**Using the IEDB to Predict Proteasomal Cleavage Events and T-cell Epitopes**

**Pre Lab**

Define the two types of immune responses against pathogens

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Distinguish between the functions of helper T-cells and cytotoxic T-cells?

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**Introduction:**

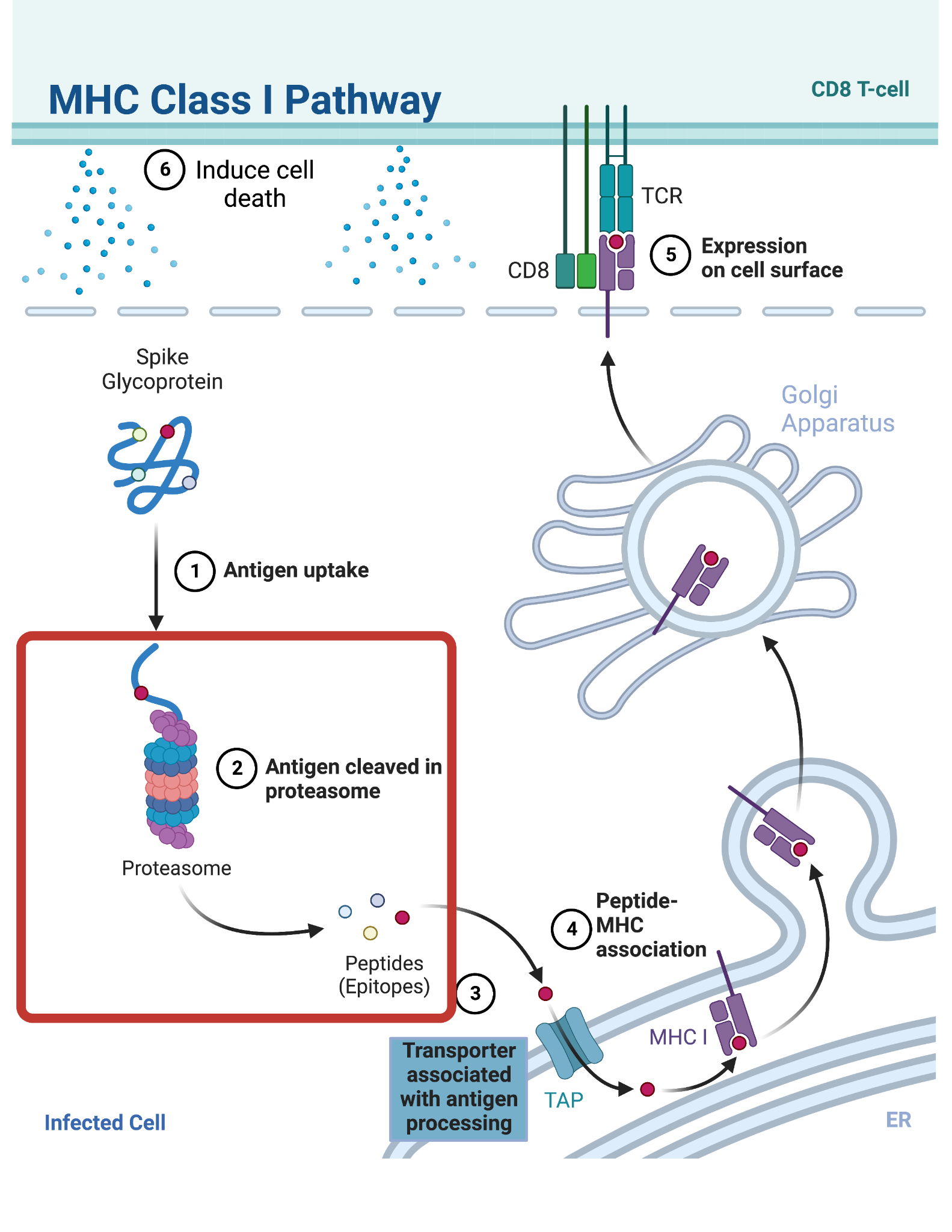
Two major immune responses defend our body against pathogens. First is the **Innate immune response** that is quick and non-specific. Microbes that enter our body are engulfed and destroyed by non-specific phagocytic cells. Microbes that escape the innate defense system face the second immune response mechanism named **adaptive immune response.**The adaptive response is a specific response against the invading microbe and involves B-cells and T-cells. With the help of a group of T-cells (**T-helper cells**), B-cells create antibody molecules that bind to specific antigens and mark them for destruction.

Microbes that escape the B-cell mediated humoral immunity by entering the cells trigger a cellular response involving another T-cell group known as **cytotoxic T-cells** (or CD8+ T cells).

Cytotoxic (CD8+) T-cells recognize and bind to the major histocompatibility complex class I (**MHC I) associated peptides** on the surface of the infected cells. This interaction induces the release of proteins from T-cells that destroy the target cell.

**MHC class I molecules**are present on the cell surface of all nucleated cells. The MHC is a structure found on antigen-presenting cells that allow T-cells to recognize if the cell is infected with a pathogen. They can recognize infection if the MHC presents a piece of a foreign **peptide,**as seen in Figure 1 part 5. These peptides are generated from the fragmentation of the ingested microbe/ viral proteins (or diseased cellular proteins) in the infected cells.

