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Treatment of Bacterial Infections with β -Lactams: Cooperation with Innate Immunity

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ABSTRACT β -Lactams are the most widely prescribed antibiotics used for the control and treatment of bacterial infections. The direct effect of β -lactams on bacteria is well studied worldwide. In the context of infections and as a consequence of their direct activity against the pathogen, β -lactams also regulate antibacterial immune responses. This knowledge has led to the theorem that the effectiveness of β -lactam treatment results from the synergy between the drug and the immune response. Key players in this immune response, with an essential role in the clearance of live and dead bacteria, are the myeloid cells. In this review, we summarize the data that shed light on how β -lactams interact with myeloid cells during bacterial infection treatment.

KEYWORDS β -lactams, bacterial infections, pathogen-associated molecular patterns, neutrophils, monocytes, proinflammatory responses, phagocytosis

The discovery of antibiotics to treat bacterial infectious diseases was a remarkable achievement in the 20th century. Unfortunately, though, the overuse and misuse of antibiotics have promoted the accelerated emergence of antimicrobial resistance that, in combination with the drying out of antibiotic discovery pipelines, has led to the postantibiotic era (1, 2). Antibiotics are drugs that stop or interfere with essential physiological processes, resulting in growth inhibition or death of bacteria. It has been proposed that antibiotic therapeutic effectiveness is also dependent on host immunity, as antibiotics are often not as effective in immunocompromised individuals (3, 4). A better understanding of the complex relationship between antibiotics and host immunity during the treatment of infection will help optimize the use of antibiotics.

The β -lactams, including penicillins, cephalosporins, monobactams, and carbapenems, constitute a major family of antibiotics. Compared to other classes of antibiotics, β -lactam drugs are safe and well tolerated (5). The β -lactams share a structure, i.e., a β -lactam ring. β -lactams inhibit peptidoglycan (PG) cross-linking by inactivating penicillin-binding proteins (PBPs), composed of synthases and hydrolases. By modifying the synthase-hydrolase balance, β -lactams disrupt cell wall integrity and lead to bacterial lysis and death (6). In turn, bacterial products, including pathogen-associated molecular patterns (PAMPs), are released and modulate antimicrobial immunity through sensing by pattern recognition receptors (PRRs) expressed by sentinel cells (4).

A β -lactam is considered highly effective when the concentration is above the minimal inhibition concentration (MIC) where it kills bacteria, leading ultimately to activation of innate immunity. When the bacteria are exposed to antibiotic concentrations below the MIC (sub-MIC), it can induce changes in bacterial gene expression, protein synthesis, metabolism, and growth properties that also influence immune responses. The goal of the present review is to provide an overview of the impact of β -lactams on innate immunity during the treatment of bacterial infections.

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INNATE IMMUNE RESPONSES TO BACTERIAL INFECTIONS

The detection of bacteria by the invaded host activates a broad range of innate immune mechanisms, i.e., effective defense responses against infection. The sensing is achieved by PRRs, receptors largely conserved through vertebrate evolution and expressed by a wide range of sentinel cells, including structural and hematopoietic cells. There are four major subfamilies of PRRs, (i) Toll-like receptors (TLRs), (ii) nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), (iii) retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and (iv) C-type lectin receptors (CLRs). More recently, the cyclic-GMP-AMP synthase (cGAS) was identified as a major STimulator of INterferon Genes (STING)-dependent cytoplasmic pathogen DNA receptor. PRRs are strategically located in the cell: they are expressed at the cell surface (CLR; TLR2, -4, -5, etc.) to recognize extracellular pathogens, within the endosome membranes (TLR3, -7, -8, and -9) or in the cytoplasm (NLR, RLR, cGAS/STING), to sense intracellular invaders. These receptors recognize conserved danger signals known as PAMPs found in bacteria, viruses, parasites, or fungi, namely, viral and bacterial nucleic acids, flagellin, PG, or PG-associated components such as teichoic acids (TA) or lipoteichoic acids (LTA), lipopolysaccharides (LPS), and carbohydrates (e.g., zymosan). PAMPs are specific for microorganisms and are required for essential functions. Binding of PAMPs to PRRs rapidly stimulates the release of immune mediators, e.g., inflammatory cytokines (interleukin 6 [IL-6], tumor necrosis factor [TNF], IL-1 β), chemokines, antimicrobial effector molecules (antimicrobial peptides, reactive oxygen species, etc.), or phagocytic processes that orchestrate anti-infectious responses (reviewed in references 7 and 8).

