

Session 6: T Cells: Development and Differentiation

Overview:

Like B cells, T cells develop in a manner that enhances the diversity of T cell receptors while preventing self-recognition. This session provides an overview of T cell development and differentiation. It covers two important mechanisms that prevent autoimmunity - central and peripheral tolerance - and characterizes the biogenesis and function of two specialized T cells, Tregs and Th17 cells.

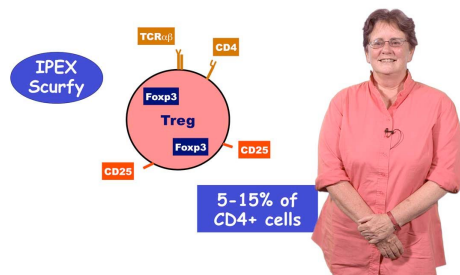
First video:

Title: An Introduction to T Cell Tolerance

Speaker: Diane Mathis

Time: 34:33

Concepts: Central and peripheral tolerance, type-1 diabetes (example of autoimmune disease), and Treg biogenesis and function

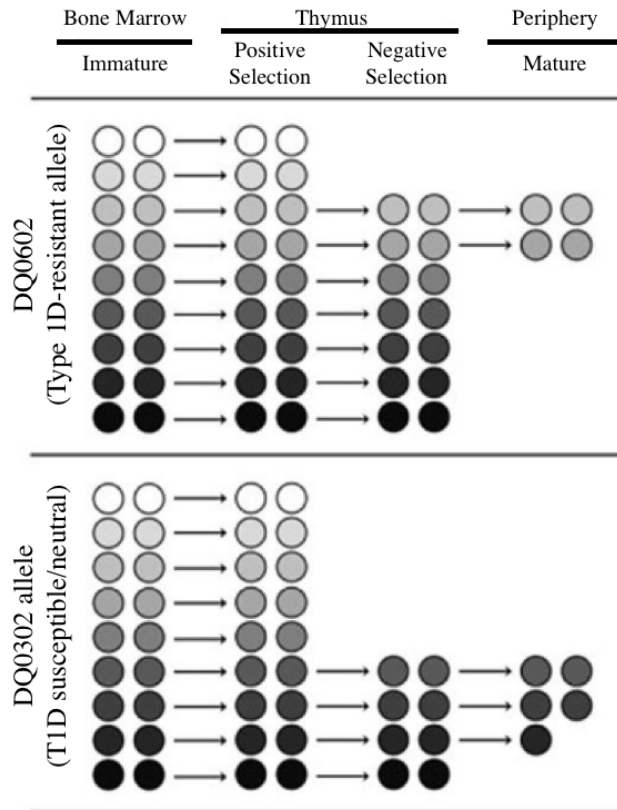


Questions for First Video:

1. Which of the following statements is true regarding T cell receptors? (Select all that apply.)
 - a. They recognize antigen-MHC complexes.
 - b. They contain variable and constant regions.
 - c. They are composed of two heavy chains and two light chains.
 - d. They are formed by the rearrangement of V, J, C, and D gene segments.
 - e. They can be found in the bloodstream independent of T cells.
2. T cell receptors
 - a. are the primary receptors for the humoral immune system.
 - b. are carbohydrates.
 - c. cannot function unless the animal has previously encountered the antigen.
 - d. are produced by plasma cells.
 - e. Are important in combating viral infections.
3. A woman goes to the doctor and is diagnosed as having rheumatoid arthritis, an autoimmune disorder. What factors likely led to her development of this disease? (Select all that apply.)
 - a. Some of her T cells were not positively selected.
 - b. Some of her T cells were not negatively selected.
 - c. Some of her T cells are recognizing and responding to her normal self-antigens.
 - d. Some of her T cells are not recognizing and associating with her MHC proteins.

