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The Unexpected Effects of the Combination of Antibiotics and Immunity

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β-lactam antibiotics and immune enzymes, including lysozyme, kill bacteria by rupturing the cell wall. Curiously, their combination can select for viable, wall-less bacteria. Kawai and colleagues describe the molecular details regarding the emergence of these forms, illustrating a novel and potentially clinically relevant mechanism by which bacteria escape killing by antibiotics.

Nothing says bacteria like peptidoglycan (PG), which is a network of long glycan strands that are cross-linked by short peptides, composing the bacterial cell wall. The synthesis and maintenance of this highly dynamic structure is accomplished by penicillin-binding proteins (PBPs) and autolysins, which build PG and hydrolyze PG, respectively. The cell wall is critically important for bacterial growth and is highly conserved, making it a robust drug target. β-lactam antibiotics bind and inactivate PBPs, preventing PG synthesis and killing the bacteria (Figure 1). Host defenses also target the bacterial cell wall; for example, the ubiquitous lytic enzyme lysozyme cleaves glycan strands to degrade PG. Interestingly, despite the importance of the cell wall, wall-less bacteria can emerge and grow under the appropriate osmotic conditions. Liquid culturing conditions in the lab are often hypotonic, but under osmoprotective conditions, both Gram-positive and Gramnegative bacteria can convert from rods into spherical "L-forms" that entirely lack cell walls and are thus resistant to β -lactams. L-forms are slower growing and are more susceptible to lysis by shear force than walled bacteria, and thus, they normally only arise under the appropriate selective pressure (Allan et al., 2009). Since the discovery of L-forms over 80 years ago, many groups have described methods for their cultivation, which typically involve adding lytic enzymes and/or antibiotics, often coupled with osmotic stabilization to prevent bacteriolysis (Klieneberger, 1935; Allan et al., 2009). For example, it is well documented that combining β-lactams and lysozyme can robustly cause L-form formation; but for unknown reasons, treatment with β-lactams alone is often detrimental to inducing L-forms (Brown et al., 1970; Gumpert et al., 1980; Innes and Allan, 2001). In this issue of Cell, Kawai and colleagues make key advances toward our understanding of how L-form bacteria emerge and describe the molecular mechanism regarding how penicillin and lysozyme combine to induce L-forms (Kawai et al., 2018).

Kawai and colleagues hypothesized that PG hydrolysis is required for inducing L-forms. If so, this would suggest that penicillin (when used alone) blocks L-form formation because it inhibits not only PG synthesis, as previously appreciated, but also PG hydrolysis. The authors used time-lapse microscopy to image bacteria as they transformed from rods to spheres, indicating loss of the cell wall. They also utilized a genetic trick to assure that the bacteria would survive oxidative stress by performing these assays with strains harboring a mutation in ispA, which limits electron transport chain-induced oxidative damage (Kawai et al., 2015). To test their hypothesis, they constructed a bacterial strain lacking all four class A PBPs, and interestingly, this strain was unable to



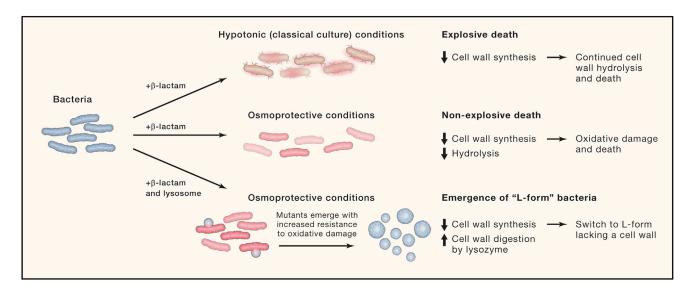


Figure 1. Bacteria Undergo Various Fates upon Antibiotic Treatment Depending on Their Environment Upon treatment with β-lactam antibiotics, hypotonic conditions (top) lead to an explosive cell death of bacteria, while in osmoprotective conditions, bacteria will eventually succumb to a slower form of cell death that is caused by the inhibition of peptidoglycan synthesis and hydrolysis (middle). However, in the presence of lysozyme (bottom), which facilitates cell wall degradation, spherical L-form bacteria can emerge that lack a cell wall and are resistant to β-lactam antibiotics.

undergo L-form formation unless treated in combination with lysozyme. These data suggest that class A PBPs are either directly or indirectly required for PG glycan hydrolysis, which is required for the switch to L-form. Future investigations will be required to determine the mechanism regarding how class A PBPs contribute to PG hydrolysis to promote the switch to L-forms.