Myeloid cells are the first line of defense against bacterial infections. Myeloid cells display an important phenotypic diversity and are mainly composed of monocytes, macrophages, neutrophils, and dendritic cells. Most tissues harbor a network of resident myeloid cells, mainly macrophages and dendritic cells, that immediately sense bacterial infection and, by producing proinflammatory molecules, further participate in pathogen elimination. The blood-circulating monocytes and neutrophils are rapidly mobilized into the tissue in response to infection (9). Neutrophils and monocytes, as well as tissue-resident macrophages, exhibit strong phagocytic and bactericidal activities, leading to clearance of bacteria, whereas dendritic cells more readily initiate pathogen-specific adaptive immunity (10). Importantly, after their migration into tissues, monocytes have the ability to differentiate into (i) inflammatory dendritic cells that further play a role in shaping the ensuing adaptive immune response, or (ii) macrophages to reconstitute the pool of tissue-resident cells (11). Neutrophils and monocytes/macrophages also contribute to resolution of inflammation (phagocytosis of cell debris, neutrophils, production of resolvins).

Through their activity on bacteria and the release of PAMPs, the β -lactams could influence the anti-infectious response of myeloid cells that, in turn, may impact the effectiveness of the treatment. Thus, the therapeutic effectiveness of β -lactam combines (i) the bactericidal effect of the antibiotic, (ii) the PAMP/toxin modulation of immune cells, and (iii) the direct effect of the antibiotic on immune cells (Fig. 1).

NATURE OF PAMPS RELEASED BY β -LACTAM-EXPOSED BACTERIA

Regarding Gram-negative bacteria, LPS (also known as endotoxin) represents a major component released after bacterial death or injury. LPS is involved in sepsis/septic shock induced by Gram-negative bacteria (12). Endotoxin release has been largely studied *in vitro* and *in vivo* upon β -lactam treatment of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* infections (13). The amount of released LPS is highly dependent on the class and concentration of β -lactams. For example, cephalosporins that are specific for targeting PBP-3 induce the release of larger amounts of endotoxin than the PBP-2-targeting carbapenems (13, 14). Moreover, concentrations of cephalosporins below or close to the MIC induce a stronger release of LPS *in vitro* and *in vivo* compared to high concentrations (50 \times MIC) (14, 15). Interestingly, elongation of *P. aeruginosa* and *E. coli* (also called filamentation), as opposed to their rod-shaped structure, is associated with a robust LPS release upon β -lactam treatment (14–16).

