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Bacterial persisters are defined as multidrug-tolerant phenotypic variants and are associated with relapsing infections, but the factors responsible for the emergence of persisters *in vivo* have been unclear. Helaine *et al.* report the use of a single-cell method to study persister formation *in vivo* and show that vacuolar acidification and nutrient deprivation induce the formation of non-replicating *Salmonella enterica* subsp. *enterica* serovar Typhimurium persisters in macrophages.

Most *in vitro* studies have suggested that entry into a reversible non-replicating state is a hallmark of persistence. Thus, the authors investigated whether non-replicating *S.* Typhimurium persisters could arise *in vivo* using a fluorescence dilution technique that distinguishes between growing and non-growing cells. In infected mice, the authors found that most *S.* Typhimurium cells entered a non-replicating state within 2 hours post-inoculation, but similarly to persisters, this growth arrest was reversible. Consistent with this, antibiotic-tolerant *S.* Typhimurium cells that were derived from antibiotic-treated mice were also growth-arrested and could resume growth *in vitro* in the absence of the antibiotic, which indicated that these cells were persisters. In mice, *S.* Typhimurium replicates within macrophages, and the authors found that internalization by macrophages resulted in a 100–1,000-fold increase in non-replicating, multidrug-tolerant persister cells. Further experiments showed that macrophage-derived persisters consisted exclusively of metabolically active cells, some of which were capable of resuming growth following internalization by naive macrophages. Thus, the authors propose that such cells might be responsible for the recurrence of *S.* Typhimurium infections *in vivo*.

So, what triggers persister formation in macrophages? Treatment of infected macrophages with bafilomycin A1, which prevents acidification of the *Salmonella*-containing vacuole, led to a significant decrease in persisters, which suggests that the

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acidic and nutritionally poor vacuolar environment induces persister formation. Furthermore, as previously reported in *Escherichia coli*, S. Typhimurium toxin–antitoxin loci were shown to contribute to the emergence of persisters. However, unlike *E. coli,* in which the Lon protease is required for antitoxin degradation to induce growth arrest and subsequent persistence *in vitro*, Lon-dependence in *S.* Typhimurium was observed only in macrophages, which suggests that this particular persistence-induction pathway is specific for the host cell environment.

This landmark study is the first to show that persisters can be directly monitored *in vivo*, and application of the technique to a wider range of bacterial pathogens should help to unravel the physiological basis of the persistence phenotype.

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ORIGINAL RESEARCH PAPER Helaine, S. *et al.* Internalization of *Salmonella* by macrophages induces formation of nonreplicating persisters. *Science* **343**, 204–208 (2014)
FURTHER READING Balaban, N. Q. *et al.* A problem of persistence: still more questions than answers? *Nature Rev. Microbiol.* **11**, 587–591 (2013)