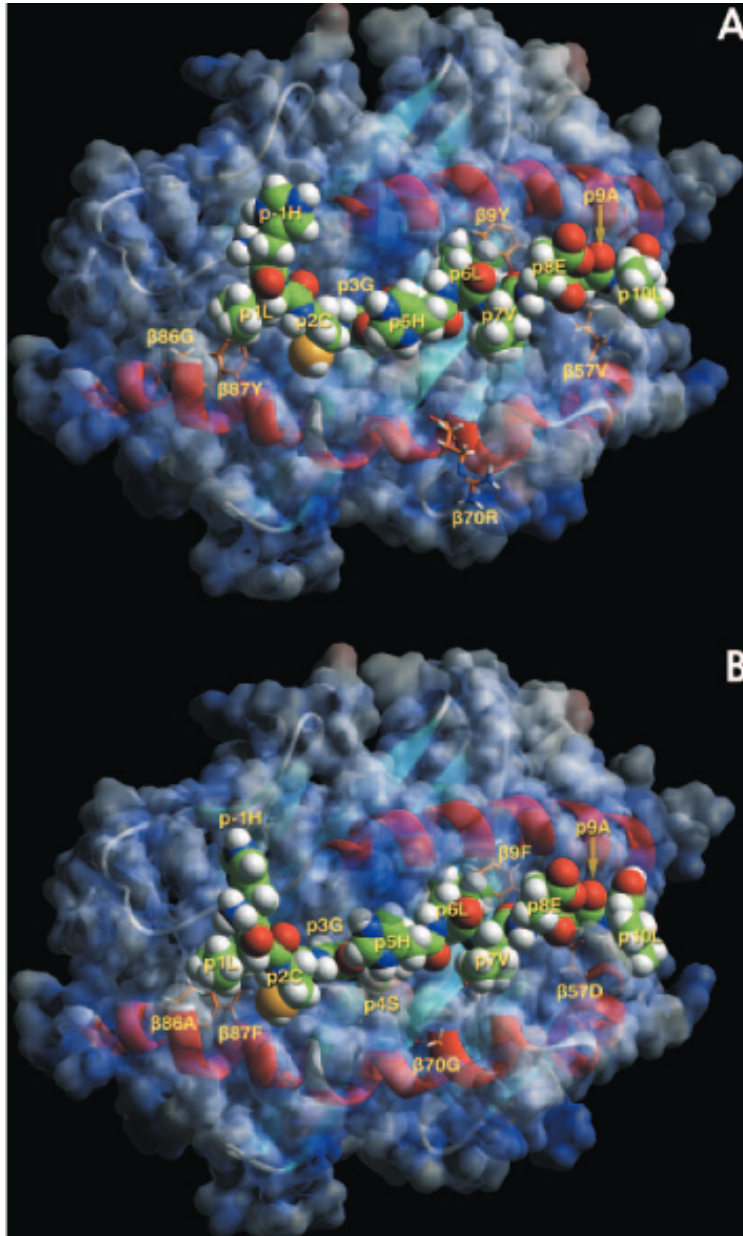
10. Polymorphisms in HLA-DQ, a component of the MHC II complex, have been linked to different autoimmune diseases, including rheumatoid arthritis and diabetes.

- a. Both positive and negative selection in the thymus contribute to form the repertoire of mature T cells in the periphery. Shown below is a representation of the thymic selection that occurs in individuals with HLA-DQ alleles resistant to disease (DQ0602) versus Type-1 diabetes susceptibility alleles (DQ0302). Briefly explain how the selection process differs in these individuals and how this could contribute to disease susceptibility.



Black circles (●) represent T cell with high affinity to self-peptides, white circles (○) represent T cells with low affinity for self-peptides, and grey circles represent T cells with intermediate affinity to self-peptides. (Morran, MP, et al. (2015) Mol Aspects Med)

- b. Shown below is a molecular simulation of an insulin-derived peptide (green) bound to two genetic variants of HLA-DQ. Shown in yellow are the amino acids that are different between the Type 1 diabetes susceptible allele versus the Type-1 resistant allele. Given that there are no major conformational differences between these two variants, briefly explain what could be a contributor to Type-1 diabetes.

