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INSIGHTS

# Breaking bad

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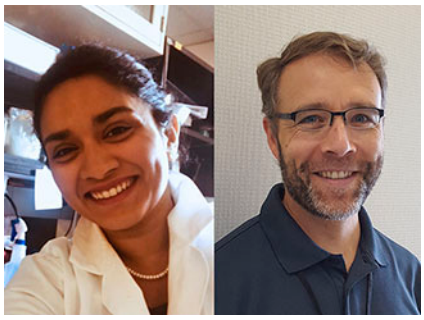
**DNASE1 (D1) and DNASE1L3 (D1L3) synergistically reduce the severity of systemic infections caused by *Staphylococcus aureus*. In this issue of *JEM*, Lacey et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221086) develop D1$^{-/-}$, D1L3$^{-/-}$, and D1$^{-/-}$D1L3$^{-/-}$ mice to show that exogenous addition of the DNase formulation Dornase alfa can facilitate removal of biofilms.**

The maintenance of homeostasis and tissue renewal often requires a rapid removal of dead and dying "self" cells through programmed cell death, a process that prevents excessive inflammation which would inadvertently present severe consequences to the host. This includes the elimination of DNA from the nucleus and mitochondrion, the two compartments that physically restrict host DNA. Under conditions of stress such as injury or infection, host DNases are responsible for eliminating self-DNA that may be released into circulation. The DNASEI family of extracellular nucleases comprises four members, namely DNASE1, DNASEIL1, DNASEIL2, and DNASEIL3. Despite being highly conserved in vertebrates, the functions of this protein family are not fully understood. While all four homologs are thought to digest extracellular DNA, DNASE1L3 plays a particularly important role in preventing deleterious immunological responses to self-DNA. Indeed, null mutations of DNASE1L3 in humans result in the development of an early antibody response against self-DNA, characteristic of monogenous systemic lupus erythematosus (SLE), which can progress into a specific anti-DNASE1L3 antibody response, thus reducing the enzymatic activity of DNASE1L3 in circulation. While the combined activities of DNASE1 and DNASE1L3 are responsible for clearing most of the DNA from circulation, the contribution of DNASE1 is poorly understood. Current and previous work has shown that deletion of DNASE1 in mice does not result in immune autoreactivity, suggesting that DNASE1 is unlikely to play a major role in SLE disease progression in humans, leaving the significance of this DNASE controversial (Keyel, 2017; Lauková et al., 2017). Additionally, while much is known about the removal of self-DNA by these proteins, their potential role in responding to DNA in the context of infection with bacterial pathogens remains unclear. Some studies indicate that DNASE1 may be important for digesting neutrophil-derived DNA released in the process of neutrophil extracellular trap (NET) formation, but beyond this the potential roles of DNASE1 and DNASE1L3 in combating infection have not been explored. Lacey et al. (2023) use genetically modified mice to examine the impact of DNASE1 and DNASE1L3 on autoimmune activity and systemic *Staphylococcus aureus* infection.

By measuring auto-antibody responses (ELISA) generated in C57BL6 mice deficient in either DNASE1 (D1$^{-/-}$), DNASE1L3 (D1L3$^{-/-}$), or both DNases (D1$^{-/-}$D1L3$^{-/-}$), Lacey et al. (2023) were able to confirm that DNASE1L3, unlike DNASE1, is required for prevention of an immune response to self-DNA. Additionally, the authors utilized Sca-1, a surface protein that is abundantly expressed in response to type 1 interferon, to verify a role for DNASE1L3, but not DNASE1, in the tolerance to self-DNA. Since nucleases have often been reported to be involved in the clearance of microbial pathogens, Lacey et al. (2023) use mice deficient in DNASE1 or DNASE1L3 in combination with mice deficient in both DNases to demonstrate that the two host DNases work synergistically to reduce the severity of systemic infection with USA300 methicillin-resistant *S. aureus* (MRSA), a problematic cause of debilitating community and hospital acquired infections. Following a high-dose ($10^7$ CFU/mouse) intravenous injection with USA300, almost all D1$^{-/-}$D1L3$^{-/-}$ mice succumbed to infection, whereas ∼50% of WT mice survived. Further studies performed with a lower bacterial dose ($7.5 \times 10^6$ CFU/mouse) yielded an ∼50% survival rate for D1$^{-/-}$D1L3$^{-/-}$ mice compared to complete survival of WT mice. In contrast and of importance, systemic infection studies on D1$^{-/-}$ and D1L3$^{-/-}$ mice, which lacked just a single DNase, showed no significant loss in survival, demonstrating that amelioration of systemic bacterial infection was a synergistic effect of both DNases. Since seminal studies have