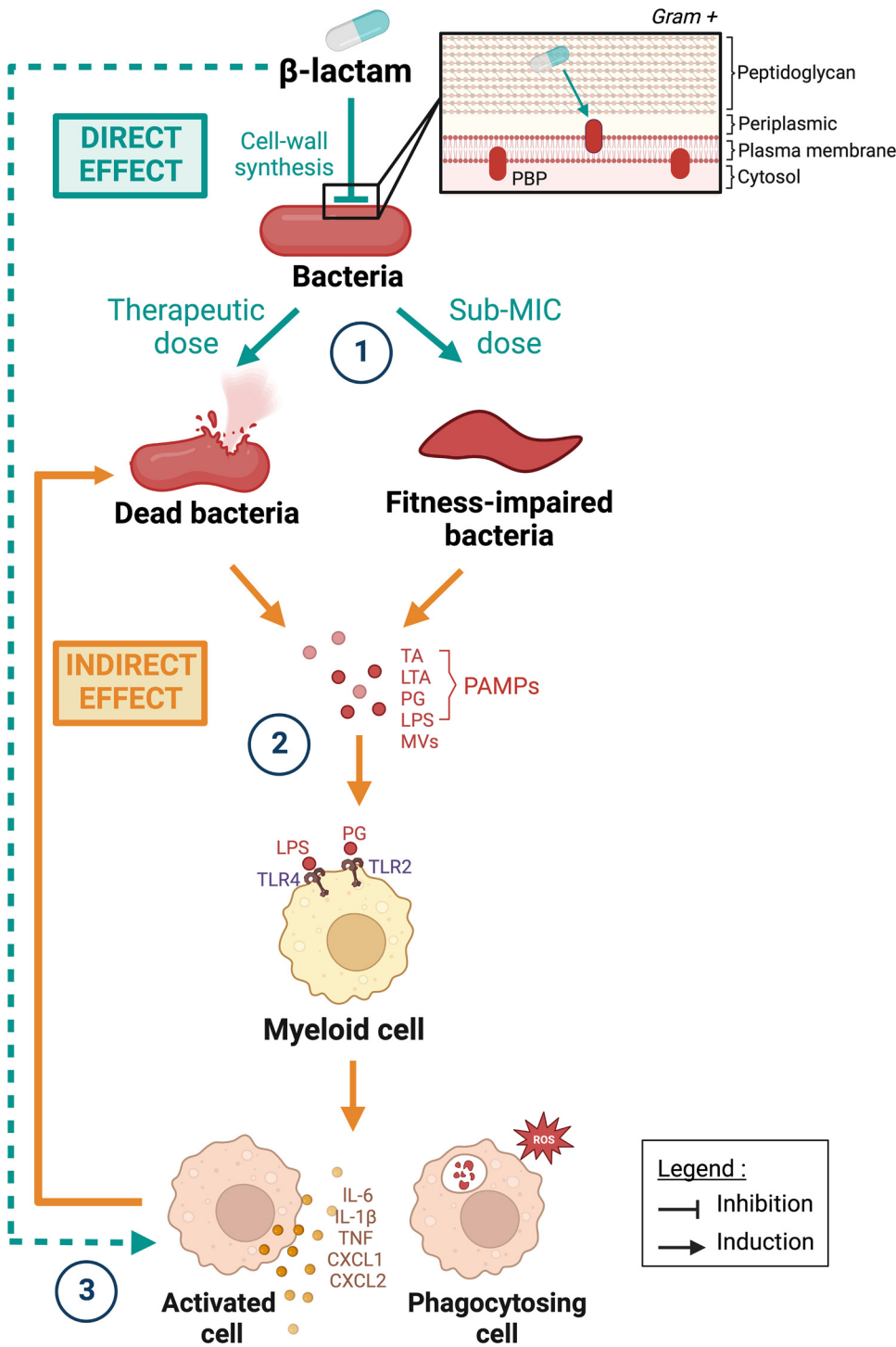


FIG 1 Direct and indirect effects of the β -lactams. β -Lactams are antimicrobial drugs that target penicillin-binding proteins (PBP) and inhibit peptidoglycan (PG) synthesis, the main component of bacterial cell walls (shown are Gram-positive [Gram⁺] bacteria). Upon β -lactam exposure, the bacterial cell wall is either disrupted and cell lysis occurs (therapeutic dose) or damaged, leading to impaired fitness (sub-MIC dosing) (1, blue arrows, direct effect). Pathogen-associated molecular patterns (PAMPs), such as teichoic acid (TA), lipoteichoic acid (LTA), peptidoglycan fragments (PG), or lipopolysaccharide (LPS) (Gram-negative bacteria), as well as bacterial membrane vesicles (MVs), are subsequently released and recognized by myeloid cells via pattern recognition receptors such as Toll-like receptor 2 (TLR2) or TLR4. Then, TLR triggering results in the release of proinflammatory cytokines and chemokines (such as IL-6, TNF, IL-1 β , CXCL1 and CXCL2) as well as reactive oxygen species (ROS) production following phagocytosis of bacteria. (2, orange arrows, indirect effect). Finally, myeloid cells could also be directly activated by β -lactams, although targets still remain not defined (3, dotted blue arrow).

Buijs et al. confirmed that clinical isolates of antibiotic-sensitive and -resistant strains of *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter baumannii* form filaments and produce a large amount of LPS when exposed to low doses of β -lactams (17). Of note, in the case of antibiotic-resistant bacteria, filament formation is extended to concentrations far above the MIC. These findings in animal models should, however, be taken with caution since clinical studies did not find any relationship between the class or concentration of β -lactams, the amount of LPS in the sera, and the disease severity in humans (18–21). In addition to LPS, carbapenem-exposed *P. aeruginosa* releases PG fragments (22). However, PG release is independent of carbapenem concentrations.

