Protein Engineering Potential Inhibitor of Detrimental Immune Responses

Nathaniel L Blalock, Liang Fang, Eric T. Boder

Chemical and Biomolecular Engineering Department of the University of Tennessee: Knoxville

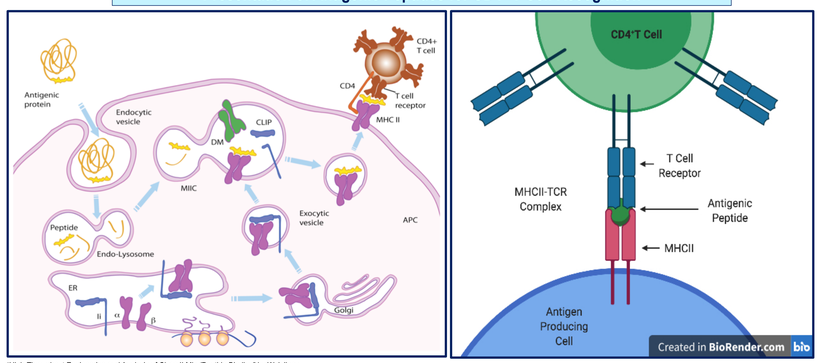
Abstract

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 with auto-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 auto-immune diseases.

1. Introduction

- Presentation of Antigenic Proteins
  1. Extracellular antigenic proteins enter immune cells through endocytosis
  2. Lysosomes digest antigenic proteins
  3. The resultant peptide fragments are loaded onto MHCII then presented on the surface of the immune cell

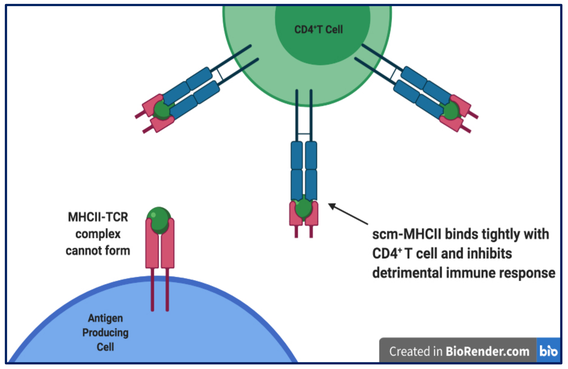
Presentation of Antigenic Peptides for CD4⁺ T Cell Recognition



"High-Throughput Engineering and Analysis of Class II Mhc/Peptide Binding" by Wei Jiang. https://repository.upenn.edu/edissertations/419/. Accessed January 21, 2020.

- CD4⁺ T cell recognition requires full-length, functional scm-MHCII to inhibit immune response by tightly binding with CD4⁺ T cells

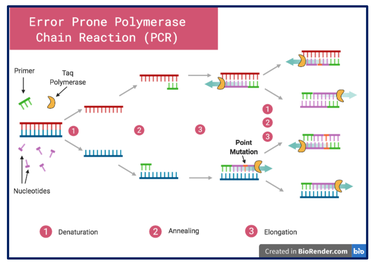
Protein scm-MHCII Inhibits Detrimental Immune Response



- 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 auto-immune diseases.

2. Creating Mutant Library

- Error-prone polymerase chain-reaction cycles produces scm-MHCII mutants



Yeast Shuttle Vector and scm-MHCII Construct

Boder, et al. Yeast surface display of a noncovalent MHC class II heterodimer complexed with antigenic peptide. Biotechnology and Bioengineering. (2005)

- Homologous recombination reintroduces scm-MHCII mutants into engineered yeast shuttle vector in EBY100 for microbial expression

