subsets from distinct activation states. Longitudinal analyses will also shed light on the influences of age on the NK cell repertoire, diurnal fluctuations in the NK cell subsets, and responses to therapeutic treatments for infectious disease and cancer.

These intriguing and unexpected results raise the more general question of what constitutes a cell 'subset'. The convention is to first identify a cell by the expression of one defining phenotype—for example, human NK cells can be defined as lymphocytes lacking cell-surface CD3 and expressing CD56—and then progressively narrow the subset by scoring cells as positive or negative for other markers such as CD94, inhibitory killer immune receptors and chemokine receptors. Much like nested Russian dolls (**Fig. 1**), as one adds more markers the subsets get smaller and smaller. However, with advances in CyTOF technology, quantitative measurement of a hundred or more parameters is foreseeable, and these parameters may go beyond the typical analysis of cell-surface proteins to include DNA, RNA (both coding and noncoding), carbohydrates, lipids and post-translational protein modifications. At this point, what really constitutes a subset? Ultimately, like snowflakes, each cell will undoubtedly be unique. The real challenge is to understand the physiological meaning of this remarkable phenotypic diversity and how it relates to the immune function of these cells.

ACKNOWLEDGMENTS

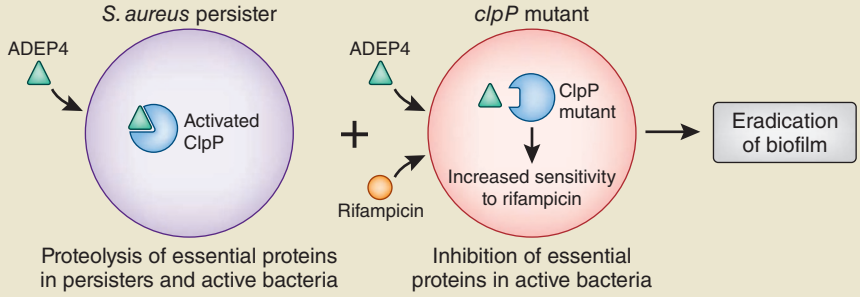
L.L.L. is an American Cancer Society Professor and funded by US National Institutes of Health grants AI066897 and AI068129.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

1. Horowitz, A. *et al. Sci. Transl. Med.* **5**, 208ra145 (2013).
2. Loken, M.R., Parks, D.R. & Herzenberg, L.A. *J. Histochem. Cytochem.* **25**, 899–907 (1977).
3. Parks, D.R., Hardy, R.R. & Herzenberg, L.A. *Cytometry* **5**, 159–168 (1984).
4. Lanier, L.L. & Loken, M.R. *J. Immunol.* **132**, 151–156 (1984).
5. Bendall, S.C., Nolan, G.P., Roederer, M. & Chattopadhyay, P.K. *Trends Immunol.* **33**, 323–332 (2012).
6. Bendall, S.C. *et al. Science* **332**, 687–696 (2011).
7. Newell, E.W., Sigal, N., Bendall, S.C., Nolan, G.P. & Davis, M.M. *Immunity* **36**, 142–152 (2012).
8. Chng, W.J., Tan, G.B. & Kuperan, P. *Clin. Diagn. Lab. Immunol.* **11**, 168–173 (2004).

A one-two punch knocks out biofilms



Biofilm formation, a tactic used by some bacterial pathogens to evade drug treatment and the human immune response, is a growing threat to human health. Now, Conlon *et al.*[1] describe a novel strategy to destroy biofilm-associated *Staphylococcus aureus*, a pathogen that can cause a range of severe diseases. They report in *Nature* that two antibiotics, when used together, eliminate *S. aureus* growing in biofilms, kill methicillin-resistant *S. aureus* (MRSA) and eradicate a deep-seated *S. aureus* infection in mice.

The two antibiotics are acyldepsipeptide 4 (ADEP4) and rifampicin. ADEP4 had been shown to kill replicating *S. aureus*[2] but it had not been tested against so-called bacterial persisters, phenotypic variants that tend to grow in biofilms. Because commonly used antibiotics target processes essential to bacterial replication, they are often ineffective against metabolically inactive persisters.

Conlon *et al.*[1] show that, whereas antibiotics such as vancomycin and ciprofloxacin are only marginally effective against *S. aureus* persisters, ADEP4 reduces their numbers exponentially within a day. ADEP4 acts by binding to ClpP, the catalytic core of a bacterial protease, activating it to degrade proteins indiscriminately[2]. A large quantitative proteomics study reveals that ADEP4-ClpP targets more than 400 proteins for degradation, and first among them are essential ribosomal proteins[1]. Conlon *et al.*[1] suggest that the efficacy of ADEP4 against persisters may be linked to its ability to uncouple ClpP from ATP dependency[2], activating the protease in dormant cells that have low energy levels.

"It's really important to find new antibiotics, particularly those that are active against *S. aureus* and against bacteria in biofilms," says Carl Nathan of Weill Cornell Medical College in New York. "The discovery that ADEP4-mediated activation of ClpP is efficacious against *S. aureus* is significant because it represents a way to kill a group of nonreplicating or slowly replicating bacteria by targeting a pathway that is not on the classical list." Conlon *et al.*[1] find that the effects of ADEP4 are impressive but transient, because bacterial growth quickly rebounds as *clpP* mutants arise that are resistant to ADEP4. But *clpP* mutants have lower fitness and are 10–100 times more susceptible to rifampicin compared with wild-type *S. aureus*. So even though rifampicin on its own is ineffective against *S. aureus* persisters, when combined with ADEP4 it reduces *S. aureus* cell counts to extremely low levels.

The problem of antibiotic-resistant biofilms is becoming increasingly acute with the rise in medical interventions such as insertions of catheters and prostheses and the paucity of new antibiotics with unconventional mechanisms of action. The efficacy of ADEP4 and rifampicin against *S. aureus* biofilms and MRSA is a tale of success, but more work is needed to determine whether this approach will work against other biofilm-forming and antibiotic-resistant pathogens. "It would be a big mistake to assume that the word 'biofilm' refers to exactly the same thing no matter what community of organisms is causing it, which anatomical site you find it in or how long it's been there," says Nathan. Similarly, "persistence is an operational definition, and we still don't know whether bacteria that persist against one antibiotic are similar to those that persist against a different antibiotic," Nathan adds.

The principle that bacterial persisters can be killed by activating an inactive target represents a promising alternative to the conventional approach of seeking to inhibit active pathways. A deeper understanding of the processes that are active and inactive in persisters and in biofilm formation will be critical to the identification of additional drug targets. While the hunt for new antibiotics continues, activating ClpP to kill *S. aureus* persisters holds promise for treatment of a dangerous human pathogen.

Irene Jarchum
Locum Assistant Editor

1. Conlon, B.P. *et al. Nature* **503**, 365–370 (2013).
2. Brötz-Oesterhelt, H. *et al. Nat. Med.* **11**, 1082–1087 (2005).

© 2014 Nature America, Inc. All rights reserved.
npg