Regarding Gram-positive bacteria, β -lactams enhance the release of LTA and PG fragments *in vitro*, as shown for *Staphylococcus aureus* and *Staphylococcus epidermidis* (Fig. 1) (23, 24). Killing of *S. aureus* is concomitant with the degradation of the cell wall and the release of LTA or PG. The discharge of PG and LTA from penicillin- or oxacillin-exposed *S. epidermidis* is in line with TNF secretion by monocytes. Interestingly, treatment of methicillin-resistant *S. aureus* (MRSA) with cefoxitin does not increase the release of PG but induces structural modification to PG, rendering it more inflammatory and thus exacerbating the immune response (25). A rapid and robust release of TA and LTA is also observed when *Streptococcus pneumoniae* is exposed to ceftriaxone or meropenem (26, 27). The LTA and TA concentrations in bacterial supernatants negatively correlate with antibiotic concentrations. Based on the mathematical model, ceftriaxone and meropenem were ranked as the most effective antibiotics with regard to LTA release per bacterium compared to quinolones or macrolides (28). The loosening of the bacterial cell wall and bacterial death upon β -lactam exposure can also result in the discharge of exotoxins from Gram-positive bacteria. Pneumolysin, a pore-forming toxin and a major virulence factor of *S. pneumoniae*, is detected in the cerebrospinal fluid (CSF) of meningitis patients, and ceftriaxone treatment further enhances pneumolysin levels in the CSF (29, 30). Similar effects are observed *in vitro* using cultures of ceftriaxone-exposed *S. pneumoniae* (28). Depending on the concentration, pneumolysin can act directly as a toxin (high dose) on mammalian cells or by stimulating the host's inflammatory response (low dose) after TLR4 engagement and/or NLRP3 inflammasome activation (see below).

Over the last decade, numerous studies have revealed a major role of bacterial membrane vesicles (MVs) in a wide range of biological processes, including virulence, antibiotic resistance, and modulation of host immunity (31). MVs are produced by almost all bacteria and contain several PAMPs, including nucleic acids, endotoxins, and fragments of PG or LTA. Exposure of *A. baumannii* to cephalosporin, in contrast to carbapenem, enhances the release of MVs. Moreover, antibiotic-conditioned MVs show high proinflammatory activity when injected into mice (32). Proteomic analysis highlighted differences in protein/virulence factor contents of MVs depending on the antibiotic used, and additional studies are underway to identify the specific determinants associated with proinflammatory activity. Interestingly, when exposed to a high dose of β -lactams, the antibiotic-resistant *Stenotrophomonas maltophilia* releases MVs packed with β -lactamases that cross-protect antibiotic-susceptible *P. aeruginosa* or *S. pneumoniae* from exposure to β -lactams (33).

CONSEQUENCES OF PAMPs ON MYELOID CELL FUNCTIONS

PAMPs are detected by PRRs expressed in sentinel cells and induce an immediate immune response. For example, LPS is recognized by TLR4, LTA by TLR2, and pneumolysin by TLR4 and the NLRP3 inflammasome (34–36). Upon β -lactam treatment, MRSA or *S. pneumoniae* releases cell wall components, such as lipoproteins, that trigger TLR2 signaling in sentinel cells (37, 38). The ultimate outcome of PRR triggering is cell activation, mainly characterized by the production of inflammatory cytokines and/or reactive oxygen species (ROS), and the increase of cytotoxic activity or phagocytosis, leading to bacterial cell death.

Effects of β -lactam-exposed bacteria on myeloid cell responses. Studies highlight that short-term β -lactam treatments stimulate proinflammatory responses (reviewed in reference 39). Interestingly, ceftazidime-exposed *E. coli* promotes inducible nitric oxide

synthase (NOS) and TNF production by mouse peritoneal macrophages, whereas imipenem does not. This correlates with a larger amount of LPS released upon ceftazidime compared to imipenem treatment. On the other hand, exposure of *S. aureus* to both antibiotics induces a similar release of the virulence factor protein A, which correlates with an enhanced TNF secretion but a decreased inducible nitric oxide synthase (iNOS) production by macrophages (40). Thus, the ensuing proinflammatory response is specifically regulated according to the class of β -lactams, the nature of the bacteria, and the amount of PAMPs released upon damage or death of bacteria. Further investigations will be needed to identify the underlying molecular mechanisms and the relative contribution of individual factors.

