Bacterial Persisters - a Thorny Problem in Clinical Antibiotic Treatment

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Uropathogenic E. coli (UPEC)

- Acute Urinary Tract Infection, **20%-30%** will have a recurrent infection within 3-4 months.

- Clinical chronic infection.

Bacterial Persisters- main reason of chronic disease

- **Bacterial Persisters**: a transiently multidrug-tolerant subpopulation of bacteria.

### Bacterial Persisters- Clinical chronic infections

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Persistent disease</th>
<th>Biologic mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic persistent infections</td>
<td></td>
<td></td>
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<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Latent tuberculosis</td>
<td>Intracellular growth, persisters</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Gastritis, gastric cancer</td>
<td>Intracellular growth</td>
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<td><em>Salmonella Typhi</em></td>
<td>Chronic carrier, gall bladder carcinoma</td>
<td>Intracellular growth, biofilm formation</td>
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<td><em>Treponema pallidum</em></td>
<td>Latent syphilis</td>
<td>Intracellular growth</td>
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<td>Symptomatic persistent infections</td>
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<td><em>Pseudomonas aeruginosa</em></td>
<td>Bronchiectasis/pneumonia in CF patients</td>
<td>Biofilms, small colony variants, persisters</td>
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<td><em>Escherichia coli</em></td>
<td>Recurrent urinary tract infections</td>
<td>Intracellular growth, biofilms</td>
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<td><em>Staphylococcus aureus</em></td>
<td>Bronchiectasis/pneumonia in CF patients; device-associated infections</td>
<td>Biofilms, small colony variants</td>
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<td><em>Hemophilus influenza</em></td>
<td>Recurrent otitis media</td>
<td>Biofilms</td>
</tr>
<tr>
<td><em>Mycobacterium leprae</em></td>
<td>Leprosy</td>
<td>Intracellular growth</td>
</tr>
</tbody>
</table>

Bacterial Persisters- Characteristics

- A minor part of a bacterial population (from $10^{-6}$ to $10^{-1}$).
- Multidrug Tolerance.
- Non-heritable phenotypic variation.
- Transient switch.
Bacterial Persisters- Formation

TA (Toxin- Antitoxin) modules

- Main model for the formation of persisters.
- Toxin: non-secreted, inhibits essential cellular functions.
- Antitoxin: normal circumstance, neutralizes the toxin, so that bacterial cell growth is unaffected.
Bacterial Persisters- Formation

Bacterial Persisters- To be or not to be

- **Triggers**: a variety of environmental conditions.
- Starvation, host environment, indole, quorum sensing, SOS response and antibiotics, etc.
Bacterial Persisters- To be or not to be

### Bacterial Persisters - To be or not to be

<table>
<thead>
<tr>
<th>Inherent variability underlying persistence</th>
<th>Trigger mechanism</th>
<th>Mechanism for noise amplification</th>
<th>Organism</th>
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<tbody>
<tr>
<td>Growth arrest</td>
<td>Starvation</td>
<td>Unknown</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Starvation</td>
<td>TA</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Starvation</td>
<td>TA threshold</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Prolonged starvation</td>
<td>Unknown</td>
<td>M. smegmatis</td>
</tr>
<tr>
<td></td>
<td>Prolonged starvation</td>
<td>Unknown</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Quorum sensing</td>
<td>Unknown</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td></td>
<td>Biofilm formation</td>
<td>TA, threshold?</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Intracellular residence</td>
<td>Unknown</td>
<td>S. enterica</td>
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<tr>
<td></td>
<td>Stringent response induction</td>
<td>Amino-acid starvation</td>
<td>M. smegmatis</td>
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<td></td>
<td>SOS response induction</td>
<td>Ciprofloxin</td>
<td>E. coli</td>
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<td></td>
<td>Efflux pumps activation</td>
<td>Intracellular residence</td>
<td>M. tuberculosis, M. marinum</td>
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<td></td>
<td>Down regulation of virulence factors</td>
<td>Intracellular residence</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>Chromatin state in cancer cells</td>
<td>Unknown</td>
<td>Mammalian cells</td>
</tr>
</tbody>
</table>

Bacterial Persisters- Experimental systems

- Persisters: focus on pathogenic bacteria, and preferably those cause recalcitrant infections.