Considering that B-lactams are widely used in medicine and that lysozyme is ubiquitous, these findings suggest that bacteria could exploit host lysozyme to escape antibiotic-mediated killing by switching to the L-form. Kawai and colleagues developed novel methods for understanding L-form switching in the context of infection. They demonstrated that when macrophage lysates and live moth larvae are inoculated with bacteria and treated with penicillin, this eukaryotic environment increases the abundance of L-forms. These in vivo conditions both limit PG synthesis (antibiotics) and facilitate PG degradation (lysozyme), and they are generally more osmoprotective than classic culture conditions, thus limiting bacteriolysis. These findings imply that the emergence of L-forms would also be increased in humans treated with antibiotics, and in agreement with their findings, L-form bacteria have indeed

been observed in antibiotic-treated patients (Domingue and Woody, 1997; Allan et al., 2009). Interestingly, bacteria that switch into L-forms can persist in the gut until antibiotics are removed, at which point there is selection for the faster-growing walled bacteria (Domingue and Woody, 1997). In regards to patient safety upon antibiotic treatment, it remains unclear whether L-forms represent a threat to the host, as both resident microbiota as well as pathogens can change to L-form. L-forms have been suggested to contribute to pathogenicity of a variety of diseases, including Crohn's disease, tuberculosis, and foodborne illnesses, but these observations remain incompletely understood (Domingue and Woody, 1997; Allan et al., 2009). The findings from Kawai and colleagues suggest that their assays in cells and in vivo could be used to better understand the role for L-form bacteria in relation to human disease.

Finally, Kawai and colleagues' findings may impact our understanding of how bacteria survive in complex microbial communities. Considering that millions of pounds of antibiotics are fed to livestock each year (https://www.fda.gov/), the microbial landscape may be shifted toward microbes that are able to survive as L-forms.

Such a massive selective pressure could drive evolution of wall-less bacteria, such as mycoplasma, as previously hypothesized (Allan et al., 2009). Indeed, L-form bacteria have been identified in cattle and other animals treated with penicillin (Owens, 1987; Allan et al., 2009). In complex microbial communities found in the environment, competing bacteria secrete lytic enzymes, including mutanolysin, lyphostatin, and type VI effectors, that degrade the cell walls of neighboring bacteria, while fungi produce PBP-inhibiting antibiotics, such as penicillin. Loss of the cell wall could potentially allow for bacteria to survive interactions with other microbial communities.

In all, Kawai and colleagues developed microbiological and tissue culture assays for investigating L-form switching and utilized the power of microbial genetics to better understand how bacteria can escape antibiotic treatment. Future research using these approaches may better determine what role L-form bacteria play upon antibiotic treatment in humans, livestock, and the environment.

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WEB RESOURCES

U.S. Food & Drug Administration, https://www.fda.gov/

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Learning from Everyday Images Enables Expert-like Diagnosis of Retinal Diseases

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Kermany et al. report an application of a neural network trained on millions of everyday images to a database of thousands of retinal tomography images that they gathered and expert labeled, resulting in a rapid and accurate diagnosis of retinal diseases.

Recent years have seen considerable increase in the development of machinelearning algorithms for medical imaging to support clinical decision making and diagnostics. Advancements in medical imaging and visualization provide medical experts with powerful tools to gain and analyze visual representation of the body. Thanks to such insights, better and faster diagnosis or triage is made possible, providing doctors with extra time to, for example, deal with difficult cases. Computed tomography, magnetic resonance imaging, ultrasound, or radiology generate high-resolution digital imaging data. Increased automatization and availability of these technologies have contributed to a great increase in the volume of imaging data, making the medical imaging field poised to

greatly benefit from machine-learning approaches. In this issue of *Cell*, Kermany et al. (2018) show how a computer vision system for classification of natural images can be successfully adapted to diagnosing common blinding retinal diseases and pediatric pneumonia, achieving performance comparable to that of human experts.

Computer processing and elements of computer vision have been used in medical imaging since the 1990s. Advanced processing and analytics help to augment work processes of human experts, providing them with image-derived scores that augment their analysis and increase their efficiency. Today, machine learning is the prime tool ready to greatly improve these workflows and perhaps completely automate diagnostic

and referral tests in the future. Kermany et al. (2018) provide a glimpse of this future. In their study, Kermany et al. demonstrate the use of deep learning for triage and diagnosis of choroidal neovascularization, diabetic macular edema, and drusen, three common retinal diseases. Authors have retrospectively analyzed optical coherence tomography (OCT) images of retina from the Shiley Eye Institute of the University of California San Diego and the Shanghai First People's Hospital, eventually yielding 108,312 OCT images curated by human experts. Leveraging these data, their deep-learning model in the form of a convolutional neural network outperformed two out of six ophthalmologists using a combined measure of sensitivity and precision. Interestingly, when their neural

