Bacterial Virulence Proteins as Tools to Rewire Kinase Pathways in Immune Cells

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Statement of Purpose: Bacterial pathogens have evolved specific effector proteins that, by interfacing with host kinase signaling pathways, provide a mechanism to evade immune responses during infection. Although these effectors are responsible for pathogen virulence, we realized that they might also serve as valuable synthetic biology reagents for engineering cellular behavior. Here, we have exploited two effector proteins, the Shigella flexneri OspF protein and Yersinia pestis YopH protein, to systematically rewire kinase-mediated responses mammalian immune cells. We show that effectors can be used in T cells, either as feedback modulators to precisely tune the T cell response amplitude, or as an inducible pause switch that can temporarily disable T cell activation. These studies demonstrate how pathogens could provide a rich toolkit of parts to engineer cells for therapeutic or biotechnological applications.

Methods: All plasmids were made by using standard cloning techniques, AarI combinatorial cloning technique1 and Gateway cloning technique. For experiment in Jurkat T cells, plasmids were transfected via electroporation. T cell receptor was activated with a Jurkat specific anti-TCR antibody, C305. For staining of phosphorylated ERK, cells were fixed, made permeable by incubation with ice-cold 90% methanol on ice for 30 minutes and stained with primary antibody to p-ERK and anti-rabbit APC secondary antibody. Resting human primary CD4+ cells were pretreated with 200 ng/ml doxycycline for 6 hours. 50, 000 cells were placed in a 96-well plate with 200 μL human growth media with activation agents added (10 ng/mL PMA + 0.5 μM ionomycin, magnetic Dynabeads coated with anti-CD3/anti-CD28). After 24 hours of incubation at 37°C, the released IL-2 in the supernatant was measured with the human IL-2 ELISA kit II. Cells labeled with CellTrace Violet dye were assayed by flow cytometry after 4 days incubation to quantitate cell proliferation.

Results: Both OspF and YopH can modify the T cell receptor (TCR) pathway. Constitutive expression of YopH and OspF in Jurkat T cells leads to severe inhibition of TCR activation, as measured by an NFAT transcriptional reporter. Given the ability of OspF and YopH to modulate T cell responses, we sought to use these proteins to build circuits that could, in principle, improve the safety of therapeutic T cells. In adoptive T cell therapy, a challenge is to limit over-activation or off-target activation of T cells that could lead to killing of host cells or to cytokine storm – a life-threatening immune response. One approach is to incorporate a safety “kill switch” into the T cells, such as the herpes simplex virus thymidine kinase (HSV TK) gene. This protein converts the pro-drug ganciclovir into an inhibitor of replication, thus killing cells expressing the gene. While HSV TK is currently being tested in a phase III clinical trial for the treatment of graft vs. host disease in bone marrow transplants, this strategy irreversibly destroys the engineered, adoptively transferred cells. Thus instead of killing the engineered cells, we sought to design circuits that would limit the amplitude of the T cell response or to temporarily pause T cell activity.

We first tested whether bacterial effectors could be used to limit the response amplitude of Jurkat T cells. Negative feedback loops can act to limit the maximal amplitude of a response, so we engineered a library of negative feedback loops in which the OspF and YopH were expressed from a series of TCR responsive promoters of varying strength (AP1 and NFAT). For further tuning of feedback parameters, we also tagged effectors with degradation sequences (PEST motif) that reduce half-life of the effectors. This series of negative feedback loops led to controlled reduction of the maximal response amplitude of T cell activation. Moreover, the amplitude could be tuned systematically by varying feedback promoter strength and effector stability.

We then created a pause switch in a clinically important cell type for adoptive immunotherapy - primary human CD4+ T cells (in contrast to the Jurkat T cell line, which does not require cytokine or TCR activation to stimulate proliferation). We showed that when OspF is induced by the addition of doxycycline, both IL-2 release and cell proliferation were inhibited in a dose-dependent manner. Activation of the TCR by anti-CD3/CD28 and antigen presenting cells can also be inhibited by expression of OspF. Moreover, after dox is removed, IL-2 release and cell division recovers within 6-18 hours. Sustained dox exposure can inhibit T cell activity over the course of several days without having any significant effect on cell viability. Thus this work provides a proof of principle for the design of a simple “pause” switch that could allow external control over the timing and level of T cell activation and cytokine release, in order to minimize adverse events associated with adoptive immunotherapy such as cytokine storm.

Conclusions: Most work on bacterial pathogen effector proteins has the long-term aim of neutralizing the pathogens’ infectious capabilities. We have shown, however, that bacterial effectors can also be valuable synthetic biology tools, because of their unique biochemical properties. We also showed that bacterial effectors can be used to flexibly tune human T cell receptor signaling dynamics, with potential application as safety switches for adoptive immunotherapy. The vast array of bacterial pathogen effector proteins, beyond those studied here, holds promise as a rich and important source of parts for the cellular engineering toolkit.

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