

Enhancing macrophages to combat intracellular bacteria

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People have taken the initiative to combat pathogenic bacteria for more than 100 years since some microbes have been found to cause diseases. The struggle between human beings and pathogens can be regarded as an arms race, in which antibacterial materials become smarter to kill bacteria, while bacteria in turn evolve to be stronger to increase survival. Antibiotic therapies, as the primary clinical treatment for bacterial infections, seem trapped in a vicious cycle because the corresponding drug-resistant bacteria emerge rapidly within a few years after the clinical application of a new antibiotic.¹ To get out of this vicious cycle, scientists have to explore new strategies to counter bacterial threats through antibiotic-free treatments or improving antibiotic utilization,² in which methods to eliminate pathogens by mobilizing immune cells draw considerable attention.

Macrophages, the representative immune cells, are able to remove pathogens *in vivo* by identification, endocytosis, sterilization, and digestion. Briefly, the Toll-like receptors (TLRs) on the macrophage membrane can recognize the pathogen-associated molecular patterns (PAMPs), resulting in the response to pathogens and inducing endocytosis thereafter. During endocytosis, the cell membrane protrudes and fuses to form a phagosome, which encloses the pathogens. In the phagosome, pathogens are inactivated by the synergistic effects of reactive oxygen species (ROS), reactive nitrogen species (RNS), free fatty acids, etc., and degraded by the hydrolases from the lysosomes fusing with the phagosome. Now that the innate macrophage can help eliminate the bacteria *in vivo*, why is there a need to develop new antibacterial strategies? That is because many bacteria have evolved to

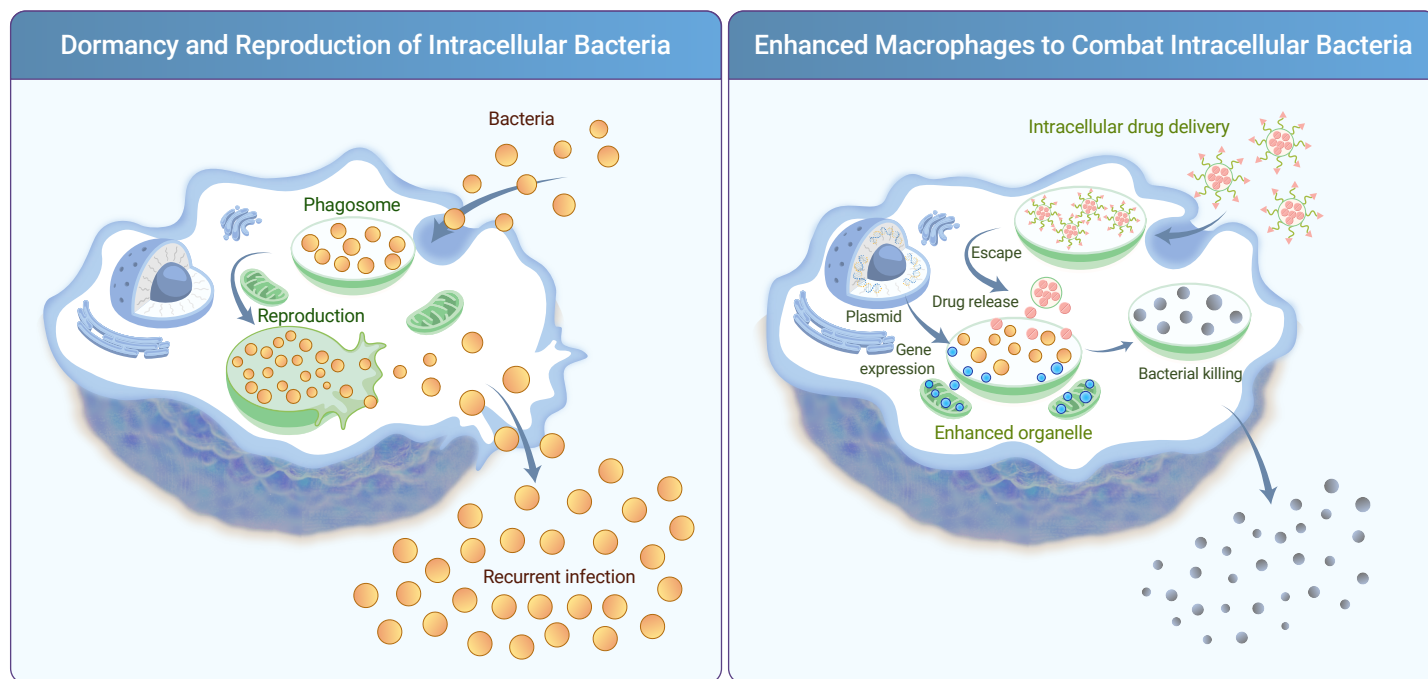


Figure 1. Schematic diagrams showing the risk of intracellular bacteria and the strategies to endow macrophages with the enhanced bacteria elimination ability.

escape from immune clearance. For example, the engulfed *S. aureus* can activate the caspase-11 (CASP11) pathway to hijack the mitochondria in macrophages, which disrupts the ROS defense system driven by mitochondrion and keeps the *S. aureus* safe from the lethal ROS.³ In this case, the macrophages somehow provide a protective shield for the intracellular bacteria to reproduce, leading to the burst release of pathogens and recurrent infection eventually. Meanwhile, under cover of the cell membrane, the intracellular bacteria can tolerate high-dose antibiotics, leading to the abuse of antibiotics and eventually aggravating the development of drug resistance. Therefore, macrophage enhancement is necessary to keep pace with bacterial evolution as well as relieve the generation of superbugs.

