Biochemistry

Viewpoint

Cite This: *Biochemistry* 2018, 57, 5923−5924

pubs.acs.org/biochemistry

A Slick Solution to a Sticky Problem

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A Viewpoint for *Biochemistry* on the article in *Cell*, "Immunomimetic Designer Cells Protect Mice from MRSA Infection"

In his 2002 essay entitled "Can a biologist fix a radio?—Or, what I learned while studying apoptosis", Yuri Lazebnik chides the biology community for its over-reliance on reductionistic experimental techniques such as genetic deletion to describe complex systems.[1] He humorously illustrates his argument by describing how a biologist might attempt to fix a radio, including the obligatory annihilation of each electrical component "at close range with metal particles" to determine its function, an approach analogous to the murine knockout experiments upon which biologists so depend. In contrast, he submits that an engineer would solve the problem handily based on a facility with standardized technical language (electrical diagramming) that fully and unambiguously describes the mechanical device.

The rub, of course, is that radios are built by engineers in the first place; cells, much less animals, are not. Indeed, a principle advantage engineers hold over biologists in terms of "handiness" derives from a hundred years of research into the fundamental principles of electromagnetism and another hundred in refining the practice of electrical engineering, which allows them to create electrical devices according to a standardized methodology. A similar position of privilege is enjoyed by modern organic chemists, who have likewise spent centuries designing and synthesizing a huge array of small molecules and natural products, including the chemical therapeutics that serve as the backbone of modern medicine, to establish a robust fundamental and technical appreciation for how molecules behave. Accordingly, it might be stated that the true understanding of a system, as achieved in areas of physics and chemistry, is indicated by the ability to construct one.

In recent years, biologists have made strides toward this lofty goal as they have endeavored to create cells, either from scratch in the case of synthetic biology or by modifying existing cells for therapeutic ends. A particularly successful example of the latter comes from immuno-oncologists, who have genetically engineered T-cells to express specific sensors for cancer antigens called chimeric antigen receptors (CARs). Ligation of these receptors elicits robust effector responses (cytokine release by CD4+ cells and direct cytotoxicity by CD8+ cells), leading to the elimination of targeted tumor cells (Figure 1). The clinical success of CAR T-cells in the treatment of advanced hematologic malignancies is unprecedented; for instance, anti-CD19 CAR-T therapy induces complete remission in ∼90% of patients with chemo-resistant B cell leukemias, in whom life expectancy would otherwise be months.[2]

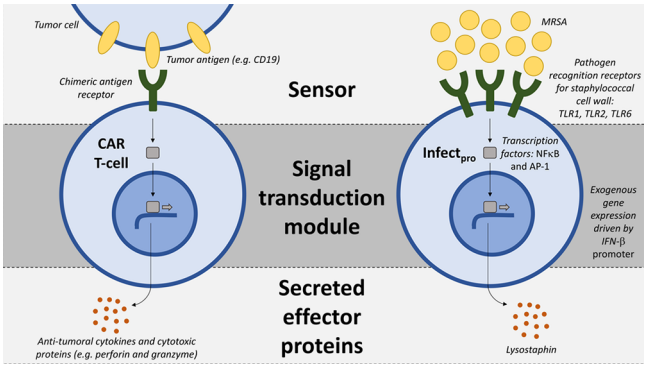

Figure 1. Tripartite design of bioengineered therapeutic cells. The three-part design includes a sensor, a signal transduction module, and secreted effectors. CAR T-cells (left) are equipped with modified surface receptors that recognize cancer cells via unique tumor antigens, leading to the production of pro-inflammatory cytokines and cytotoxic proteins. Infect$_{pro}$ cells are equipped with pathogen recognition receptors that recognize *Staphylococcus aureus* via bacterial cell wall components, leading to generation of lysostaphin, which clears MRSA infections. Abbreviations: CAR, chimeric antigen receptor; MRSA, methicillin-resistant *S. aureus*; TLR, Toll-like receptor; IFN, interferon.

Now, Liu et al. apply this engineering principle to develop a novel therapeutic for infectious disease.[3] They target methicillin-resistant *Staphylococcus aureus* (MRSA), a Gram-positive bacterium that is responsible for more deaths in the United States than any other bacterial pathogen, and can infect virtually any tissue, including blood, lung, and skin. A particularly feared form of MRSA infection is establishment of biofilms on implanted hardware, such as prosthetic joints or heart valves, which almost invariably necessitates surgical removal of the foreign material due to resistance of the associated biofilms to antibiotics.