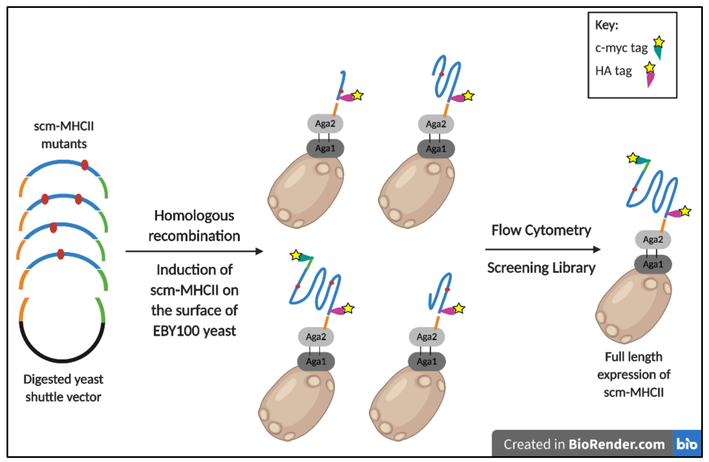
3. Characterizing Library

- Mutant library contains 2-5x10^5 scm-MHCII mutants
  - Significance: library includes all possible single amino acid change 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

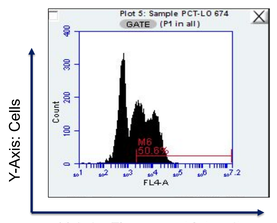
- During yeast surface display, a flow cytometer quantifies the intensity of fluorescent antibodies bound to scm-MHCII displayed by induced EBY100.
- Fluorescence indicates the partial or complete display of simplified HLA-DR4 depending on the specific fluorophore detected.

Homologous Recombination and Yeast Surface Display

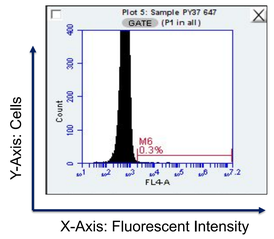


- Establishing positive and initial scm-MHCII display with histograms

Positive display with PCT302

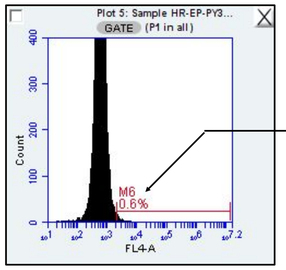


Initial scm-MHCII Display



5. Improving scm-MHCII Stability

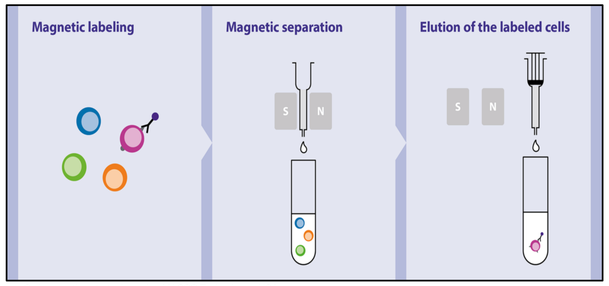
- Yeast surface display quantifies improvement of scm-MHCII mutants



There is a possible increase in stability indicated by the slightly more substantial "shoulder" of cells.

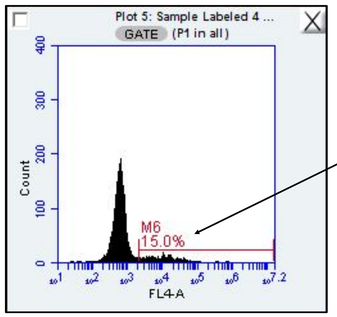
6. Screening Library for Full Length Expression

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



Cell separation strategies using MACS® Technology - USA.

After 4 Rounds of MACS



There is a significant increase in stability in the cell population from the screened library indicated by the tail of cells with a substantial increase in fluorescent intensity.

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
- Plate mutants and sequence monoclonal colonies
- Identify desirable mutations
- Investigate combinations of desirable, characterized mutations
- Engineer stabilized scm-MHCII fto possess a high affinity for CD4⁺ T cells

Acknowledgements

- Genomic Core, University of Tennessee
- National Institute of General Medical Sciences 1R15GM122326

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