When mouse macrophages are exposed to ceftriaxone prior to any interaction with bacteria, an increased adhesion of *Salmonella enterica* serovar Typhimurium to cells is observed, concomitant with an enhanced expression of IL-1 β and TNF compared to untreated macrophages (41). This major finding points out that an antibiotic alone can enhance the functions of immune cells in the absence of any bacteria. However, the molecular mechanisms and targets of antibiotics on immune cells remain to be defined. Although β -lactams are ineffective at killing β -lactam-resistant bacteria, they achieve sub-MICs and can thus enhance the proinflammatory response of myeloid cells, as shown for MRSA exposed to ceftiofuran (25). However, the resulting immune responses in the absence of any bacteriostatic or bactericidal effects are deleterious and further enhance immunopathology.

Regarding antibacterial activity of myeloid cells, reports show that the cephalosporin cefodizime stimulates phagocytosis of macrophages or neutrophils under *in vitro*, *ex vivo*, and *in vivo* conditions (42). For example, in patients with severe infections caused by *E. coli*, *S. pneumoniae*, *Neisseria meningitidis*, and *S. enterica* serovar Typhi, both cefodizime and ceftriaxone treatments trigger the phagocytic activity of neutrophils *ex vivo*, a phenomenon correlated with a higher generation of ROS (43). The ROS-dependent killing potency of neutrophils is increased rapidly after therapy initiation and returns to baseline within 3 to 7 days. The mechanisms involved in the potentiation of phagocytosis and bactericidal activities by cephalosporins may be used as targets of adjunct therapeutics to improve cure of infection. Increased phagocytic/killing activities of human neutrophils are also observed after *S. aureus* exposure to flucloxacillin, a penicillin-type antibiotic (44). The driving force of myeloid cell maturation is the LTA released by antibiotic-exposed bacteria, and it is specific to β -lactams, as macrolides or fluoroquinolones have no effect. Finally, β -lactams may also impact neutrophil cell death after bacterial killing (45). In the case of *E. coli*, imipenem treatment promotes neutrophil apoptosis, whereas ampicillin induces neutrophil necrosis. Although the underlying mechanisms remain unknown, apoptosis versus necrosis is dependent on the LPS released by *E. coli*; thus, high concentrations of LPS are closely associated with neutrophil necrosis.

Effectively, when exposed to β -lactams, bacteria further stimulate proinflammatory and phagocytic/killing activities of myeloid cells, although these functions are differentially regulated depending on the class of β -lactams.

Myeloid cell responses in sub-MIC dosing of β -lactams. As optimal antibiotic dosing is difficult to achieve in infected tissue, exposure of bacteria to subinhibitory doses of antibiotics is a frequent occurrence. The major effects of sub-MICs of antibiotics on bacterial physiology and proinflammatory responses were recently reviewed (4). Subinhibitory doses of antibiotics initiate a microbial stress response characterized by an altered expression of virulence factors promoting bacterial persistence and a sustained proinflammatory environment. For example, exposure of MRSA to sub-MIC of oxacillin modifies expression of virulence factors that enhance IL-6 and TNF production by mouse macrophages and aggravate the outcome of infection (37). Along the same lines, morphological alterations of the *Streptococcus suis* capsule are observed at sub-MIC levels of amoxicillin, and they further increase production of IL-1 β , IL-8, and IL-6 by human macrophages (46). In the case of *S. pneumoniae*, sub-MIC levels of penicillin enhance the production of TNF by human

macrophages, which is a finding of note, considering that the TNF cytokine has been associated with greater meningitis severity (47). Thus, optimization of β -lactam antibiotic treatment is essential to avoid the risk of underdosage, particularly in patients with comorbidities, that could lead to a detrimental inflammatory response.

