CellPress
Cell Host & Microbe
Previews

Antibiotic Education: Not Just Another Brick in the Cell Wall

Inka Sastalla[1] and Sandip K. Datta[1,*]
[1]Bacterial Pathogenesis Unit, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, USA
*Correspondence: dattas@niaid.nih.gov
http://dx.doi.org/10.1016/j.chom.2015.10.019

Methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to β-lactam antibiotics, which inhibit bacterial cell wall synthesis. In this issue of *Cell Host & Microbe*, Müller et al. (2015) show that β-lactam treatment of MRSA leads to synthesis of an altered cell wall that increases inflammasome activation and immunopathology during skin infection.

Staphylococcus aureus is both a human commensal that persistently or intermittently colonizes the skin and nasal mucosa of a significant proportion of the human population, and a leading human pathogen that can cause infections at a variety of superficial and invasive body sites. A master of adaptation, *S. aureus* has evolved strain-specific virulence factors and has acquired resistance to a multitude of antibiotics, including widely used β-lactam antibiotics such as methicillin. Historically, methicillin-resistant *S. aureus* (MRSA) strains were predominantly acquired in hospitals; however, in the last 20 years MRSA strains have become a prevalent cause of infection in otherwise healthy individuals in the community.

MRSA are resistant to β-lactam antibiotics, which are substrate analogs of the essential bacterial penicillin-binding proteins (PBPs). PBPs play a pivotal role during synthesis of the bacterial cell wall, crosslinking peptides between the glycan chains that form the skeleton core of the peptidoglycan (PGN) cell wall. The *mecA* gene of MRSA encodes an alternative PBP, PBP2a. PBP2a is induced and bound by β-lactam antibiotics but evades their inhibitory action via a distorted active site that inhibits conformational changes required for acylation-induced inactivation (Lim and Strynadka, 2002). During antibiotic-mediated inhibition of other PBPs, MRSA depends exclusively on the β-lactam-resistant PBP2a, although the resulting cell wall is not as heavily crosslinked (Snowden and Perkins, 1991). In this issue, Liu and colleagues (Müller et al., 2015) present intriguing data showing that the poorly crosslinked PGN that results from exposure of MRSA to β-lactam antibiotics induces a hyperinflammatory response in human and murine macrophages characterized by increased IL-1β production (Figure 1). Similar effects were seen with *mecA*-expressing *Staphylococcus epidermidis* and with methicillin-sensitive *S. aureus* exposed to sublethal doses of β-lactam antibiotics.

IL-1β is critical for immunity to *S. aureus*. Its antistaphylococcal role has been best studied in models of skin infection, where it triggers the influx of neutrophils, which are essential for abscess formation and bacterial clearance (Miller et al., 2007). Production of active IL-1β in macrophages is dependent on two signals: (1) the sensing of an extracellular threat, classically perceived by Toll-like receptors (TLRs), that initiates transcription and translation of a precursor pro-IL-1β protein, and (2) the detection of an intracellular danger signal by cytosolic receptors, such as the Nod-like receptors (NLRs), that mediate the assembly of multi-protein complexes called inflammasomes, which ultimately trigger cleavage of pro-IL-1β into its active form via the cysteine protease caspase-1 (Martinon et al., 2009).

In the current study, Liu and colleagues advance their previous observation that reversing the intrinsic resistance of the *S. aureus* cell wall to lysozyme, a host enzyme that hydrolyses the glycan backbone of PGN, increases inflammasome activation and IL-1β release (Shimada et al., 2010). This earlier study was an indication that a structurally sound cell wall helps *S. aureus* dampen inflammatory responses. Now, the authors find that the induction of *mecA* expression after exposure of MRSA to β-lactam antibiotics triggers PBP2a-mediated synthesis of a structurally altered cell wall containing poorly crosslinked PGN, which in turn induces significantly higher levels of secreted IL-1β than the conventional cell wall (Figure 1). These results were recapitulated using enzymatically digested PGN, or by using MRSA mutants with altered levels of peptide crosslinking of the cell wall. Phagocytic uptake of the poorly crosslinked PGN from antibiotic-exposed MRSA was not altered compared to that from unexposed bacteria. Of note, MRSA with poorly crosslinked PGN was more susceptible to macrophage killing. The subsequent release of IL-1β after exposure to either native or antibiotic-exposed PGN remained dependent on the phagolysosomal processing of the PGN within macrophages, on the cytosolic inflammasome component NLRP3 (NACHT, LRR, and PYD domains-containing protein 3), and on caspase-1. NLRP3 can be activated by a variety of danger signals, and it remains unclear what phagolysosomal or cytoplasmic molecules may be involved in upstream detection of PGN to activate NLRP3. Intriguingly, macrophages that underwent NLRP3 inflammasome activation and IL-1β release in response to PGN from either *S. aureus* treated or not treated with β-lactams did not enter the inflammatory pyroptotic cell death pathway that is characteristic of classical inflammasome-activating signals such as ATP and nigericin. Pyroptotic cell death after inflammasome-mediated activation of caspase-1 involves cellular pore formation that causes osmotic swelling and ultimately leads to lysis and the release of cellular components into the milieu. The release

