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BACTERIAL PATHOGENESIS

CXCRs mark the spot

> LukED specifically targets CXCR1 and CXCR2 on neutrophils to kill these leukocytes

Staphylococcus aureus produces different toxins that target and kill host leukocytes to evade immunity and to enhance pathogenesis. One of these toxins, leukotoxin ED (LukED), was recently shown to specifically recognize CC-chemokine receptor 5 (CCR5) on the surface of T cells and macrophages. However, CCR5 targeting could not explain LukED-mediated killing of neutrophils. Reyes-Robles *et al.* now report that LukED also targets CXC-chemokine receptor 1 (CXCR1) and CXCR2, which, in contrast to CCR5, are expressed on neutrophils.

Leukotoxins are porins that consist of two components (for example, LukE and LukD for LukED) that bind and are inserted into the plasma membrane of target cells. This process depends on surface receptors that are expressed on target cells, such as CCR5 on T cells, macrophages and dendritic cells. Neutrophils, which are the most important cell type for the control of acute *S. aureus* infection, do not express CCR5. Nevertheless, Reyes-Robles *et al.* found that LukED efficiently kills neutrophils that have been isolated from human blood.

Furthermore, in CCR5-knockout mice, wild-type *S. aureus* replicated more efficiently than *S. aureus* lacking LukED, which indicates that LukED has additional targets besides CCR5. To identify these targets, the authors expressed different chemokine receptors in human embryonic kidney cells. As expected, LukED killed CCR5-expressing cells; however, both CXCR1- and CXCR2-expressing cells were also susceptible to LukED. CXCR1 and CXCR2 are closely related receptors that are expressed on neutrophils and that respond to CXC-chemokine ligand 8 (CXCL8). Indeed, the addition of CXCL8 blocked the LukED-mediated killing of human neutrophils in a dose-dependent manner, which confirms that LukED and CXCL8 bind to the same receptors.

To further characterize LukED–chemokine receptor interactions, the authors incubated neutrophils with GFP-fusion proteins of the two toxin components. GFP–LukE showed specific neutrophil binding and, again, this interaction could be blocked by CXCL8, whereas GFP–LukD showed only non-specific background binding. LukE contains five regions that are highly divergent compared to other leukotoxins. The authors mutated these divergence regions (DRs) and observed that neither DR4- nor DR5-mutated LukE could kill neutrophils when combined with LukD but only DR5-mutated LukE abolished killing of CCR5-positive cells. These results indicate that DR5 is essential for killing, whereas DR4 determines CXCR1 and CXCR2 specificity.

In accordance with these binding specificities, all mice that were infected with an *S. aureus* strain in which LukED was deleted — and thus, in which leukocyte targeting was inhibited — survived. Complementation of LukED made this strain highly pathogenic, as shown by the death of all infected mice. By contrast, complementation with DR4-mutated LukE and LukD did not restore full pathogenicity, and most mice that were infected with this *S. aureus* strain survived. Taken together, these results show that LukED specifically targets CXCR1 and CXCR2 on neutrophils to kill these leukocytes, which are crucial for protecting the host from fatal *S. aureus* infections.

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ORIGINAL RESEARCH PAPER Reyes-Robles, T. *et al. Staphylococcus aureus* leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. *Cell Host Microbe* **14**, 453–459 (2013)