To tackle this problem, Liu et al. construct an elegant genetic network consisting of three principal components (Figure 1): (i) a sensing mechanism for constituents of the *S. aureus* cell wall, namely, lipoproteins and wall teichoic acids, which are detected via the cell-surface pathogen recognition receptors TLR1, TLR2, and TLR6, along with the co-receptor CD14; (ii) a highly optimized signal transduction module based on the immune transcription factors NFκB and AP-1 and the promoter for interferon-β; and (iii) a response element leading to expression of proteins that function as either diagnostics (secreted alkaline phosphatase) or therapeutics

Received: August 30, 2018
Published: October 5, 2018

DOI: 10.1021/acs.biochem.8b00916
Biochemistry 2018, 57, 5923−5924

(lysostaphin, an enzyme that digests peptidoglycan by cleaving pentaglycine cross-bridges). Lysostaphin has long been touted as a potential treatment for refractory *S. aureus* biofilms given its rapid bactericidal effect (even against the slow-growing cells characteristic of biofilms), relative nonsusceptibility to drug resistance, minimal effects on gut microbiota (as it is degraded by intestinal proteases), and specificity for staphylococcal peptidoglycan (which is based on the bacteria's unique expression of pentaglycines).[4] However, several obstacles have precluded its clinical translation, including high costs and immunogenic potential.

Here, the researchers hypothesize that local, pathogen-elicited lysostaphin production would eradicate MRSA biofilms in a murine model of implant infection. To test this, they express their synthetic gene network in HEK-293 cells and find that the modified cells, dubbed $Infect_{pro}$, are indeed able to detect *S. aureus in vitro* and respond with robust production of lysostaphin, resulting in rapid bacterial killing. Next, they show that prophylactically delivered alginate-encapsulated $Infect_{pro}$ cells are able to prevent infection produced by subcutaneous implantation of biofilm-coated Teflon tissue cages in mice. Finally, they demonstrate near-complete eradication of tissue cage biofilms after injection of $Infect_{pro}$ cells into established implants. Most impressively, they find that the "sense-and-destroy" cells outperform both conventional antibiotic therapy with vancomycin and systemic administration of lysostaphin.

Further directions might include transfecting the synthetic gene network into host leukocytes, which would circumvent rejection of orthologous cells and also allow tissue homing of $Infect_{pro}$ cells to infected sites if the cells are delivered intravenously. Combination treatment with vancomycin and $Infect_{pro}$ is another logical option for maximizing therapeutic efficacy, as suggested by prior studies demonstrating synergism between antibiotics and lysostaphin.

As the post-antibiotic era ineluctably nears, the need for creative nontraditional solutions to infectious disease intensifies. Vaccines represent an important preventive modality, but for established infections with multi-drug-resistant organisms, few options exist. Bacteriophages, which actually predate antibiotics in the history of antimicrobial therapeutics, represent a potentially powerful option for salvage therapy, as dramatically demonstrated by a report showing their efficacy in the treatment of a patient with antibiotic-refractory pseudomonal infection of an aortic graft.[5] Immunomimetic cells like the one reported here may well be another. Beyond infectious disease, one can imagine a myriad of potential applications for a designer cell capable of delivering protein therapeutics selectively at their desired site of action, for instance, intraclot secretion of tissue plasminogen activator in patients with pulmonary embolism.

Ultimately, one might envision a modular genetic cassette consisting of a biological sensor, a signal transduction system, and secreted effector protein that is optimized for easy expression in host cells and customizable to suit a patient's specific pathology, be it infectious, oncologic, or other. Such a practical, engineering-inspired method for creating therapeutic cellular devices would represent a significant departure from traditional small molecule-based therapy, a step toward personalized medicine, and an answer to Lazebnik's prescient call for a codified, generalizable system for solving biomedical problems.

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Funding

S.G. was supported by National Heart, Lung and Blood Institute Grant T32-HL007778.

Notes

The authors declare no competing financial interest.

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DOI: 10.1021/acs.biochem.8b00916
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