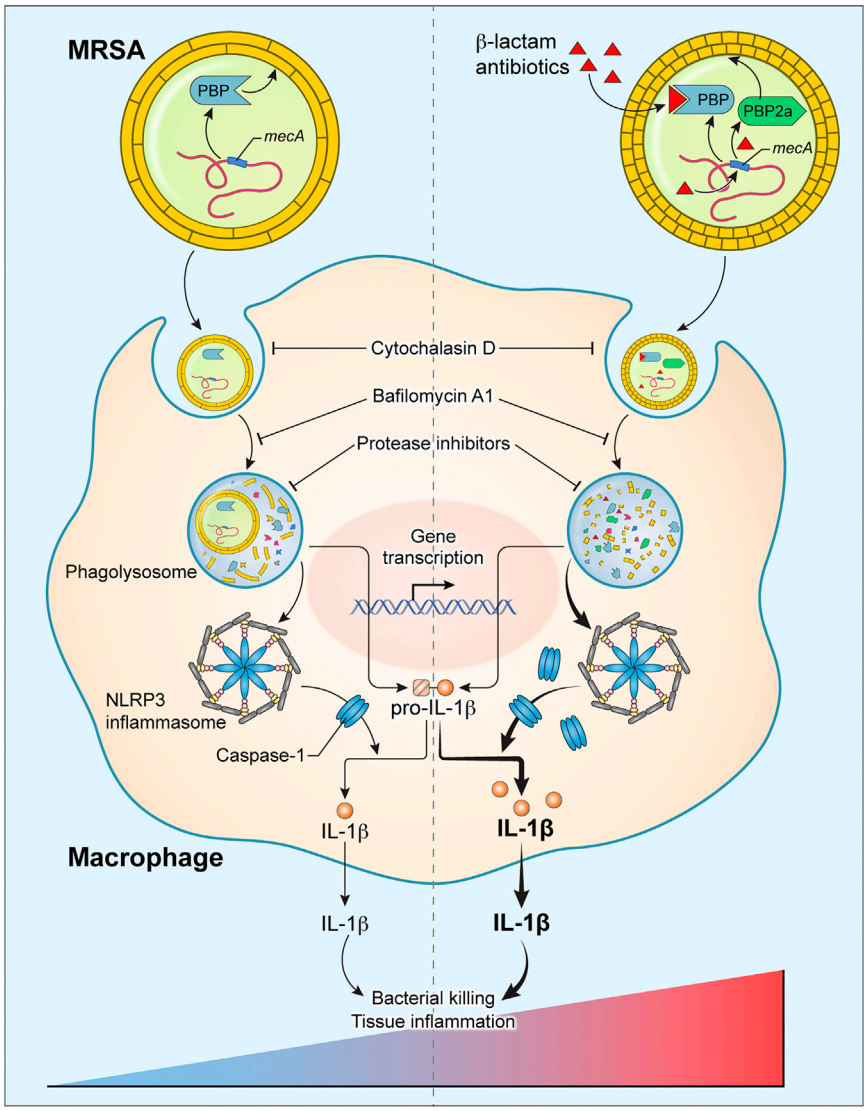

Figure 1. β-lactam Antibiotics Alter Inflammatory Activity of *Staphylococcus aureus* Peptidoglycan

Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA), use a number of penicillin-binding proteins (PBPs) to form a cell wall containing highly crosslinked peptidoglycan (PGN), depicted here as long golden bricks (top left). β-lactam antibiotics, such as methicillin, inactivate these native PBPs. The *mecA* gene in MRSA encodes PBP2a, which is resistant to inactivation by β-lactam antibiotics. In this issue, Müller et al. (2015) show that, compared to untreated MRSA (left), induction of PBP2a in MRSA treated with β-lactam antibiotics (right) results in poorly crosslinked PGN, depicted here as shortened golden bricks (top right). Stimulation of macrophages by PGN from either untreated or antibiotic-treated MRSA is dependent on phagocytosis (inhibited by cytochalasin D), acidification of the phagolysosome (inhibited by bafilomycin A1), and phagolysosomal proteases. Both PGN forms equivalently induce transcription of pro-IL-1β. However, MRSA expressing the poorly crosslinked peptidoglycan are more effectively killed in the macrophage phagolysosome, and this PGN form more potently activates NLRP3 inflammasome-mediated cleavage by caspase-1 of pro-IL-1β to active IL-1β. This increased secretion of IL-1β by macrophages, and presumably other phagocytic cells, leads to enhanced bacterial control in vivo at the potential expense of increased tissue inflammation.

of mature IL-1β and cell rupture can occur independently of each other, since osmotic protectants prevent lysis but not cytokine release (Fink and Cookson, 2006). The mechanisms by which PGN-mediated inflammasome activation and IL-1β release are uncoupled from cell death remain to be explored.