INTERACTIONS BETWEEN β -LACTAMS AND IMMUNITY IN CLINICALLY RELEVANT TREATMENT OF INFECTIONS

Infections with Gram-positive bacteria. Curative treatment with β -lactams first stimulates proinflammatory responses and is then followed by reduced systemic inflammation and pathology. In *S. pneumoniae*-infected patients, blood concentrations of proinflammatory cytokines (TNF, IL-1 β , IL-6, IL-8, IL-10, and IL-1RA) decrease over time upon treatment with ceftriaxone (48). A recent study revealed that β -lactam treatment of patients with *S. aureus* bacteremia generates a favorable immune response related to enhanced IL-1 β and decreased IL-10 production, two cytokines strongly associated with favorable clinical outcome (49). In contrast, the ceftriaxone-related ceftazidime has a reduced therapeutic effectiveness when *S. aureus* is located in abscesses (50). The unfavorable outcome correlates with infiltrating neutrophils that are unable to kill *S. aureus*. When ceftazidime is combined with an additional β -lactam, i.e., ertapenem, treatment effectiveness is restored (51): The protective effect could be related to the sensitization of the bacteria to antimicrobial peptides (AMPs) and/or an enhanced IL-1 β production (51, 52).

Most clinical studies performed in patients with acute and severe diseases relevant to critical care, e.g., community-acquired pneumonia (CAP), point to the beneficial effects of combining β -lactam antibiotics with macrolides (53–55). It was reported that the β -lactam-macrolide therapy decreases the mortality of patients with pneumococcal CAP and is more effective than the β -lactam-fluoroquinolone combination (53). The beneficial effect of macrolides may be linked to their immunomodulatory effects (reviewed in reference 56). A recent retrospective study on hospitalized CAP patients points out the beneficial effect of combining β -lactams with tetracyclines (57). Apart from their antimicrobial and immunomodulatory effects, the tetracycline protective effect may be driven by activity on tissue repair and/or liver metabolism reprogramming (58–60).

In line with human studies, β -lactam-macrolide combined therapy is also effective in the treatment of primary and secondary pneumococcal infections in mice (61–63). In primary infection, the protective effect is associated with an upregulation of MCH II and CD86 in dendritic cells and macrophages and a downmodulation of the expression of CTLA-4 and PD-1 on CD4-positive (CD4⁺) T lymphocytes. Although further investigations are needed to decipher the mechanisms, the activation of dendritic cells and macrophages and the decreased expression of immune checkpoint molecules could lead to an effective and accelerated immune response recovery. In secondary (postinfluenza) pneumococcal infections, β -lactam-macrolide combination therapy protects 90% of animals compared to 50% with stand-alone β -lactam treatment (61). The treatment with β -lactam enhances the inflammatory response, which could account for the lowered efficiency, whereas addition of macrolide dampens the proinflammatory response (62). The protection by the combination of β -lactam-macrolide is associated with a strong reduction of neutrophil infiltration into the lungs. A possible mechanism for protection would imply myeloid-derived suppressive cells (MDSC). Indeed, it was recently shown that the macrolide clarithromycin protects mice from secondary pneumococcal pneumonia through the expansion of CD11b⁺ Gr1⁺ MDSC-like cells and STAT3-dependent IL-10 production (64).

Several reports highlighted the beneficial effects of combining β -lactams with anti-inflammatory molecules. In *S. pneumoniae*-infected animals, the combination of ceftriaxone with the phosphodiesterase-4 inhibitor rolipram dampens inflammatory responses and enhances macrophage phagocytosis, bacterial clearance, and survival compared to monotherapy with the antibiotic (65). The protection is associated with

an enhanced expression of annexin A1, which is known to be involved in the resolution of inflammation. Ibrutinib, a potent and irreversible inhibitor of Bruton's tyrosine kinase, improves protection of *S. pneumoniae*-infected animals treated with ceftriaxone (66). Ibrutinib decreases the number of pulmonary and blood monocytes-macrophages and neutrophils as well as the activation threshold of cells but has no intrinsic bactericidal or bacteriostatic activity. Of note, ibrutinib inhibits inflammatory responses in a model of LTA acute lung inflammation. Thus, combination therapies, including (i) a β -lactam that clears bacteria, and (ii) drugs that regulate inflammatory responses, appear largely beneficial to combat β -lactam-sensitive Gram-positive bacterial infections, although underlying cellular/molecular off-targets are not defined.

