**Antigen Presentation of p15 Peptides**

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**Introduction**

When the body is infected by a pathogen or a virus, the innate immune response consisting of multiple physical barriers, phagocytic leukocytes, dendritic cells, natural killer cells, and proteins will activate to eliminate the foreign particles. However, if the pathogens are not able to be successfully destroyed by the innate immune response, the adaptive immune system, consisting of B-cells in humoral immunity and T-cells in cell-mediated immunity, is activated to ‘adapt’ to the presence of the pathogen and create potent antibodies to destroy them.3

Effective vaccination against many infectious diseases such as human immunodeficiency virus/acquired immunodeficiency disease syndrome (HIV/AIDS) and tuberculosis (TB) critically relies on the application of both the humoral and cellular responses of the adaptive immune system, more specifically antigen processing and presentation. One of the difficulties in developing a T cell-targeted vaccine would be locating specific epitopes that are presented by the MHC molecules during early infection and throughout the process of infection.2

Antigen processing and presentation are complex processes that occur during adaptive immune responses. In the beginning of the process, proteins are fragmented in the cytosol by proteosomes or other proteases. In the endogenous pathway, the proteins range from 8-11 amino acids long and is used to present cellular peptide fragments on the cell surface of MHC class-I molecules in all cells whereas in the exogenous pathway, the proteins range from 12-18 amino acids long and is used to present cellular peptide fragments on the cell surface of MHC class-II molecules in a limited group of cells, which includes antigen-presenting cells: macrophages, dendritic cells, and B cells. After the peptides are degraded, the fragments are transported differently through the two MHC class molecules in order to be presented at the surface of the infected cell. In the endogenous pathway, a protein called transporter associated with antigen processing (TAP) transports the peptides into the endoplasmic reticulum, where many chaperone proteins help synthesize the peptide to form an MHC complex that is then transported to the cell surface by the Golgi apparatus. In the exogenous pathway, the alpha, beta, and invariant chains of MHC class-II are assembled in the endoplasmic reticulum and transported through the Golgi apparatus to digest. The peptide fragments from the exogenous protein associate with the class-II MHC molecules and finally transported and presented to the cell surface.4

Dendritic cells and macrophages are two types of antigen presenting cells that express class-II MHC molecule. Dendritic cells transport antigens to the T cell areas of the lymph nodes and spleen and are the most effective antigen-presenting cells. Macrophages, on the other hand, perform phagocytosis and are not as effective in presenting antigens to naive T cells as activating memory T cells. After antigen processing and presentation, naive and/or memory T cells will recognize and respond to foreign protein antigens by self MHC restriction, where they recognize the MHC on the presenting cells as self MHC. Helper T cells recognize class II self MHC while cytolytic T cells recognize class I self MHC.4

**Purpose**

The original goal of this project was to compare antigen processing and presentation between macrophages and dendritic cells due to the many applications in tuberculosis research. However, due to the availability of p15 peptides that has not been studied thoroughly in the Los Alamos database, the new goal was to focus on antigen processing and presentation on these peptides.

**Materials and Methods**

***HIV Peptide Degradation***

Purified epitope-containing HIV peptides (1.5nmol) from Massachusetts General Hospital donors were digested with 15*μ*g CD4+ T cell extracts at 37°C in degradation buffer (50 mM Tris-HCl, 137 mM potassium acetate, 1 mM MgCl2, and 1 mM ATP) at pH 4 and 7)). Aliquots were taken at a time point of 1 hour, and the reaction was stopped with 2*μ*L 100% formic acid. Peptide fragments in the mix were purified by 10% TCA precipitation followed by centrifugation at 14,000 rpm at 4°C for 30 min to isolate the digestion products in the supernatant.

***Mass Spectrometry Analysis***

The identity of the peptides in the digestion mix was determined by in-house mass spectrometry analyses. Equal amounts of peptide degradation samples at different time points were injected into a Nano-HPLC (Eksigent) in line with an Orbitrap massspectrometer (LTQ Orbitrap Discovery; Thermo) with a flow rate of 400 nL/min. A Nano cHiPLC trap column (200 *μ*m × 0.5 mm ChromXP c18-CL 5 μm 120Å; Eksigent) was used to remove salts from samples, and peptides were separated on a Nano cHiPLC column (75 *μ*m × 15 cm ChromXP c18-CL 5 μm 300Å; Eksigent) over a gradient of 2–40% buffer B (buffer A, 0.1% formic acid in water; buffer B, 0.1% formic acid in acetonitrile) and electrosprayed in the mass spectrometer. Mass spectra were recorded in the range of 370–2000 Da. In tandem mass spectrometry mode, the eight most intense peaks were selected with a window of 1 Da and fragmented. The collision gas was helium, and the collision voltage was 35 V. Tandem mass spectrometry spectra were searched against custom-made source peptide databases with Sequest and Proteome Discoverer (version 1.3; Thermo Scientific). The integrated area under a peak generated by a given peptide is proportional to the abundance of that peptide. Each degradation time point was run on the mass spectrometer at least twice.

**Statistical Analysis**

Using our mass spectrometry data, we analyzed protein degradation through size, size intensity, size % intensity, N- and C-terminal peptide cuts, and coverage. We also quantified intracellular peptide presentation and surface peptide presentation.1

**Results/Conclusion**

*Refer to PowerPoints.*

**Reflection**

Overall, the program has helped me have a much better idea of what the various paths are in academia, the industry, and even the business world. This exposure has allowed me to be assured that there is not only one straight path but many different roads that may lead to eventually a similar place. Before this program, I had my mind set on majoring on biomedical engineering and getting a Master’s degrees, potentially a Ph.D. However because of the many personal career stories that I’ve heard through the lectures and one-on-one conversations, I definitely will consider going into the industry. Along with thinking about future plans, I also spent much of my time learning and gaining wet lab experience. For my protein degradation project, I grew extremely appreciate of the data analysis process and realized that there is such a huge part that bioinformaticians play, especially those with computer science knowledge. Therefore, I might consider taking some classes in college to gain some computer science skills, which would be able to give me an opportunity to explore more avenues in research. In my past experience in wet lab work, I was not so heavily focused on analyzing experiments in various ways and rather analyzed previous experiments through simple proliferation or viability assays. However, I am extremely glad that I had the chance to learn how to use macros in order to create graphs. It allowed me to become more familiar with hands-on statistical work instead of only graphing out points in a graph that have come from, for example, a spectrophotometer. It has opened my eyes to not only the wet lab experience, but also the computerized version of research, which has caused me to consider bioinformatics as a potential major or minor in college. I am extremely grateful for this wonderful opportunity to be a part of this program at the Ragon Institute. It allowed me to be exposed to something new albeit my previous experience in wet lab work. Although I had initially believed that I would be doing cell culture work, which was what I was more familiar with, I am glad that I was introduced to something different because it was an opportunity for me to be able to explore different pathways that I was previously not familiar with; it was essentially a gateway towards my new passion in statistical analysis; even though it was a lot more tedious than the wet lab work itself, I was able to understand and appreciate the beauty of computational programs that could be used to study patterns in databases. Overall, my experience with the Ragon Institute was a highly positive influence in my future decisions for majoring in computational biology and possibly going into the industry.

**References**

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