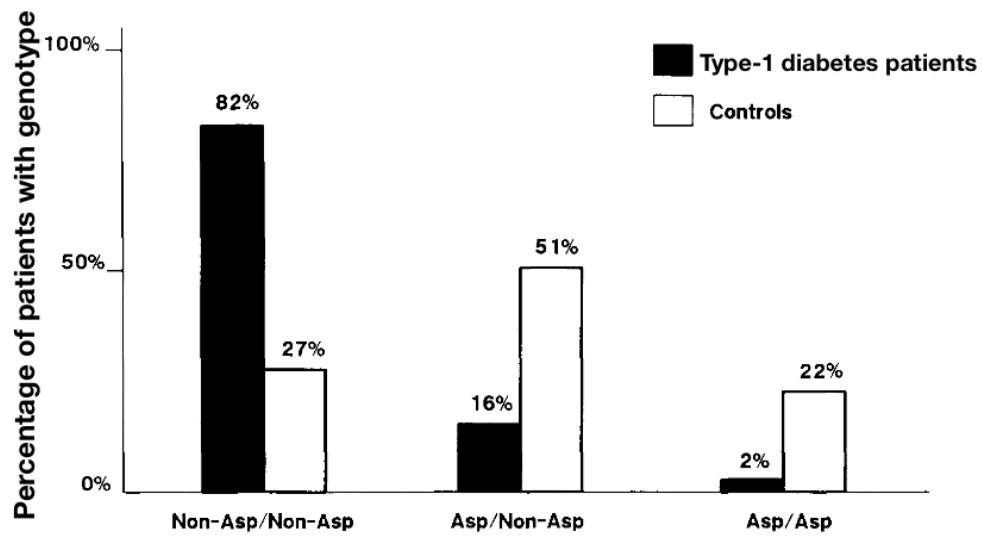


DQ0604 allele
(T1D susceptible/neutral)

DQ0602
(Type 1D-resistant allele)

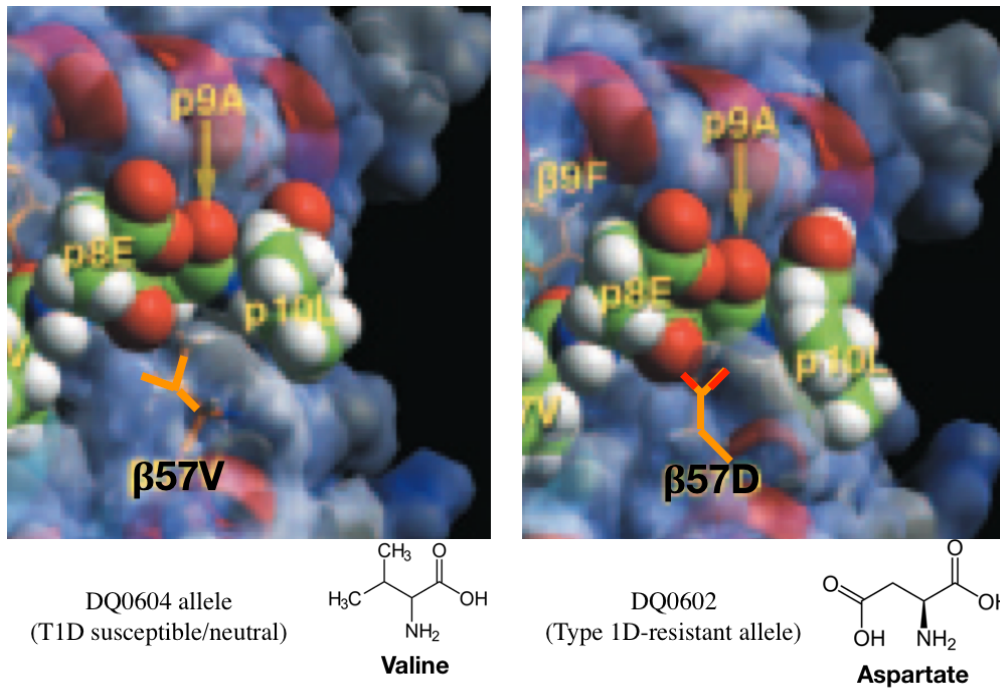
Legend: α -Helix in red, random coil in cyan, and β -sheet in blue
Ettinger, RA. et al. (2006) J Immunol

- c. A study investigated the frequency of aspartate at position β -57 in HLA-DQ (shown below). What would you conclude from these results? Briefly explain.



