COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Host-pathogen Interactome Analysis Identifies Key Pathways For Host Targeted Anti-toxin Therapies

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Background information.
Biological toxins are produced by several bacterial species. Common pathways of intoxication in the so-called short-trip toxin pathway. In the short-trip pathway, toxins are internalized via the endocytic pathway and require acidification of the endosome to initiate translocation into the cell cytoplasm where they have unique mechanisms of action. Since toxins have unique receptors mechanisms of action, the shared translocation step is an attractive target for development of anti-toxin therapies.
Purpose.
Develop broadly efficacious host-targeted anti-toxin therapies using PPMO or AAV knockdown vectors to temporarily inhibit toxin translocation.
Objective.
Identify the global host-gene requirements for toxin resistance and identify specific host-factors involved in maturation of the endosome to the endolysosome that result in toxin resistance.
Rationale of the research.
Using anthrax toxin as a model short-trip toxin, identify genes whose knockout results in toxin resistance. Validate the role of key players and test other toxins or infections for efficacy of the host-targeted intervention.
Relationship to other areas of study.
These host-targeted therapies should be tested in MPS, organoid, and small animal models.
Methods.
Whole genome CRISPR knockout pools produced in mouse macrophages were challenged with toxigenic spores. Whole genome sequencing was performed weekly after challenge. String network analysis was used to identify interconnected protein nodes. A gene showing consistently high selection was knocked out in mouse macrophages for further study. Toxin survival assays were used to measure toxin resistance.
Preliminary results.
Key protein networks identified in the screen confirmed the endosomal maturation components required for short-trip cellular intoxication. Later resistance determinants revealed the major anthrax toxin receptor (Antxr2) and a second gene (Ccdc115) experiencing the highest selection at the end of the study. Knock out of the regulator gene Lamtor4 did not affect viability. Clones knocked out using different sgRNAs were significantly resistant to anthrax toxin mediated cell death.
Preliminary conclusions.
The short-trip toxin pathway shares many waypoints with virus internalization, including acidification of the endosomal compartment. Coxiella burnetii requires acidification of the endosomal vesicle to shift from the small to large cell variant. Lamtor4 may modulate acidification of the endosomal compartment. One of the strongest negative signals was Ccdc115 mutation. Ccdc115 mutations have been shown to over acidify the endosomal compartment and degrade IAV and VSV before infection and presumably results in degradation of the anthrax toxin before translocation. Aside from anthrax toxin and these pathogen agnostic applications, other short-trip toxins include BoNT, TeNT, iota toxin, C2 toxin, large Clostridial toxins, and diphtheria toxin.
Impact to the DTRA JSTO mission and the Joint Force.
Targeting the endolysosomal pathway for host therapies can provide broad temporary protection against several toxin, viral, and bacterial threats. PPMOs and AAVs can be applied via the inhalational or oral routes to rapidly protect warfighters. sgRNAs and Cas9 enzymes can be encoded in liposome encapsulated RNA treatments to take advantage of RNA-based therapeutics.