The combination of antibiotics and AMPs, effector molecules of the innate immune response, represents a promising approach to combat antibiotic-resistant bacteria (67). In the case of MRSA, β -lactams sensitize bacteria to killing by AMPs such as the cathelicidin LL-37 produced by neutrophils (68). The study by Sakoulas et al. suggests that β -lactams are highly efficient at rendering antibiotic-resistant bacteria susceptible to host innate immune effectors like AMPs *in vivo*. Along the same lines, our own research has focused on developing novel therapies, which combine β -lactams with stimulators of the innate immune response, to combat pneumonia caused by *S. pneumoniae*. In a mouse model of primary or secondary pneumonia caused by *S. pneumoniae*, the therapeutic efficacy of associating a low dose of the β -lactam amoxicillin with stimulators of TLRs, i.e., the TLR4 agonist, monophosphoryl lipid A (MPLA), or the TLR5 agonist, flagellin, was assessed (69–71). A synergistic effect of MPLA or flagellin combined with amoxicillin in the treatment of bacterial pneumonia was shown. Of note, the amoxicillin-flagellin combined therapy is also effective in infections caused by antibiotic-resistant strains of *S. pneumoniae*. Both MPLA and flagellin rapidly trigger pulmonary production of inflammatory molecules, as well as an innate immune response associated with efficient protection. The underlying mechanisms of protection of both combined therapies are under investigation and might involve myeloid cells such as monocytes (MPLA/amoxicillin) or neutrophils (flagellin/amoxicillin).

Infections with Gram-negative bacteria. Regarding Gram-negative bacteria, very little information on the relationship between β -lactams and the immune response during the treatment of bacterial infection is available. Using a mouse model of *K. pneumoniae* lung infection, it was reported that doripenem, in contrast to imipenem and meropenem, enhances IFN- γ and IL-6 blood levels, both being crucial for activation of killing properties of macrophages and neutrophils (72). Moreover, the number of alveolar macrophages is enhanced upon treatment, whereas the lung neutrophil population is decreased, a process associated with a rapid resolution of infection/inflammation. Doripenem pharmacokinetics in blood and lung may differ from other carbapenems, leading to an inflammatory response that improves clinical outcomes; however, further studies are needed to elucidate the mechanisms of doripenem protective response and specificities compared to other carbapenems.

There is also a growing interest in combination therapies for the treatment of infections caused by Gram-negative bacteria in humans (73) that are often difficult to treat. The curative failure may be due to the pathogenicity of the bacteria or the increasing development of resistance to high concentrations of multiple antibiotics. For instance, β -lactams are relatively unsuccessful compared to aminoglycosides in the treatment of common urinary tract infections (UTIs), mainly due to increased β -lactam resistance of *E. coli*, one of the main causative agents of UTIs (74, 75).

All combined therapies include a β -lactam due to its bactericidal activity, its broad spectrum, and its safety profile. As reported for Gram-positive bacteria, exposure of *S. enterica* serotype Newport to β -lactams enhances its susceptibility to killing by LL-37 and human neutrophils, a promising approach to treat serious infections such as *Salmonella* meningitis (76). Two recent studies also highlight the beneficial effect of combining imipenem with molecules showing anti-inflammatory properties, such as the inhibitor of cholesterol biosynthesis, atorvastatin, and the NF- κ B inhibitor,

rographolide (a water-soluble form of a natural diterpenoid herb extract) (77, 78). In acute lung infection caused by *K. pneumoniae*, both combinations improve mouse survival and are associated with a decrease of (i) TNF, IL-6, CXCL1, and CCL12 production; (ii) lung injury; and (iii) inflammatory cell recruitment in the lungs. Atorvastatin also enhances the phagocytic activity of macrophages and neutrophils (77). Similarly, in acute lung infection caused by *P. aeruginosa*, the codelivery of ceftazidime and resolvin D1 by using nanovesicles dramatically decreased the inflammation and the bacterial load, the latter being associated with an enhanced phagocytosis of neutrophils and macrophages (79).

