazidime increased mortality in comparison to untreated animals (90). Similarly, in sepsis models of *E. coli* and *P. aeruginosa* infection in D-galactosamine-treated rats, imipenem therapy resulted in lower levels of circulating endotoxin and TNF and reduced mortality compared to ceftazidime treatment (127).

Aminoglycosides. In rabbits suffering from *E. coli* sepsis, gentamicin released approximately 10-fold less endotoxin than moxalactam despite equal killing of bacteria. The reduction in endotoxin release, however, did not lead to an increase in survival (150).

As in meningitis, the present data do not allow the uncritical use of this concept in clinical routine but strongly suggest a large clinical trial in strictly selected sepsis patients. The benefit of treatment with bacterial protein synthesis inhibitors is probably greatest in patients with a high bacterial load.

Other Infections

Peritonitis. The intraperitoneal injection of *P. aeruginosa* with imipenem resulted in rounding of the bacteria and strong phagocytosis by peritoneal cells. Administration of *P. aeruginosa* with ceftazidime induced the formation of long filaments and resulted in a decreased number of phagocytizing peritoneal cells and an increased number of viable bacteria recovered from the peritoneal cavity (186).

Plague. In a mouse model of pneumonic plague, early treatment (24 h after infection) with ceftriaxone (250 mg/kg every 6 h) produced 100% protection. At this time point, septicemia was absent. When treatment was initiated 42 h after infection (at this time, mice were bacteremic), mortality was 97.5%. Mice receiving ceftriaxone and other β-lactam antibiotics (cefotetan, cefazolin, ceftazidime, ampicillin, and aztreonam) beginning 42 h after infection died earlier than saline-treated control animals. Although proinflammatory bacterial components were not measured, the authors suggest that the poor performance of β-lactam antibiotics in advanced pulmonic plague may be due to endotoxin release. Consistently, approximately 60% of mice receiving streptomycin, netilmicin, gentamicin, ciprofloxacin, and ofloxacin beginning 42 h after infection survived (27).

RELATIONSHIP BETWEEN PROINFLAMMATORY COMPOUND CONCENTRATION AND OUTCOME IN HUMANS

There are no prospective randomized studies on well-defined severe forms of human sepsis with a sufficiently high number of patients to address the question of whether bacterial protein synthesis inhibitors can decrease mortality. Considering the large number of negative trials in sepsis, a clinical trial addressing the concept of bacterial protein synthesis-inhibiting antibiotics should include a sufficiently high number of patients and aim at a homogeneous study population (i.e., include only patients with a high bacterial load).

Urosepsis. In a small study of patients with urosepsis (two groups of 35 patients), the use of imipenem as the primary antibacterial significantly shortened the interval to defeverescence compared with ceftazidime. After 4 h of treatment, the blood endotoxin levels decreased in patients receiving imipenem, whereas they increased above the pretreatment level in half of the patients receiving ceftazidime. Overall outcome was not different (133). It should be noted, however, that another small study in gram-negative urosepsis comparing imipenem ($n = 14$) and ceftazidime ($n = 19$) found no differences in plasma or urine endotoxin, plasma IL-6 and TNF, and urine IL-6 and IL-8 levels between the two treatment groups (103).

