Antibiotic-evading bacteria

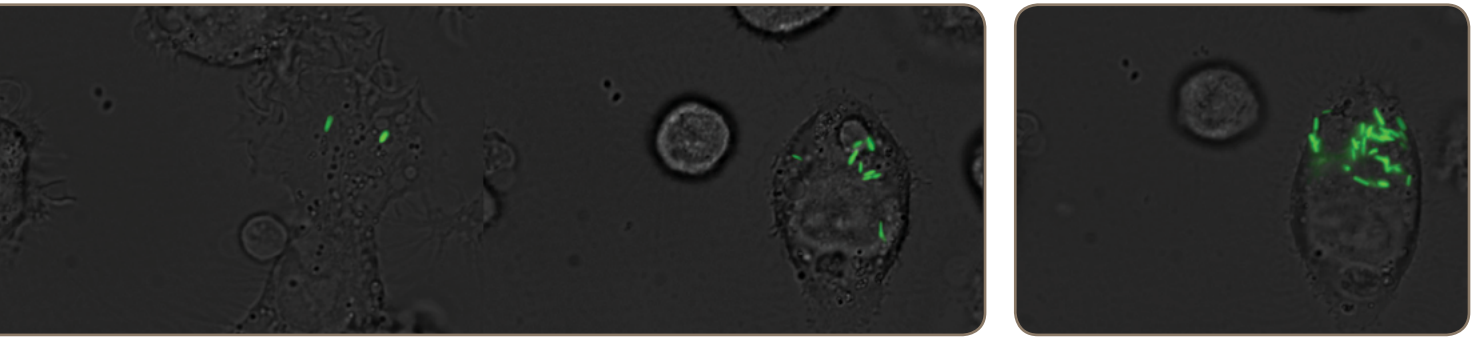

Image: *Salmonella* coloured green growing in macrophages.
Credit: MRC Centre for Molecular Bacteriology and Infection, Imperial College London.

Researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI) at Imperial College London are studying dormant 'persister' cells produced by *Salmonella* bacteria. These cells are formed by bacteria when they are exposed to stresses such as antibiotics. By studying persister cells, the researchers hope to understand the link between these dormant cells and antibiotic resistance, as well as develop treatments that target persister cells directly.

Most antibiotics act only on active bacteria. But nearly all bacterial pathogens produce a small sub-population of dormant cells that can evade antibiotics. These cells — called persisters — tolerate antibiotics and other environmental stresses, such as nutrient depletion or host cell acidity. Once the stress has been removed, for example, by the completion of a course of antibiotics, the dormant cells are able to revert back to the active, disease-causing form. These cells are thought to be the cause of many persistent or recurrent infections.

This antibiotic 'tolerance' is temporary and reversible, unlike resistance, which is caused when the bacteria acquire stable genetic traits. However, it is thought that prolonged and repeated treatment of persistent infections may lead to genetic drug resistance[1], and so it is important that the mechanisms behind this evasion are identified to help develop appropriate strategies to treat these persisters. Despite their discovery by Joseph Biggar more than 70 years ago[2], persister cells are still poorly understood.

Persisters and resistance

Up until now, persister cells have only been studied in test tubes. However, in 2014, a team led by Professor David Holden at the CMBI used a technique they had developed to visualise persisters for the first time at the single-cell level as they are consumed by white blood cells[3].

Using a fluorescent protein, Professor Holden and colleagues showed that the bacteria produced persister cells when consumed by white blood cells at a much greater rate than when grown in laboratory media. The researchers demonstrated that the bacteria formed persisters immediately after being attacked and consumed by the host's white blood cells in response to the levels of acidity and lack of nutrients inside the cells.

These stresses also cause some bacterial cells to start replicating rather than form persister cells, and this dual response allows bacteria to 'hedge their bets' to gives them a selective advantage.

Professor Holden is now hoping to use these approaches to study how persister cells might lead to resistance.
"It is widely thought that the multiple courses of antibiotics made necessary by persistent infections leads to resistance. However, this has not been tested experimentally. Since the genetic basis of persister formation has been worked out in recent years, we

can make bacterial mutants with enhanced or reduced persister frequency and use these in conjunction with our techniques to determine if and how persisters contribute to emergence of resistance during infection," says Professor Holden.

Surviving adverse conditions

Part of Professor Michael Barer's research at the University of Leicester looks at the transmission and persistence of *Mycobacterium tuberculosis*. In 2008 he, together with colleagues at the MRC unit, The Gambia, demonstrated that the tuberculosis bacteria in samples of sputum — the mucus and other matter brought up from the lungs by coughing, and which helps transmit the disease between people — were likely to be in their persistent state. These samples contained a fat called triglyceride, produced by the bacteria when they form persister cells. This suggests that formation of the persisters might help the bacteria survive the adverse conditions that *M. tuberculosis* encounters when it is transmitted between people[4].

Targeting persisters

Pyrazinamide is the only drug that specifically targets persister cells. In 1970 researchers at the MRC Tuberculosis and Chest Diseases Unit[5] demonstrated for the first time that the inclusion of pyrazinamide in an antibiotic regimen for the treatment of *Mycobacterium tuberculosis* substantially reduced the relapse rate[6]. Certain antibiotics however do have limited action against the persisters and REMoxTB, a clinical trial involving several MRC researchers, is currently underway to determine whether the inclusion of the antibiotic moxifloxacin can shorten the duration of treatment[7].

Enhancing the host's immune response is another method of targeting persister cells[8]. The bacillus Calmette-Guerin (BCG) vaccine has limited success as a preventative measure. However, researchers at the MRC's National Institute of Medical Research (NIMR) showed that the use of the *M. tuberculosis* Hsp60 DNA vaccine, in combination with antibacterial treatment, was successful in treating heavily infected mice. The DNA vaccinations can switch the immune response from one that is relatively inefficient to one that kills these persistent bacteria[9].

"Another possibility is to work out what triggers the persister cells to start growing again — give someone with a persistent infection a drug that induces this — and then attack the bacteria as they come out of hiding," says Professor Holden.

References

1. Zhang Y. Persisters, persistent infections and the Yin–Yang model. *Emerging Microbes & Infections* (2014) 3, e3; doi:10.1038/emi.2014.3
2. Hobby GL, Meyer K, Chaffee E. Observations on the mechanism of action of penicillin. *Proc Soc Exp Biol NY* 1942; 50: 281–285.
3. S. Helaine et al. 'Internalization of Salmonella by Macrophages Induces Formation of Nonreplicating Persisters.' *Science*, 10 January 2014. Vol. 343 no. 6167 pp. 204-208 DOI: 10.1126/science.1244705
4. Garter J et al. Cytological and Transcript Analyses Reveal Fat and Lazy Persister-Like Bacilli in Tuberculous Sputum. *PLOS Medicine* Published: April 01, 2008 DOI: 10.1371/journal.pmed.0050075
5. Closed in 1986.
6. Fox et al. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946–1986, with relevant subsequent publications. *The International Journal of Tuberculosis and Lung Disease*, Volume 3, Supplement 2, October 1999, pp. S231-S279(49)
7. *http://clinicaltrials.gov/ct2/show/NCT00864383*
8. Zhang Y et al. Targeting Persisters for Tuberculosis Control *Antimicrob. Agents Chemother.* May 2012 vol. 56 no. 5 2223-2230. doi: 10.1128/AAC.06288-11
9. Lowrie DB et al. Therapy of tuberculosis in mice by DNA vaccination. *Nature*. 1999 Jul 15;400(6741):269-71.