- Persisters: pay attention to the disparity between in vitro and in vivo experiments.
Internalization of *Salmonella* by Macrophages Induces Formation of Nonreplicating Persisters

Sophie Helaine,* Angela M. Cheverton,† Kathryn G. Watson,† Laura M. Faure, Sophie A. Matthews, David W. Holden*

Many bacterial pathogens cause persistent infections despite repeated antibiotic exposure. Bacterial persisters are antibiotic-tolerant cells, but little is known about their growth status and the signals and pathways leading to their formation in infected tissues. We used fluorescent single-cell analysis to identify *Salmonella* persisters during infection. These were part of a nonreplicating population formed immediately after uptake by macrophages and were induced by vacuolar acidification and nutritional deprivation, conditions that also induce *Salmonella* virulence gene expression. The majority of 14 toxin-antitoxin modules contributed to intracellular persister formation. Some persisters resumed intracellular growth after phagocytosis by naïve macrophages. Thus, the vacuolar environment induces phenotypic heterogeneity, leading to either bacterial replication or the formation of nonreplicating persisters that could provide a reservoir for relapsing infection.

Many studies, mostly focusing on bacteria grown in laboratory media, have shown that persisters are an unstable, nongrowing, multidrug-tolerant subpopulation that result from phenotype switching (1–3). In contrast to antibiotic-resistant bacteria arising from heritable mutations, the progeny of persisters are mainly antibiotic-sensitive cells. Most studies on persistent infections are based on the assumption that a proportion of the bacterial population is nonreplicating (4); however, this consensus was challenged recently in studies that showed replicating antibiotic-
Activated ClpP kills persisters and eradicates a chronic biofilm infection

B. P. Conlon¹, E. S. Nakayasu²†, L. E. Fleck¹, M. D. LaFleur³, V. M. Isabella¹, K. Coleman³, S. N. Leonard⁴, R. D. Smith², J. N. Adkins² & K. Lewis¹

Deep-seated mouse thigh infection model.

Chronic infections are difficult to treat with antibiotics but are caused primarily by drug-sensitive pathogens. Dormant persister cells that are tolerant to killing by antibiotics are responsible for this apparent paradox. Persisters are phenotypic variants of normal cells and pathways leading to dormancy are redundant, making it challenging to develop anti-persister compounds. Biofilms shield persisters from the immune system, suggesting that an antibiotic for treating a chronic infection should be able to eradicate the infection on its own. We reasoned that a compound capable of corrupting a target in dormant cells will kill persisters. The acyldepsipeptide antibiotic (ADEP4) has been shown to activate the ClpP protease, resulting in death of growing cells. Here we show that ADEP4-activated ClpP becomes a fairly nonspecific protease and kills persisters by degrading over 400 proteins, forcing cells to self-digest. Null mutants of clpP arise with high probability, but combining ADEP4 with rifampicin produced complete eradication of Staphylococcus aureus biofilms in vitro and in a mouse model of a chronic infection. Our findings indicate a general principle for killing dormant cells—activation and corruption of a target, rather than conventional inhibition. Eradication of a biofilm in an animal model by activating a protease suggests a realistic path towards developing therapies to treat chronic infections.
# Bacterial Persisters- Experimental systems

## Table 1. Experimental models to study bacterial persisters during infection of their host

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Bacterial species</th>
<th>Tools to analyse persisters&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured host macrophages</td>
<td><em>Mycobacterium tuberculosis, Salmonella Typhimurium</em></td>
<td>CFU, microscopy, and FD</td>
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<tr>
<td>Zebrafish larvae</td>
<td><em>Mycobacterium marinum</em></td>
<td>CFU and microscopy</td>
</tr>
<tr>
<td>Isoniazid-treated mice</td>
<td><em>M. tuberculosis</em></td>
<td>STM and CFU</td>
</tr>
<tr>
<td>Mouse urinary tract-inserted catheters</td>
<td><em>Escherichia coli</em></td>
<td>CFU</td>
</tr>
<tr>
<td>Murine subcutaneous biofilm</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>CFU</td>
</tr>
<tr>
<td>Mouse thigh infection</td>
<td><em>Staphylococcus aureus</em></td>
<td>CFU</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: CFU, colony-forming unit; FD, fluorescence dilution; STM, signature-tagged mutagenesis.

Bacterial Persisters- Eradication

Mathematical modeling:

1. Periodic dosing of antibiotics.

2. Prolong treatment with current antibiotics.
Bacterial Persisters - Eradication

- **Sugars**: led to increased uptake of aminoglycosides.

- **Weak electrochemical currents** and **quorum sensing inhibitors**.

- **Oxygen**: Reactive Oxygen Species (ROS).

- **Lytic phages**: lyse bacteria when growth is resumed.
Small molecule compounds: ADEP4 activates and corrupts ClpP, results in over 400 various functional types of proteins degradation.
Persisters may result in the enrichment of multidrug resistance bacteria.

Persisters formation is very complex and under multigenic control.

Persisters formation include bacterial species, host environment, and treatment regimens.
Bacterial Persisters- Further Direction

- Design successful drugs.

1. Metabolite-enabled eradication of bacterial persisters: awake or inhibit.

2. pro-antibiotic: nonspecifically, affect all bacterial cells.

References

References

Thank you!