Protein Engineering Potential Inhibitor of Detrimental Immune Responses

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Abstract

On the surface of immune cells, class II major histocompatibility complex proteins (MHCII) present antigenic peptides for CD4+ T cell recognition, which initiate a variety of antigen-specific immune responses such as antibody response or cytotoxic T cell activation. In people with autoimmune-immune diseases including but not limited to type 1 diabetes, multiple sclerosis, and rheumatoid arthritis, detrimental immune responses occur after the presentation of antigenic peptides. A single-chain, minimal MHCII (scm-MHCII) has been designed to retain its function as an antigen-presenting protein with a simplified structure that can be easily produced and manipulated in a laboratory by recombinant microbial expression. By applying directed evolution and selection for protein stability quantified using yeast surface display (YSD), we have engineered a mutant library which may contain highly stable mutants capable of functioning as a highly specific inhibitor of T cell-mediated immune responses with the potential to be applied to treating a variety of autoimmune-immune diseases.

1. Introduction

Goals

1. Apply directed evolution to engineer stable scm-MHCII mutants.
2. Isolate and characterize stable mutants demonstrating potential as a highly specific inhibitor of T cell-mediated immune responses to treat a variety of autoimmune diseases.

2. Creating Mutant Library

- Error-prone polymerase chain-reaction cycles produces scm-MHCII mutants
- Homologous recombination reintroduces scm-MHCII mutants into engineered yeast shuttle vector in EBY100 for microbial expression

3. Characterizing Library

- Mutant library contains 2.5x10^5 scm-MHCII mutants
- Successful mutagenesis: average 4.5 nucleotide mutations per 700 base pairs of scm-MHCII obtained from Sanger sequencing of clones

4. Quantifying Stability

- During yeast surface display, a flow cytometer quantifies the intensity of fluorescent antibodies bound to scm-MHCII displayed by induced EBY100.
- Fluorescence indicates the partial or complete display of simplified HLA-DR4 depending on the specific fluorophore detected.

5. Improving scm-MHCII Stability

- Yeast surface display quantifies improvement of scm-MHCII mutants

6. Screening Library for Full Length Expression

- Magnetic-activated cell sorting (MACS) isolates full length expression of scm-MHCII mutants in the library magnetically binding to the c-myc tag

Conclusions

- The combinatorial library contains the diversity required to represent all single amino acid change mutants.
- After 4 rounds of MACS screening, the more stable mutants have been isolated.

Future Considerations

- Use fluorescent-assisted cell sorting (FACS) to isolate the most stable mutants from MACS screens
- Identify desirable mutations
- Investigate combinations of desirable, characterized mutations
- Engineer stabilized scm-MHCII fts possess a high affinity for CD4+ T cells

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