The representative strategies to strengthen macrophages can be classified into external assistance and endogenous intervention (Figure 1). The former aims to endow macrophages with helpful “weapons” for bacterial elimination. Hence, how to deliver antimicrobials into macrophages precisely is the first thing to consider. In a paper published in 2022, Feng et al. used mannose to decorate antibiotic-encapsulated nanoparticles to lead to

macrophage-specific uptake by mannose receptor-mediated endocytosis.⁴ After entering the macrophages, the acidic condition in phagolysosomes cleaved the Schiff-based bonding between mannose and nanoparticles, consequently releasing the nanoparticles into the cytoplasm to target the methicillin-resistant *Staphylococcus aureus* (MRSA) hidden in the privileged intracellular compartments. In that study, antimicrobial was delivered precisely into the macrophages and targeted the latent MRSA to assist the dysfunctional macrophages, thus producing excellent eradication of intracellular bacteria.

In addition to external assistance, another potential way to combat intracellular bacteria is to improve the antibacterial performance of endogenous organelles. For instance, although some bacteria have evolved immune evasion, lysosomes still retain their innate tendency to fuse with the bacteria-containing phagosome and provide endogenous antimicrobials. In a paper published in 2020, Hou et al. introduced the antimicrobial peptide (AMP) to the lysosomes in macrophages to enhance the bactericidal ability.⁵ Briefly, the vitamin C lipid nanoparticles with AMP-linked cathepsin B (CatB) mRNA

were transfected in macrophages to produce protein precursors by translating the CatB mRNA in the cytoplasm. The CatB precursors were transferred to lysosomes and processed to CatB proteins, during which the binding between AMP and CatB was cleaved to obtain the macrophages with AMP-rich lysosomes. In treating the immunocompromised septic mice, these macrophages with AMP-enhanced lysosomes exhibited superior elimination of intracellular bacteria.

In the local treatment of implant-related infections, *in situ* macrophage enhancement is more favorable as it avoids complicated steps to collect, modify, and re-administrate the immune cells. A recent study, titled "Surficial nano-deposition locoregionally yielding bactericidal super CAR-macrophages expedites periprosthetic osseointegration," describes a promising strategy to facilitate the bactericidal ability of periprosthetic macrophages.³ In this study, the plasmid DNA-encapsulated peptide nanoparticles (pPNP) were loaded on the titanium surface *via* layer-by-layer self-assembly with poly-L-lysine. As the peptide contained a macrophage-targeting sequence and nuclear localization signal, the pPNP could be precisely engulfed by macrophages and transported the plasmid DNA (pDNA) into nuclei. In the nuclei, the *S. aureus*-specific chimeric antigen receptor (CAR) gene in the pDNA was transcribed to the CAR mRNA related to the specific surface protein on bacteria, and the CASP 11 short hairpin RNA (shRNA) sequence was exposed to produce the small interfering RNA (siRNA) of CASP 11 pathway. The expression of the CAR gene resulted in the formation of the *S. aureus*-specific CAR structures on the macrophage membrane, which significantly enhanced the ability of macrophages to identify and internalize *S. aureus*. Meanwhile, since the suppression of the CASP 11 pathway reduced mitochondrial hijacking, mitochondria were found to accumulate around the bacteria-containing phagosomes, thus killing the intracellular bacteria by sufficient mROS production. Due to the enhanced bacterial recognition and intracellular sterilization, the periprosthetic macrophages treated by pPNP showed an inspiring efficacy in countering the *S. aureus* threat in the mouse model with hematogenous implant infection.

Overall, although evasive bacteria have evolved to conceal inside the immune cells, the ability of macrophages to identify and internalize pathogens cannot be ignored during infections. Therefore, enhancing macrophages to combat intracellular bacteria is valuable because it makes maximum use of the endogenous power. Unlike the exposed bacteria, elimination of intracellular bacteria needs to overcome the barrier from the

macrophage membrane. This explains why nanomaterials are consistently applied as illustrated by the aforementioned research articles. In the future, nanomaterials in the form of either carriers or functional parts may still act as the leading force to combat intracellular bacteria. On the heels of recent advances in physiology, proteomics, and genomics, the interactions between pathogens and immune cells can be profiled and the related biomacromolecules have been identified gradually. Undoubtedly, current and future interdisciplinary discoveries will continue to guide scientists to design workable routes to combat bacteria hidden in macrophages.

REFERENCES

1. Dickey, S.W., Cheung, G.Y.C., and Otto, M. (2017). Different drugs for bad bugs: Antivirulence strategies in the age of antibiotic resistance. *Nat. Rev. Drug Discov.* **16**: 457-471. DOI: 10.1038/nrd.2017.23.
2. Wu, Y., Liu, P., Mehrjou, B., and Chu, P.K. (2023). Interdisciplinary - inspired smart antibacterial materials and their biomedical applications. *Adv. Mater.* **36**: 2305940. DOI: 10.1002/adma.202305940.
3. Li, Z.Y., Zhang, S.C., Fu, Z.P., et al. (2023). Surficial nano-deposition locoregionally yielding bactericidal super CAR-macrophages expedites periprosthetic osseointegration. *Sci. Adv.* **9**: eadg3365. DOI: 10.1126/sciadv.adg3365.
4. Feng, W.L., Li, G.F., Kang, X.X., et al. (2022). Cascade-targeting poly(amino acid) nanoparticles eliminate intracellular bacteria *via* on-site antibiotic delivery. *Adv. Mater.* **34**: 2109789. DOI: 10.1002/adma.202109789.
5. Hou, X.C., Zhang, X.F., Zhao, W.Y., et al. (2020). Vitamin lipid nanoparticles enable adoptive macrophage transfer for the treatment of multidrug-resistant bacterial sepsis. *Nat. Nanotechnol.* **15**: 41-46. DOI: 10.1038/s41565-019-0600-1.

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DECLARATION OF INTERESTS

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