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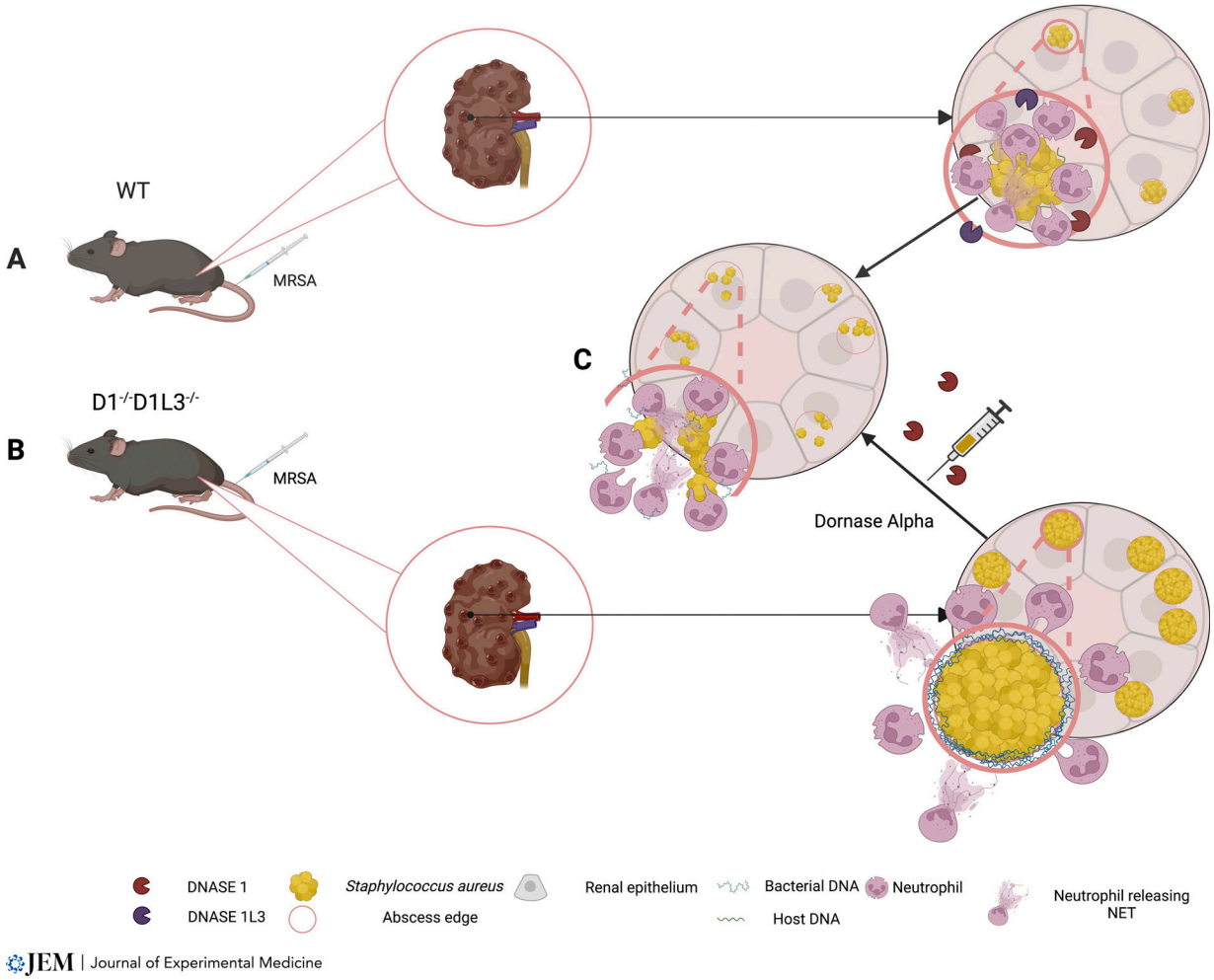