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| **Figure 1**- Antigen processing and display in infected Antigen Processing Cell (APC). **1)**Microbial/ viral proteins in the cytosol of the infected cell are first marked by ubiquitination which serves as a signal for destruction by the proteasome. **2)** **Proteasome**is a complex with proteolytic activity that breaks down the marked proteins into small peptides. The cleaving is not random, and it follows a pattern. When a cell is under an infection, the proteasome can be changed to a form called immunoproteasome, changing the cleavage design  to make the presentation of that protein easier to the T cells. **3)** The peptides are then selectively translocated into the endoplasmic reticulum (ER)by the **transporter associated with antigen processing (TAP)**. TAP is a complex protein belonging to the endoplasmic reticulum,  and its primary function is selecting cytosolic peptides to be transported into the ER. TAP also selects peptides with a better binding affinity to the MHC and facilitates MHC I folding and peptide binding. **4)** MHC I peptide complexes are transported via transport vesicles to the Golgi apparatus and the plasma membrane. T-cells bind the MHC-associated peptide on the cell's surface by their **TCR**(T Cell Receptor) and CD8 coreceptor. **5)**The peptide associated with the MHC I molecule and recognized by T-cells is known as a **T-cell epitope**[[1]](#_msocom_1) [[2]](#_msocom_2) . 5) CD8 T-cell releases cytotoxic protein to induce apoptosis. Made in Biorender. |



The recent increase in new and emerging infectious diseases underscores the need for rapid methods to develop effective **vaccines**. Vaccines are non-pathogenic forms of the microbe/ virus that induce an adaptive immune response and protect individuals from future infections. Traditional vaccines use inactivated whole organisms or large surface proteins from the organism to stimulate an immune response. **Peptide vaccines**, on the other hand, contain short amino acid sequences that constitute epitopes from the larger antigen that can induce both B-cell and T-cell responses. The advantage of peptide vaccines over whole organisms or proteins is that they eliminate extra allergic reactions to the large antigens. Other vaccines developed in recent years are made of DNA and mRNA molecules carrying the genetic coding for the immunogenic epitope. Identification of immunogenic B and T cell epitopes on the pathogen’s proteins help in the design and development of effective vaccines.

The **IEDB**(Immune Epitope Database and Analysis Resource[[1]](#_msocom_1) ) is an example of a bioinformatic tool that can be used to predict the sequence and location of both linear (T-cells) and discontinuous (B Cell) epitopes in a protein structure. In this activity, we will learn how to predict CD8+ T-cell epitopes using the various tools available on the IEDB, using the SARS-CoV-2 Spike glycoprotein as an example.

In this activity, we will predict and evaluate the initial cell organelle interaction with a foreign virus protein. This organelle is the proteasome; its function is to break down proteins into peptides of 8-11 amino acids.

**Test your knowledge:**

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| Q1 What is the purpose of antigen presentation?   |  | | --- | |  |   Q2. Describe the proteasome and their function.   |  | | --- | |  |   Q3. What is an epitope? What does it interact with?   |  | | --- | |  |   Q4. What is the general length of a peptide after being processed in the proteasome?   |  | | --- | |  | |
| Q5. Compare and contrast the structural differences between linear and discontinuous epitopes?   |  |  |  | | --- | --- | --- | | **Type of Epitope** | **Linear Epitope** | **Discontinuous Epitope** | | Similarities |  | | | Differences |  |  | |
| Q6. Why are T-cell epitopes linear and B-cell epitopes more likely to be conformational (discontinuous)? |