To investigate whether the effects of antibiotic-exposed PGN on macrophage inflammasome activation have implications for in vivo infection and therapy, Liu and colleagues used a mouse model of cutaneous *S. aureus* abscess formation, in which IL-1β plays a critical role in the neutrophil-mediated protective response (Miller et al., 2007). Consistent with their in vitro observations, the authors found that injection of heat-killed MRSA pretreated with β-lactam antibiotics induced a more robust local IL-1β response in mice in comparison to injection of non-treated MRSA. The higher IL-1β levels correlated with increased abscess size and weight. Since the use of heat-killed bacteria precluded bacterial proliferation or differential toxin production, the increased abscess size suggested increased inflammatory cell recruitment. β-lactam treatment after infection with live MRSA produced similar results: the treated animals developed larger lesions and had increased neutrophil infiltration despite fewer live bacteria in the abscess, indicating that the effects were caused by inflammation rather than bacterial proliferation. This in vivo immunopathology was shown to be IL-1-dependent using mice deficient for the IL-1 receptor.

This study highlights the complex interplay between host and bacterial factors that determines the outcome of host-bacteria interactions. The relatively noninflammatory nature of normally crosslinked *S. aureus* PGN may be viewed as a cloak from host detection mechanisms. In a seeming paradox, many secreted toxins of *S. aureus*, such as alpha-hemolysin, Panton-Valentine leukocidin, and LukAB, induce NLRP3 inflammasome activation and release of IL-1β, which would be predicted to lead to increased bacterial clearance but may be counterbalanced by specific roles of these toxins in virulence (Vandenesch et al., 2012). The potential for an excessive host inflammatory response is evident not only in this study involving mouse skin abscesses but also in the neutrophil-mediated inflammation seen in a rabbit model of pneumonia (Diep et al., 2010), a site that is likely less tolerant of inflammation than the skin. In contrast, as cited by Müller et al. (2015), a few studies have suggested potential benefit to using β-lactam antibiotics concurrently with MRSA-active antibiotics during bloodstream and endocarditis infections, sites where the bactericidal effects of the inflammatory response may outweigh its detrimental effects on the host. The optimal level of inflammation

to balance bacterial control and tissue inflammation likely depends on a number of factors, including site of infection, bacterial virulence, and host immune milieu. The current study by Müller et al. (2015) lends mechanistic credence to the possibility that use of antibiotics without conventional antimicrobial activity may still alter inflammatory responses and affect clinical outcomes.

ACKNOWLEDGMENTS

The authors are supported by the Intramural Research Program of the NIH, NIAID. We thank Alan Hoofring (NIH Medical Arts) for assistance with the figure artwork.

REFERENCES

Diep, B.A., Chan, L., Tattevin, P., Kajikawa, O., Martin, T.R., Basuino, L., Mai, T.T., Marbach, H., Braughton, K.R., Whitney, A.R., et al. (2010). Proc. Natl. Acad. Sci. USA *107*, 5587–5592.

Fink, S.L., and Cookson, B.T. (2006). Cell. Microbiol. *8*, 1812–1825.

Lim, D., and Strynadka, N.C. (2002). Nat. Struct. Biol. *9*, 870–876.

Martinon, F., Mayor, A., and Tschopp, J. (2009). Annu. Rev. Immunol. *27*, 229–265.

Miller, L.S., Pietras, E.M., Uricchio, L.H., Hirano, K., Rao, S., Lin, H., O'Connell, R.M., Iwakura, Y., Cheung, A.L., Cheng, G., and Modlin, R.L. (2007). J. Immunol. *179*, 6933–6942.

Müller, S., Wolf, A.J., Iliev, I.D., Berg, B.L., Underhill, D.M., and Liu, G.Y. (2015). Cell Host Microbe *18*, this issue, 604–612.

Shimada, T., Park, B.G., Wolf, A.J., Brikos, C., Goodridge, H.S., Becker, C.A., Reyes, C.N., Miao, E.A., Aderem, A., Götz, F., et al. (2010). Cell Host Microbe *7*, 38–49.

Snowden, M.A., and Perkins, H.R. (1991). J. Gen. Microbiol. *137*, 1661–1666.

Vandenesch, F., Lina, G., and Henry, T. (2012). Front. Cell. Infect. Microbiol. *2*, 12.