Overall, most of the literature highlights the enhanced efficacy of therapies combining β -lactams with macrolides or anti-inflammatory drugs in the treatment of bacterial infections. There is also an increasing interest in developing alternative therapeutic strategies, such as targeting innate immunity to improve β -lactam efficacy, against bacterial infections. Triggering the proinflammatory response by means of TLR agonists represents a promising approach for a rapid bacterial clearance and the resolution of inflammation.

A DIRECT EFFECT OF β -LACTAMS ON IMMUNE CELLS?

Studies on the direct effects of β -lactam on myeloid cell functions, i.e., without bacteria, remain rare. The lack of known off-target activities of β -lactams makes these antibiotics safe. Short-term treatment of healthy volunteers with amoxicillin (and clavulanic acid as a β -lactamase inhibitor) did not significantly impact neutrophil phagocytosis or TNF production by monocytes (80). However, long-term treatment of infected patients with β -lactams can cause severe neutropenia (reviewed in reference 81). The mechanisms of neutropenia remain unclear and might be secondary to direct toxicity of β -lactams on host tissues. A recent study suggests that neutropenia could be associated with loss-of-function polymorphisms in the multidrug resistance protein 4 gene (*MRP4*), encoding a drug efflux transporter (82). This transporter is expressed on renal proximal tubule cells and stem cells in the bone marrow. *MRP4* is also a known protective factor against drug-induced toxicity of hematopoietic cells. As β -lactams are substrates of *MRP4*, a loss-of-function polymorphism may enhance the plasmatic concentration of antibiotics that may induce damage to stem cells. Of note, there is no report on monocyte and/or macrophage depletion during long-term β -lactam therapies.

Direct interferences of β -lactams with phagocyte functions *in vitro* have been reported: the main impact is the enhancement of phagocytic activity, cell survival, activation, and/or oxidative burst by human and/or mouse neutrophils, either unstimulated or stimulated with opsonized particles/zymosan or purified LTA from *S. aureus* (83–85). Moreover, amoxicillin can increase the generation of neutrophil extracellular traps by mitogen-activated human neutrophils (86). Using the LPS-conditioned human monocytic cell line THP-1, Bode et al. showed that piperacillin stimulates higher IL-1 β and IL-6 production, as opposed to macrolides, tetracyclines, and fluoroquinolones (87). In contrast, piperacillin inhibits the phagocytic activity of monocytes. A combination of two β -lactams was shown to stimulate IL-1 β secretion by peripheral blood mononuclear cells in the absence of bacteria (52). The IL-1 β production was exacerbated in the presence of *S. aureus*. These studies highlight possible off-target effects of β -lactams, at least *in vitro*, and additional investigations would be necessary to unravel the mechanisms and identify the molecular target(s) that interfere with phagocyte functions. A direct effect of β -lactams on myeloid cells could be of major importance in patients with delayed-type hypersensitivity (DTH) reactions to amoxicillin. The antibiotic could directly operate on dendritic cells similarly to a danger signal and promote partial functional maturation, characterized by strong surface expression of MHCII, CD83, CD80, and CD86 molecules but low endocytic properties (88, 89). When combined with TLR agonists *in vitro*, dendritic cells from allergic patients undergo a complete functional maturation, i.e., secretion of IL-12 and stimulation of T helper 1-biased T cells (90). Thus, identifying potential off-target activities of β -lactams could be of major importance for the treatment of patients with DTH reactions to antibiotics.

CONCLUSIONS

Although β -lactams are direct antibacterial agents, growing evidence suggests that β -lactams also interact with host innate immunity to provide potent indirect effects that enhance bacterial clearance and may result in more rapid and complete cure of infections. Whereas antibiotic treatment of infections is currently compromised worldwide by the emergence of multidrug-resistant bacteria, understanding innate immunity-antibiotic relationships is of prime interest to improve treatment effectiveness. Indeed, innate immune defenses against pathogenic bacteria mobilize a broad range of antibacterial mechanisms. Facing the variety of antibacterial effectors and cells produced by innate immunity signaling, it is expected that host defenses will be active against multidrug-resistant bacteria and that the development of resistance to such defenses by bacteria will be infrequent. Thus, targeting host innate immunity together with an appropriate antibacterial drug may limit the emergence of resistance to antibiotics, leading to successful treatment and thereby overcoming some of the impediments to antibiotic therapies.

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