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

Selecting Antibodies Against The Y. Pestis Lipid Membrane In A Nanodisc Format

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Antibodies are highly effective therapeutics and diagnostics because of their high affinity for protein-based antigens. However, proteins are species-specific and prone to mutation, which are limitations when designing countermeasures against future, unknown pathogens. To create broadly protective countermeasures, we need antigens that are conserved among pathogens and antibodies that block key interactions between the pathogen and host. Lipids are attractive antigen candidates for their conservation and their important roles in host–pathogen interactions. To address the need for broadly protective medical countermeasures, we are developing antibody selection protocols for lipid-specific, nanodisc-based antigen format. We hypothesize that a lipid-targeting antibody could block host-pathogen interactions and have a protective effect. Initial protocol development was performed using nanodiscs containing lipopolysaccharide (LPS) of Escherichia coli. Our current work focuses on selecting antibodies from the lipids of Yersinia pestis, the causative agent of plague.

LPS nanodisc selection details, including antigen format, antibody library preparation, and selection protocols will be presented. Due to the “sticky” nature of lipids, we were forced to overcome many obstacles, which we combated with selection strategies such as excising fragmented sequences, competitive binding, preclearing nonspecific binders, subtracting nonspecific binders, and alternating antigen formats, which will also be discussed. Using these methods, we successfully identified one peptide and one antibody with LPS-specific binding. The binding characteristics of the peptide, such as affinity and specificity along with the error prone PCR based affinity maturation and fluorescence-activated cell sorting (FACS) of the antibody will be detailed.

For our current work on Y. Pestis selection, the Los Alamos National Laboratory chemistry team has recently developed a novel nanodisc-based method that enable us to pursue lipid antigens from select agents, such as Y. Pestis. Our team isolates surface and internal lipids from Y. Pestis and embeds them in a well characterized nanodisc format. This allows us to select antibodies against a bacterial membrane—agnostic of its lipid profile—in a stable format at a low biosecurity level (BSL1). Details of selection protocol and results for Y. Pestis lipid antibodies will be presented. Future work on this project could include testing of selected antibodies in the IgG format for efficacy as a broadly protective medical countermeasure against plague.

In addition, the nanodisc format has been shown to be physiologically relevant to eliciting an immune response. This presents an opportunity to evaluate Y. Pestis lipid nanodiscs as a possible vaccine candidate. To test this hypothesis, future work would include in vitro immunoassays using peripheral blood mononuclear cells (PBMCs) to evaluate the ability of Y. Pestis nanodiscs to elicit an immune response, followed by animal testing. This process and these methods can be further expanded to create effective protection across a variety of bacterial threats, their evolutions and their new strains.

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