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Modification and Stabilization of MHC Class I Molecules for Use in DNA Vaccine

Gregory Cresswell
Southern Illinois University Carbondale

John Martinko
Southern Illinois University Carbondale

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Gregory Cresswell
Department of Microbiology
Dr. John Martinko

Southern Illinois University Carbondale

Abstract

Major Histocompatibility Complex class I molecules present antigens to the immune system. A mouse MHC class I gene, H-2Kb, was modified by linking individual subunits together with a peptide antigen to form a single chain trimer (SCT). A disulfide bond, termed the disulfide trap, was added to stabilize the preloaded antigen (Figure 1 and Figure 2). The original antigen peptide, SIINFEKL, was modified to test the disulfide trap for stabilization of MHC class I SCT with a modified antigen. The SIINFEKL antigen was modified to SIINHEKL and SIINYEKL. DNA primers were designed to mutate the phenylalanine (F) amino acid to histidine (H) or tyrosine (Y). This was done through site directed mutagenesis, transformation, and transfection into tissue culture cells. Modifications of the peptide were tested using B3Z T cells that specifically recognize the SIINFEKL-Kb epitope. Cells expressing the disulfide trap proved to be very stable and presented a recognizable antigen to the T cells even when the original peptide had been modified. SCTs without the disulfide trap were weakly presented to the T cells. SCTs containing the mutant SIINYEKL or SIINHEKL peptides lacking the disulfide trap were not as stable and did not fold or present peptides well. In particular, the SIINHEKL modification showed that the disulfide trapped SCT was recognized by the T cells. However, the non-trapped SIINHEKL showed a significant reduction in epitope expression. The SIINYEKL mutants showed similar results. This indicates that peptides of our design can be presented efficiently to the immune system with disulfide trap stabilization. Further application of this research may allow construction of DNA vaccines expressing custom antigens.

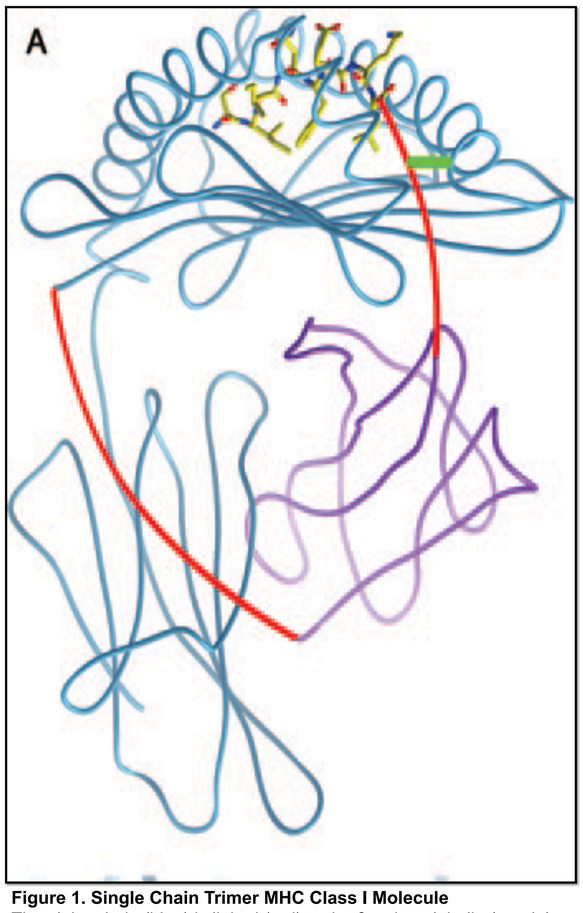

Figure 1. Single Chain Trimer MHC Class I Molecule
The alpha chain (blue) is linked (red) to the β_2-microglobulin (purple) and the peptide is connected to the linker (red) on the β_2-microglobulin. The disulfide trap is in green.
Image taken from Journal of Immunology 2007.

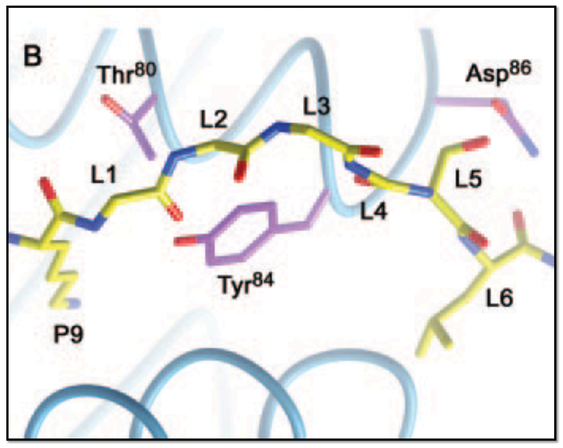

Figure 2. The disulfide trap site. Tyr^{84} is replaced with cysteine in the disulfide trap.
Image taken from Journal of Immunology 2007.

Methods

Site-Directed Mutagenesis
DNA primers were designed to mutate the original SIINFEKL peptide to the new peptides SIINHEKL and SIINYEKL. Both disulfide trap and non-disulfide trap models were created.

Transformation
The mutated strands were then transformed into *E. coli* for amplification of the DNA. The plasmid carrying the DNA carries genes for ampicillin resistance. The *E. coli* were grown on LB agar with 50 µg/mL of ampicillin. Once grown on plates, resistant colonies from the plates were transferred to LB broth with 50 µg/mL of ampicillin. Ten liquid cultures were made and then used for DNA extraction.