**Activity: Using the Immune Epitope Database**

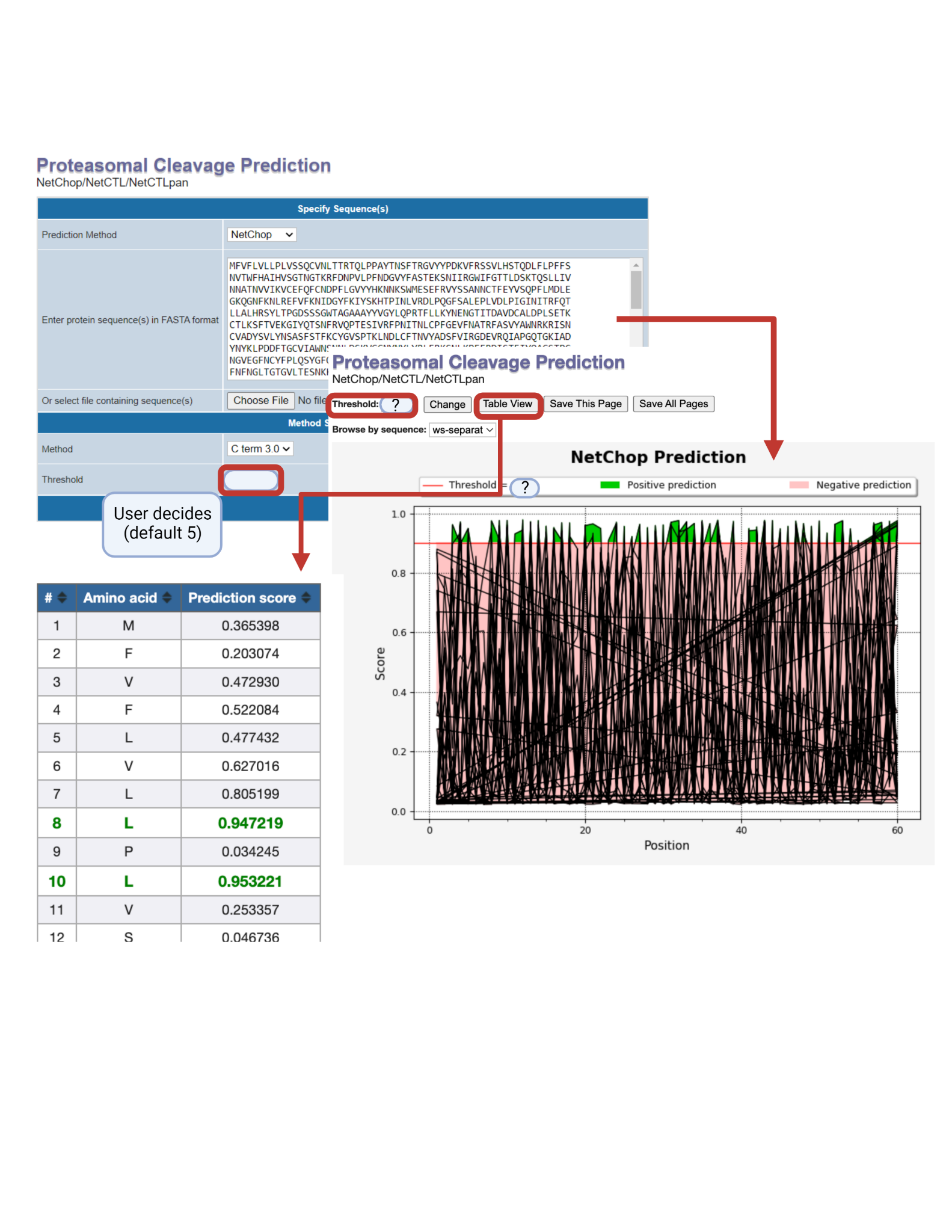
Let’s simulate how cells process foreign antigens using a tool from the [IEDB](https://www.youtube.com/watch?v=JRoWhD3I8ko)

1. Go to the [Uniprot website](https://www.uniprot.org/). Search for “SARS Cov2 spike glycoprotein”
2. Click on entry Spike\_SARS2 (Entry: P0DTC2)
3. Find the download prompt and download the “FASTA canonical” file.
4. Go to the [IEDB home page](https://www.iedb.org/).
5. From the “Analysis Resources” drop-down menu on the top of the page choose “T-cell epitope prediction tools”.
6. here are several prediction tools available on IEDB. We will use a combined tool to predict the entire antigen pathway and identify **linear epitopes** on the antigen that may be candidates for vaccine development.

Choose the “Neural network based prediction of proteasomal cleavage sites”.

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| **Figure 2.** The IEDB Epitope analysis resource has utilized real data gathered in the database to create prediction tools for researchers. Within the T Cell epitope prediction tools we can find tools to analyze amino acid sequences to anticipate the probability these events will occur. |

1. First choose the Prediction method: “NetChop”
2. Insert the protein (FASTA) sequence. Leave the parameters as the default selections.



**Figure 3.** Predicting proteasomal cleavage prediction of SARS-CoV2 Spike Glycoprotein (P0DTC2). The amino acids shown in green are events that the user indicates passes the criteria for the percent likelihood a successful cleavage will occur.

1. Take a quick look at the chart. The X-axis represents the positions of the protein’s amino acid residues. The peaks represent the likelihood that the protein is cleaved at that position by the proteolytic activity of the proteasome. The green represents sites that are more likely to be cleaved whereas pink means the positions that are less likely to be cleaved. You can play with the threshold number and see how the chart changes.

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**Apply Your Knowledge**

Input spike glycoprotein FASTA sequence P0DTC2:

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| **Threshold value** | **How many positive events are there?** |
| 0.5 (default) |  |
| 0.9 |  |
| 0.2 |  |

Q7. Assess how the changes in threshold can influence protein cleavage predictions?

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Q8. Select a threshold value that will yield only high cleave prediction values (>95%). Find two cleavage events with an appropriate amount of amino acids to create a peptide and report your findings in the table below and take a screen capture of the excerpt of the table view sequence.

Do you think this peptide is a good candidate for designing a vaccine for the virus? Explain your answer.

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| **Threshold Value** | **Peptide Sequence** | **Residue Position numbers** | **Length (number of amino acids)** | **Prediction score** |
| --- | --- | --- | --- | --- |
|  |  | Cleavage 1: |  | Cleavage 1: |
| Cleavage 2: | Cleavage 2: |
| Paste screen capture of sequence here | | | | |