Summary of major findings by Lacey et al. (2023). Tail vein injection of WT C57 BL6 mice with MRSA results in systemic bacterial infection resulting in renal pathology characterized by abscess formation. Small clusters of bacteria are surrounded by host DNA and infiltrated by neutrophils. Two host DNases, DNASE1 and DNASE1L3, break down host DNA (A). C57 BL6 mice deficient in DNASE1 ($D1^{-/-}$) and DNASE1L3 ($D1^{-/-}D1L3^{-/-}$) infected with MRSA similar to panel A develop worsened renal pathology with higher numbers of large biofilm-like clusters surrounded by bacterial DNA. Neutrophils are unable to penetrate these clusters, resulting in higher biofilm biomass and increased bacterial burden (B). The activity of DNASE1 and DNASE1L3 in WT mice, or the exogenously added DNASE1 formulation Dornase alfa in $D1^{-/-}D1L3^{-/-}$ mice, results in breakdown of host (WT) and bacterial ($D1^{-/-}D1L3^{-/-}$) DNA and removal of biofilm-like clusters, reducing renal pathology and disease severity (C).

identified the development of renal abscesses, with immune infiltration and inflammation as a characteristic feature of murine models of systemic *S. aureus* infection, and identified the kidneys as a preferred niche for *S. aureus* persistence following systemic infection, Lacey et al. (2023) characterized the innate immune responses generated in infected WT and $D1^{-/-}D1L3^{-/-}$ mice (Thammavongsa et al., 2013). In agreement with previous reports, the authors observed increased influx of neutrophils in both WT and $D1L3^{-/-}$ mice, with greater influx occurring in $D1^{-/-}D1L3^{-/-}$ mice. Despite the greater neutrophil influx in DNASE-deficient mice, there was no significant difference in the levels of inflammatory cytokines generated by WT and $D1^{-/-}D1L3^{-/-}$ mice in response to infection. Since the studies noted above, as well as others, identified NETs as one of the hallmarks of severe *S. aureus* mediated pathology, NET-associated markers were quantified by ELISA. These measurements ruled out any significant difference in this neutrophil defense in infected WT and $D1^{-/-}D1L3^{-/-}$ mice, and suggested involvement of an additional DNase, other than D1 or D1L3, in the elimination of NET-associated DNA generated in response to *S. aureus* infection.

Biofilms are structured communities of bacteria, prominent during infection, in which bacteria are encased in an extracellular matrix consisting of protein, polysaccharide, and DNA, with the extracellular DNA (eDNA) potentially being of both bacterial and host origin. *S. aureus* is a prolific biofilm-forming pathogen that relies in large part on negatively charged DNA for interbacterial attachment as well as adherence to biotic and abiotic surfaces (Hall-Stoodley et al., 2004). *S. aureus* biofilms have been reported to induce NETosis and utilize their own nucleases to break down NET DNA, a process that results in the dispersal of biofilm bacteria, and the addition of exogenous DNASE1 has been shown to disrupt biofilms in vitro (Tetz et al., 2009; Thammavongsa et al., 2013; Bhattacharya et al., 2020). Given these observations, Lacey et al. (2023) explored the possibility that the decreased survival of $D1^{-/-}D1L3^{-/-}$ mice might be a consequence of increased formation of *S. aureus* biofilm communities within their kidneys. Using electron

microscopy, they were able to observe significantly more *S. aureus* biofilm aggregates in the kidneys of D1$^{-/-}$D1L3$^{-/-}$ mice in comparison of those of WT mice, thereby confirming a role for the host DNases in reducing in vivo biofilm formation within the kidney. While DAPI staining, which does not differentiate between host- and bacterial-derived eDNA, indicated that the bacterial aggregates in the kidneys of both WT and D1$^{-/-}$D1L3$^{-/-}$ mice were rich in eDNA, immunostaining for 5-methylcystosine, which is specific for host-derived eDNA, was higher in WT mice than D1$^{-/-}$D1L3$^{-/-}$ mice, suggesting that in the absence of the host DNases the bacterial aggregates contain predominantly bacteria-derived eDNA. Although the frequency of biofilm-like clusters was higher in D1$^{-/-}$D1L3$^{-/-}$ mice compared to WT, the authors use 5-methylcytosine to stain for DNA around these communities and provide a contrast of host DNA commonly observed in WT infected mice, from bacterial DNA that was prominent in D1$^{-/-}$D1L3$^{-/-}$ infected mice. While neutrophils were present around the site of all abscesses, the authors demonstrated that unlike WT infected mice, neutrophils were unable to infiltrate biofilm-like communities encased in bacterial DNA that were observed in D1$^{-/-}$D1L3$^{-/-}$ mice. Lastly, in agreement with previous work as well as in vitro studies performed here, the authors are able to exogenously treat WT and D1$^{-/-}$D1L3$^{-/-}$ infected mice with Dornase alfa, a commercially available formulation of human DNASE1, to reduce the pathology caused by *S. aureus* infection.