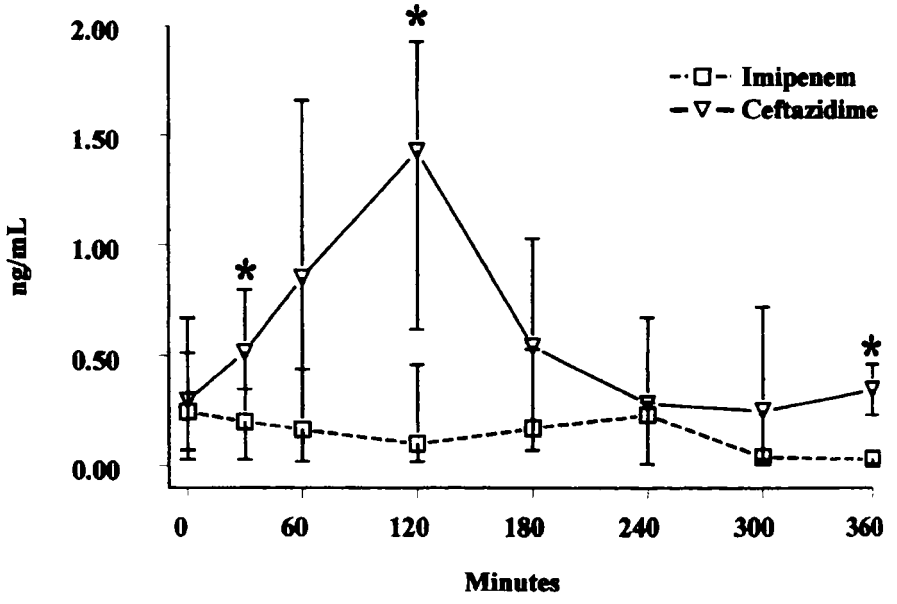

FIG. 6. Plasma endotoxin concentrations after the initial dose of imipenem or ceftazidime ($n = 34$ each) in patients with acute septicemic melioidosis (medians, 25th / 75th quartiles). Reprinted from reference 154 with permission of the publisher. Note the increased endotoxin levels during ceftazidime treatment. *, $P \leq 0.05$.

Melioidosis. In severe melioidosis caused by *Burkholderia pseudomallei*, ceftazidime-treated patients ($n = 34$) had greater systemic endotoxin levels than patients treated with imipenem ($n = 34$) (Fig. 6). However, the difference in endotoxin release did not have a significant impact on survival in these patients (154).

Septic trauma. A post hoc analysis of a randomized trial designed to test the efficacy of gamma interferon yielded a mortality of 17% in patients treated with antibacterials preferentially binding to PBP-3 versus a mortality of 8% in patients treated by antibiotics not binding to PBP-3 ($P = 0.02$) (115).

Invasive *Streptococcus pyogenes* infections. Patients with deep infection were more likely to have a favorable outcome when initial treatment included a protein synthesis-inhibiting antibiotic (mainly clindamycin) than when treated exclusively with cell wall-inhibiting agents (189). The authors of the latter study hypothesize that protein synthesis inhibitors such as clindamycin may work by decreasing the production of extracellular bacterial compounds which play a role in the pathogenesis of systemic toxicity or tissue destruction (189).

Meningitis. In children with *H. influenzae* meningitis, initial total endotoxin concentrations in CSF correlated with the severity of the disease and with the number of febrile hospital days. Ceftriaxone treatment induced a pronounced increase in free endotoxin in CSF, which was associated with a rise in CSF lactate and a decrease in CSF glucose levels (4). Increased endotoxin and IL-1 concentrations in CSF and poor outcomes have been reported in infants treated with intraventricular gentamicin compared with infants receiving intravenous antibiotics alone for meningitis caused by gram-negative enteric bacilli (119). This has been attributed to the rapid release of endotoxin by high aminoglycoside concentrations in CSF. In adults with *S. pneumoniae* meningitis, clinical outcome was correlated with the LTA concentration in the CSF drawn upon admission (146).

IMMUNOMODULATORY AND TISSUE-PROTECTIVE ACTION OF ANTIBACTERIALS

A variety of immunomodulatory actions of antibacterials suggesting direct effects of these compounds on eukaryotic cells have been noted, but these have not always been reproducible. The exact underlying mechanism(s) and clinical significance of these observations frequently remain to be determined.

Rifampin. Immunosuppression, including inhibition of T-cell activity, has long been noted during rifampin therapy (62). The compound has been claimed to bind at glucocorticoid receptors, thereby acting as an immunosuppressant. Recent studies in human alveolar and neuroblastoma cells and in mouse hippocampal cells, however, have found no evidence of activation of glucocorticoid receptors by rifampin (69, 81).

Minocyclin. Minocyclin inhibits caspase-1 and caspase-3 expression, thereby delaying mortality in a transgenic mouse model of Huntington disease (31). In a rat model of ischemic stroke, minocycline (45 mg/kg intraperitoneally twice on the first day and then 22.5 mg/kg for the subsequent 2 days) reduced the infarct size when started before and up to 4 h after the onset of ischemia (188).

Fosfomycin. In a mouse model of gut-derived *P. aeruginosa* sepsis, treatment with an isomer of fosfomycin without antibacterial activity significantly increased the survival rate in comparison to saline-treated mice. Apparently, the fosfomycin isomer possessed immunomodulatory activity inducing protection against *P. aeruginosa* bacteremia (109).

Quinolones. Trovafloxacin reduced the in vitro production of TNF-α, IL-1β, and IL-6 by LPS-stimulated human monocytes (87). In vivo, trovafloxacin (100 mg/kg), ciprofloxacin (250 mg/kg), and tosufloxacin (100 mg/kg) protected 75, 25, and 50%, respectively, of mice challenged with a lethal dose of *E. coli* LPS against death (86). The mechanism of this effect

remains unclear, and in alveolar macrophages TNF production after LPS stimulation was not decreased by ciprofloxacin (124).