(Rønningen, KS. et al. (1989) Hum Immunol)

- d. A closer look at position $\beta 57$ (shown below; V= Valine; D = Aspartate) may explain disease susceptibility. Does this result help to explain your conclusions in question (c)? Briefly explain.

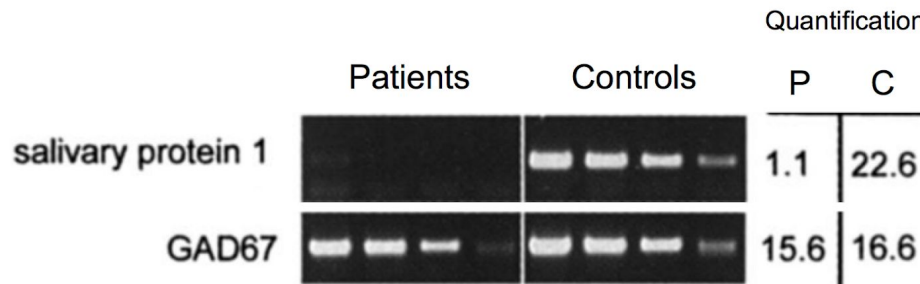


ABOVE: The atoms in insulin-derived peptides are shown as spheres (oxygen atoms are in red). The side chains of the amino acids at position $\beta 57$ within the HLA-DQ variants are shown in stick configuration.

11. Why, evolutionarily, might it make sense that women prefer the smell of men with different MHC alleles? Briefly explain.

12. Compare and contrast T cell central and peripheral tolerance.

17. Four patients with a T-cell mediated autoimmune disorder of the upper digestive tract were evaluated and compared to four individuals without the disorder. The patients' immune cells attack cells that express a protein known as salivary protein 1 (SP1). It was determined that the patients had altered expression of SP1 in a certain tissue, as indicated below. GAD67 is a tissue-specific housekeeping protein and is shown as an endogenous control.



- What is the difference in expression of SP1 between patients and controls?
 - Which tissue - bone marrow, thymus, lymph nodes, esophageal epithelium - was evaluated in the figure above? Choose only one.
 - Explain the reasoning for your answer.
18. Not all self-antigens are presented in the thymus during negative selection (for example, antigens from the eye). Therefore, some mature T cells are potentially self-reactive. How does the body prevent autoimmunity in these instances?

Second video:

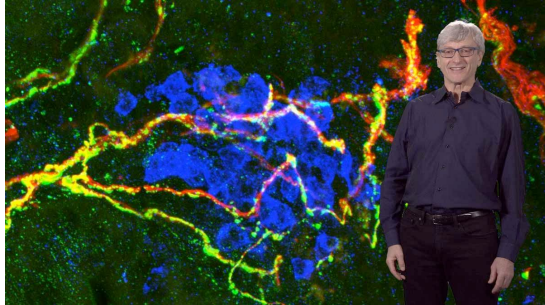
Title: Th17 Cells and Innate Lymphoid Cells in Barrier Defense and Inflammatory Diseases

Speaker: Dan Littman

Time: 29:28 min

Concepts: T cell differentiation, characterization of CD4+ cells, and review of Th17 role in autoimmune diseases

Link to Original video: ([Link](#)) *Please watch this video from time 1:55 to 31:25.*



Questions for Second Video:

