Courtesy: Dr Mukta, UCMS

BACTERIAL PERSISTENCE

Within a population of bacteria there exists a subgroup of cells that do not grow at the normal rate but exists in a quiescent, nongrowing or slow-growing state, known as **persister cells. Bacterial persistence** is the capacity of bacteria to tolerate exposure to lethal concentrations of bactericidal antibiotics. In the case of antibiotic treatment, persister cells are able to survive because an important action of antibiotics relies on disrupting translation of the mRNA code to polypeptide chains, and this process does not occur in non-growing cells

Clinical implications of persisters: antibiotics might not sterilize infections and remaining remaining bacteria could later cause recurrence once treatment ended

Mechanism of persistence

They are **transiently refractory to killing**, without having acquired resistance through genetic modification. Hence when the antibiotic pressure drops, the cells will give rise to a population that is as susceptible as the original one, and that again possesses a similarly small proportion of persister cells. This discriminates persister cells from resistant mutants, which exhibit stable, inheritable drug insensitivity. In contrast to resistance, the tolerance of persisters to antibiotics might function by preventing target corruption by a bactericidal agent through the blocking of the antibiotic target(s)

More than one way to make a persister

- **Heterogenous Growth-** non-growing and dormant cells are more likely to persist antibiotic treatment than actively growing cells.
- **Nutrient limitation-** persisters predominantly form as cultures approach and enter stationary phase and they are lost from cultures that have been serially passed through rich media conditions to promote early exponential-phase growth
- **SOS response-** The SOS response, a major stress response system in bacteria that is induced by DNA damage , has also been implicated in persistence

Persisters have been observed in Esch coli, M tb, Ps aeruginosa , S aureus, T pallidum, S typhi, H pylori and C albicans

Genetic mechanisms implicated in persistence: The simplest strategy to trigger entry into dormancy would be to overproduce proteins or toxins that inhibit cellular processes and growth. Mechanisms include toxin–antitoxin modules. The two most prominent TA loci involved in persistence are hipBA and tisAB

Isolation of persister cells: Done by-

1. Expose log phase cultures of *E. coli* to ampicillin till lysis (about 3 h) and collect the surviving cells (persisters) by centrifugation
2. By inserting a green fluorescent protein (GFP) reporter gene at the at site of the *E. coli* chromosome and sorting out the cells into bright green (normally growingcells) and dim green types (slow-growing subpopulations of cells)

Other features:

Persister cells are highly enriched in biofilms, which are complex and highly organized surface-attached communities of microbes embedded in a polymeric matrix. Treatment with antibiotics eliminates the planktonic cells and the majority of biofilm-associated cells except the persisters. Once the antibiotic is withdrawn, the persisters inside the biofilm grow and repopulate the niche leading to secondary infection. Secondary infection, in turn, generates antibiotic-sensitive cells as well as antibiotic-tolerant persisters. The infection is perpetuated in this manner in spite of prolonged therapy. Antibiotic-tolerant persisters are often shielded from the host's immune defence systems as well, as they apparently 'hide' in various niches such as CNS, macrophages or granulomas, biofilms, stomach and gall bladder.

With most of the currently available chemotherapeutic agents targeting exponentially growing cells, our therapeutic arsenal is ineffective in eradicating these dormant persister cells. The prospect that persisters are responsible for the persistence of chronic infections and, more gravely, recalcitrance of disseminating cancers have identified these culprit cells as viable targets for new therapies

Anti-persister strategies

1. *Metabolite enabled eradication of bacterial persisters by Aminoglycosides*:**-** As protein translation occurs in persisters, persisters should be susceptible to aminoglycoside antibiotics, which are

ribosome-targeting, bactericidal antibiotics. Persisters are however not susceptible to aminoglycosides, and this could be owing to decreased proton-motive force(PMF), as PMF is required for aminoglycoside uptake. Through specific metabolic stimuli (e.g. mannitol, fructose), PMF was induced in persisters, thereby enabling aminoglycoside uptake and killing.

2. Utilizing methods promoting peptidoglycan synthesis or autolysin activity for the beta-lactams
3. By harnessing methods promoting DNA replication.
4. Employing methods inducing reactive oxygen species (ROS) or inhibiting ROS-protective genes for all bactericidal antibiotics
5. By combining a conventional antibiotic, such as a fluoroquinolone, and an inhibitor of an essential persister protein.
6. Development of sterile surface materials by covalently attaching the non-toxic antiseptic to antimicrobial molecule & by linking the antimicrobial compound to a long, flexible polymeric chain that is covalently anchored to the surface of a material
7. Development of pro-antibiotic compound- pro-antibiotic compound is benign, but a bacterial enzyme converts it into a reactive antiseptic compound in the cytoplasm.

Chronic infectious diseases are a major challenge in present-day global health care. The problem of antibiotic resistance is compounded by the presence of persister cells, which, although formed in small numbers and only transiently so, are virtually impossible to kill with conventional drugs. Their importance in treatment failure is increasingly being recognized, fuelling efforts to develop specific anti-persister drugs. This will undoubtedly enhance our understanding of microbial infections and, hopefully, result in better treatment options in the future