Emergent Enterococcus toxins

Enterococcus is a large genus of bacteria that is found in human and animal microbiomes, and multidrug-resistant enterococci (for instance, Enterococcus faecalis and Enterococcus faecium) have emerged as important nosocomial pathogens. Unlike many other Gram-positive bacteria, potent protein toxins targeting human and animal cells have not been described in Enterococcus species. Now, Xiong et al. uncover a new family of Enterococcus pore-forming toxins (Epxs) that use human leukocyte antigen class I (HLA-I) or major histocompatibility complex class I (MHC-I) as receptors.

The authors collectively identified eight Epxs (Epx1–8) in the genomes of E. faecalis, E. faecium and Enterococcus hirae strains isolated from diverse sources around the world, including animal meat, wastewater, healthy humans, infections and even a 40,000-year-old mammoth gut. Genomic analyses revealed that these toxins are 40–89% identical to each other and are phylogenetically distinct from other pore-forming toxins (PFTs).

Next, focusing on Epx1–4, the authors purified Epxs produced in Escherichia coli and confirmed that they are cytotoxic when added to a range of human and animal cell lines. Structural analyses of Epx1 and Epx4 revealed that these toxins form a sub-class of the haemolysin family, with an overall feature of Epx pore architecture is similar to other β-barrel PFTs such as γ-haemolysin structural features similar to other MHC-I and homologous MHC-I of equine, bovine and porcine (but not murine) origin. The authors were able to specifically map the binding site for Epx2 (the region involved in antigen presentation and T cell receptor binding) by switching domains between human HLA-I and murine MHC-I. Treatment with interferon-γ, which upregulates MHC-I expression, significantly increased the sensitivity of human cell lines and intestinal organoids to Epx2- and Epx3-induced cytotoxicity.

Last, the authors co-cultured E. faecium DIV0147 harbouring Epx2 with human cell lines, human peripheral blood mononuclear cells (PBMCs) or intestinal organoids. Co-culture with E. faecium DIV0147, but not a control strain, led to significant cell line death and damage to PBMCs and intestinal organoid monolayers. The addition of an Epx2 antibody abrogated the toxic effects of E. faecium DIV0147.

Altogether, these data demonstrate toxin-mediated virulence by enterococci encoding Epxs with a potential role in immune suppression and epithelial barrier disruption during infection.

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**RELATIONED ARTICLE** Harvey, E. & Holmes, E. C. Diversity and evolution of the animal virome. Nat. Rev. Microbiol. https://doi.org/10.1038/s41579-021-00665-x (2022)