R1. A PROTEIN PLATFORM FOR THE RAPID BIOENGINEERING OF MULTI-EPITOPE ANTIGENS AND MULTI-VALENT BIOMARKERS

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INTRODUCTION Recombinant technology has greatly advanced immunology-based solutions to infectious diseases. Prior to the use of recombinant proteins, reagents were generated directly from preparations of target pathogens. Such preparations have the distinct advantage of including all antigens to elicit or recognize immune system responses. However, they require the manipulation of pathogens, which can be costly and dangerous. Utilization of recombinant proteins can overcome this disadvantage, but robustness and sensitivity can often be decreased by the reduction in the epitopes present. Enhancements have been achieved by combining multiple pathogenic biomarkers into single recombinant proteins, but practical limitations have restricted the complexity of these chimeras and, therefore, the number of pathogen proteins represented.

OBJECTIVE To improve the immunologically relevant representation of a pathogen in a single recombinant protein, a protein platform was engineered to receive multiple short amino acid sequences (<20 aa) without altering the behavior of the resulting chimeric protein compared to the unaltered platform.

METHODOLOGY A naturally occurring protein sequence was identified that demonstrated a capacity to accommodate the insertion of extraneous sequences at various locations along the coding sequence. Through molecular biology, the sequence was modified to contain multiple insertion points spread throughout the coding region. Each point was defined by a unique restriction site that allows the incorporation of peptide sequences in frame to the platform protein. Four pathogens were selected to generate examples of multiepitope antigens and multi-valent biomarkers using elements of the pathogens that were identified as capable of delivering desired performance

profiles. Chimeric proteins were expressed in *E. coli* using the pET expression system. The performance of multi-epitope antigens will be tested for their ability to elicit an immune response towards pathogen specific sequences while multi-valent biomarkers were analyzed for reactivity to patient sera by *Western Blot*, ELISA and carbon printed electrodes.

RESULTS The expression profiles of the five chimeric proteins showed similar patterns with a 3 hour induction period sufficient to produce target levels of 50-100 μg/mL. Identical protocols permitted the purification of each to high purity (>95%). The performance of the individual multi-valent biomarkers showed high reactivity to sera from patients with the corresponding infection without significant cross reactivity to other infections. Evaluation of the immune response against the multi-epitope antigen is in progress.

CONCLUSION The protein platform utilized in these studies shows great promise as a method to develop immunological tools for combating infectious agents. The results suggest that this approach can rapidly (<8 weeks) generate multiepitope antigens or multi-valent biomarkers with desired performance properties through standardized protocols.

KEYWORDS biomarkers, pathogens, rapid test.