Plasmid Preparation
A plasmid preparation was used extract the DNA from the *E. coli* liquid cultures.

DNA Gel Electrophoresis
The purified DNA from the plasmid preparation was then cut with the BamH1 restriction enzyme. This cuts the DNA into fragments for visualization on the gel.

Transfection
293T cells were used to generate viral particles carrying the mutated DNA strands. Those viral particles were then used on LM18 mice cells to create a new cell line expressing SIINHEKL or SIINYEKL. The plasmid tranfsected into the cell lines carried genes for hgyromycin resistance. This was used as the selective agent to eliminate non-transfected cells.

The cell lines were then analyzed for expression of the SCT MHC Class I through a B3Z Assay.

B3Z Assay
B3Z T cells carry the *lac* Z operon and have T cell receptors specific for the SIINFEKL-Kb epitope. These cells were used to measure expression of the SCT in transfected cell lines. B3Z cells that bound the SCTs were activated and cleaved MUG (a lac Z substrate) to generate a color change. This was quantitatively measured to determine expression.

Results and Discussion

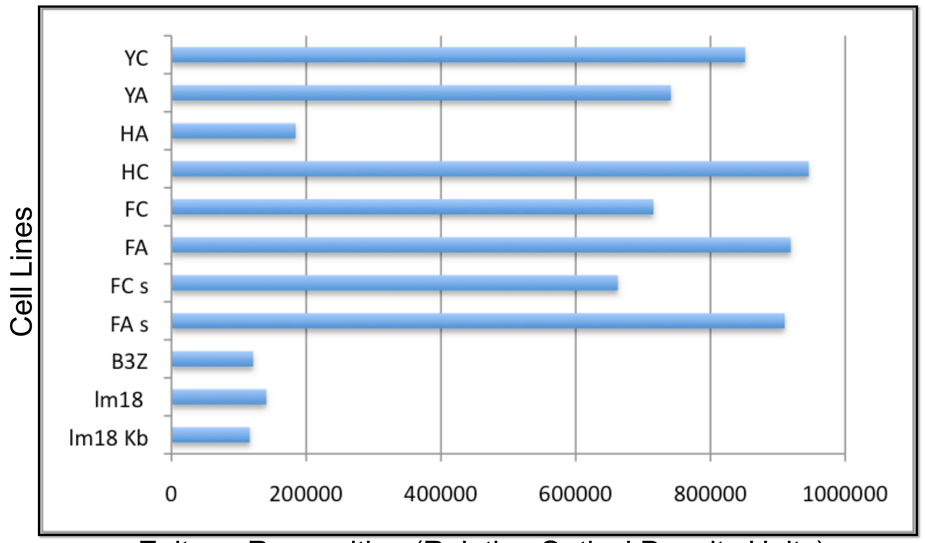

Figure 3. T cell epitope assay of SCT MHC proteins. B3Z T cells, specific for the SIINFEKL–Kb MHC I epitope were used to assay the expression levels of both natural and mutated versions of the epitope in transfected cell lines. YC: SIINYEKL with the disulfide trap. YA: SIINYEKL without the disulfide trap. HC: SIINHEKL with disulfide trap. HA: SIINHEKL without disulfide trap. FC: SIINFEKL with disulfide trap. FA: SIINFEKL without disulfide trap. S: Cell line that has been kept under selective pressure. B3Z are the B3Z cells alone. Lm18 is a cell line that is not expressing the Kb epitope. Lm18 Kb is a cell line expressing only the Kb epitope and not the SCT.

We showed that an SCT model of the MHC class I molecule can be generated and expressed in cell lines. All class I MHC SCT molecules generated in this study are recognized by the B3Z T cell, indicating that the mutations introduced to generate the disulfide trap to alter the major anchor residues in SIINFEKL did not destroy the T cell epitope: with or without the disulfide trap, the native SIINFEKL SCT and the SCTs containing the SIINHEKL and SIINYEKL peptides maintained T cell epitope expression. The B3Z T cells specific for the SIINFEKL peptides recognized the altered peptides and disulfide trapped constructs in every case.

With the native SIINFEKL peptide, the disulfide trap partially reduced expression of the T cell epitope (Figure 3). In the both cases where the native peptide was altered, however, the disulfide trap provided increased T cell epitope stability; both SIINYEKL and SIINHEKL disulfide trapped molecules were recognized better than the non-trapped molecules (Figure 3). This was especially evident for SIINHEKL where the non-disulfide trapped molecule showed significantly reduced expression of the T cell epitope, relative to all other SCT constructs. The disulfide-trapped SIINHEKL molecule, on the other hand, showed enhanced expression of the T cell epitope, indicating significant stabilization of the epitope due to the disulfide trap.

The native SIINFEKL-Kb SCT is shown with and without disulfide trap along with cell lines that were kept under antibiotic selective pressure to enhance expression of the SCT DNA. There were no observable difference in SCT expression between cells kept under selection and those that were not kept under selection; the comparison between selected and unselected cell lines did not significantly alter surface recognition of the T cell epitope, indicating that selective pressure is not needed for the cells to maintain and express the transfected DNA. Thus, the SCT disulfide trapped MHC is very stable once it has been introduced into the cell. This stability is further proof of concept for application in DNA vaccines.