There are three major implications of these studies to the field of bacterial pathogenesis. First, these discoveries are a conclusive and elegant demonstration of the importance of host hemostatic proteins during infection. The primary requirement for the elimination of pathogens from the human body is the ability to differentiate "self" from "non-self" or "harmful." When this differentiation collapses, as is the case in patients with autoimmune diseases, microbial pathogens are known to flourish at the expense of the host. Indeed, almost all known autoimmune disorders are associated with at least one pathogen (Kivity et al., 2009). Nasal colonization by *S. aureus* has been demonstrated to be a predisposing factor for severe infections in patients with autoimmune conditions including SLE and rheumatoid arthritis (Terui et al., 2022). DNase deficiencies alone are associated with anemia, cataracts, and parakeratosis, all of which tip the scales in favor of opportunistic pathogens like *S. aureus* (Kivity et al., 2009; Terui et al., 2022). Therefore, while current studies by Lacey et al. (2023) focus specifically on the role of host DNases in response to *S. aureus*, these findings have strong potential for a much broader impact across pathogens and conditions of immune dysfunction.

Second, the discoveries made in this manuscript nicely tie together major findings in the host response to *S. aureus*, while utilizing a well-established in vivo model of severe bacterial infection. Studies establishing renal abscesses as a hallmark of septic *S. aureus* infections demonstrate subsequent NET formation and exclusion of macrophages from the abscess. Specifically, NET degradation by *S. aureus* results in the formation of cytotoxic deoxyadenosine that was reported to cause macrophage apoptosis. It would therefore be of significant interest to understand the effect of these DNases on additional aspects of the host immune response (Thammavongsa et al., 2013; Winstel et al., 2018). Importantly, these findings provide a greater appreciation for the ability of bacteria to switch between planktonic and biofilm lifestyles, with each phenotypic manifestation demonstrably resulting in local (neutrophil infiltration) and systemic (bacterial burden, survival) consequences to the host. Although biofilms are ubiquitous to chronic infections, understanding many of their characteristics including the response to the host immune system has been challenging. By demonstrating the presence of biofilm-like clusters in renal abscesses and visualizing the DNA present in the surrounding environment, the authors provide further evidence of the importance of these communities in vivo. *S. aureus* is particularly notorious for encasing in biofilm matrices with varied composition, often depending on raw materials available in the tissue environment (Paharik and Horswill, 2016). Extending these techniques to understand the role of DNases in clearing additional non-systemic, localized infections associated with biofilms (chronic wounds, keratitis, prosthetic joint, etc.) would further exemplify the impact of these findings. For example, previous studies describe the release of NETs from biofilm-like clusters formed in chronic wounds (Bhattacharya et al., 2018). The consequence of NETosis to the host and the mechanism of clearance however remains to be identified. Additionally, while the authors cleverly utilize a combination of 5-methylcytosine and increased wall teichoic acid staining to confirm the presence of biofilm-like clusters, these studies further emphasize a need for markers that define a biofilm, in the study of infectious diseases.

Lastly, this work describes the use of the clinically approved DNase formulation Dornase alfa as a means to control *S. aureus* infections. This is of great significance to therapeutic applications since *S. aureus* has proved extremely proficient at acquiring resistance to most antibiotics in current use against the pathogen. Furthermore, the authors provide evidence for their ability to prevent the development and assist the dispersal of biofilm-like infections. Biofilms can be recalcitrant to up to 20 times the concentrations of therapeutics (including antibiotics) often used for "planktonic" infections, making their eradication a challenging hurdle (Bhattacharya et al., 2015). This work provides clear evidence for the potential of these agents in vivo. The findings of Lacey et al. (2023) therefore greatly broaden our understanding of the host response to *S. aureus* and open new avenues for the development of therapeutic strategies against bacterial biofilms, an area that currently lacks effective clinical options.

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