Macrolides and ketolides. Erythromycin and other macrolides inhibit cytokine production (TNF-α and IL-6) in leukocytes in vitro. In contrast, upon stimulation with heat-inactivated *S. pneumoniae*, erythromycin treatment increased the release of neutrophil degranulation products bactericidal/permeability-increasing protein, neutrophil elastase, and lactoferrin in human blood (147). From 5 to 80 μg of clindamycin per ml reduced TNF-α production by monocytes upon LPS stimulation by 30 to 40% (158). The ketolide HMR3004 reduced the release of IL-6 and IL-1β into bronchoalveolar lavage fluid, leukocyte infiltration, and lung edema after challenge with heat-inactivated pneumococci (45).

β-Lactam antibiotics. The cephalosporin cefodizime enhanced immune parameters such as natural killer and phagocytic activity in immunocompromised animals. In a mouse pneumonia model, it upregulated the early *Klebsiella*-induced secretion of TNF-α and the number and phagocytic efficacy of alveolar macrophages (11). Prophylactic administration of cefodizime increased the survival of some mouse strains after infection with *Toxoplasma gondii* or *Candida albicans* (101). In contrast, cefodizime inhibited the LPS-stimulated release of TNF and IL-1 from human monocytes (140) and TNF-α and IL-6 secretion into the bronchoalveolar lavage fluid after intranasal challenge with heat-killed fluorescein isothiocyanate-labeled pneumococci (12).

In healthy rats, an intravenous bolus of 30 mg of ceftazidime per kg led to a substantial increase in IL-6 and TNF-α concentrations in serum (1). This observation suggests that the increase in parameters of inflammation occurring after initiation of ceftazidime therapy may be a consequence not only of the release of proinflammatory bacterial compounds, but also of a direct immunostimulatory action of ceftazidime. In alveolar macrophages stimulated with *P. aeruginosa* LPS, however, ceftazidime, imipenem, aztreonam, amikacin, and ciprofloxacin did not influence TNF release (124).

Clinical significance. Low-dose macrolide therapy has greatly increased survival in patients with diffuse panbronchiolotis, a chronic inflammatory airway disease that is relatively frequent in the Far East, with a high mortality during conventional treatment (98). This has led to investigations concerning macrolide use in cystic fibrosis, bronchiectasis, and asthma. Preliminary results in patients with cystic fibrosis are encouraging. Randomized studies in patients with cystic fibrosis, bronchiectasis, and asthma, however, have not been completed yet, i.e., routine use of macrolides for these indications cannot be recommended at this time (80). For other indications, at present there is no place for attempts to modulate the immune response by antibacterials in clinical routine. Several effects, however, deserve attention for future research in humans.

CONCLUSIONS

Bacterial products stimulate monocytes, macrophages, and endothelial and glial cells and can exert direct cytotoxicity. Initiation of treatment by cell wall-active antibiotics causes a rapid release of proinflammatory or toxic compounds from both gram-negative and gram-positive bacteria. This phenomenon is less pronounced with PBP-2 than with PBP-3-specific β-lactam antibiotics and can be absent when antibiotics inhibiting bacterial protein synthesis are employed. High antibiotic concentrations release smaller amounts of bacterial compounds than doses close to the MIC. Quantitative differences in the release of proinflammatory bacterial compounds correlate with antibiotic-initiated morphological changes in the bacteria.

With a variety of bacteria, the release of mediators of inflammation by cultured cells and in experimental animals depends on the mode of action and the dose of the antibiotic employed. In several animal models of infection, antibiotic regimens aimed at reducing proinflammatory bacterial compounds improve outcome compared to standard therapy. The clinical importance of this strategy, however, is still unclear.

Sufficient in vitro and experimental animal data to validate this concept have been accumulated to justify large clinical trials for bacterial meningitis and sepsis. In randomized trials available for septicemia and other systemic infections, the number of patients was too small and the mortality in the control group was too low to detect a decrease in mortality by antibacterials that reduce the release of bacterial compounds with sufficient statistical power. For the planning of appropriate trials, it has to be considered that in the past all major studies of immunomodulators in sepsis have yielded disappointing results despite having shown promise during preliminary clinical studies (10), for several reasons. The studies used the sepsis syndrome as an entry criterion. This allowed the inclusion of a substantial number of intensive-care patients in clinical trials, but accepted a very heterogeneous study population. Also, although several hundred patients were included, the heterogeneity of the study population impeded the detection of a significant benefit of experimental therapies.

A properly conducted study on the possible benefit of bacterial protein synthesis inhibitors versus β-lactam antibiotics will require both stricter selection and a larger number of patients. We hypothesize that the benefit of the approach discussed will be greatest in patients with a high bacterial load in the bloodstream or in the CNS.

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