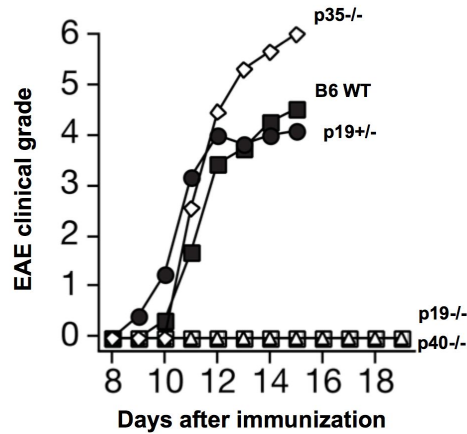
1. For the following T cells, provide two characteristics that defines the lineage.
 - a. Treg:

 - b. T_H17:

 - c. Cytotoxic T cells:

2. State whether the following statements are true or false. If false, explain why.
 - a. Once a T cell is differentiated into a T helper cell, it can no longer become a Treg cell.
 - b. T helper cells become activated when their TCR binds peptide:MHC II complexes.
 - c. ROR γ t is exclusively expressed in T_H17 cells.
 - d. Depletion of ROR γ t protects against experimental autoimmune encephalomyelitis (EAE) due to the decrease in production of T_H17 cells.
 - e. T cells in the periphery must be replenished by cells from secondary lymphoid organs.
 - f. Interleukins are comprised of more than one subunit.
 - g. All interleukin receptors signal through STAT3.
 - h. Naive T cells encounter antigen in the periphery.

8. Researchers sought to identify which cytokines are necessary for mediating a type of experimental autoimmunity (abbreviated as EAE) in mice. To address their question, they attempted to induce the autoimmune phenotype in mice strains lacking a range of cytokine subunits, as shown below. They measured the progression of the autoimmune phenotype over several days after induction.



- Which cytokine subunit(s) are necessary for mediating the EAE phenotype?
 - Which cytokine subunit(s) are not necessary for mediating the EAE phenotype?
 - How would you explain the phenotypic difference between p19^{+/-} and p19^{-/-} mice?
 - Based on the data, what do you think is the role of p35 in this phenotype?
 - How would you test your prediction in d)?
9. Th1 and Th2 cell differentiation each rely on a single cytokine, while differentiation into Th17 and Tregs relies on signals from several cytokines. Predict why this is the case.

10. Summarize the role of each of the following sites in terms of T cell development, activation, or function. Use one sentence for each site.

a. Thymus.

b. Secondary lymphoid organs (i.e., lymph nodes).

c. Periphery (i.e., skin).

Answers for Session 6:

Questions for First Video:

1. Which of the following statements is true regarding T cell receptors? (Select all that apply.)
 - a. **They recognize antigen-MHC complexes.**
 - b. **They contain variable and constant regions.**
 - c. They are composed of two heavy chains and two light chains.
 - d. **They are formed by the rearrangement of V, J, C, and D gene segments.**
 - e. They can be found in the bloodstream independent of T cells.
2. T cell receptors
 - a. are the primary receptors for the humoral immune system.
 - b. are carbohydrates.
 - c. cannot function unless the animal has previously encountered the antigen.
 - d. are produced by plasma cells.
 - e. **Are important in combating viral infections.**
3. A woman goes to the doctor and is diagnosed as having rheumatoid arthritis, an autoimmune disorder. What factors likely led to her development of this disease? (Select all that apply.)
 - a. Some of her T cells were not positively selected.
 - b. **Some of her T cells were not negatively selected.**
 - c. **Some of her T cells are recognizing and responding to her normal self-antigens.**
 - d. Some of her T cells are not recognizing and associating with her MHC proteins.
4. You are a doctor examining a patient's blood test results. The patient's T cell count is low, and T cells that are present are not working properly. You examine scans of the patient's thymus. It is underdeveloped, which means the T cells in the blood _____.
 - a. will be present in lower numbers because the thymus is where T cells are formed.
 - b. **will be present in lower numbers because the thymus is where T cells mature.**
 - c. will have not undergone selection against self-recognition, and the patient will be more susceptible to autoimmune diseases.
 - d. None of the above.
5. For the following statements, briefly describe if it is likely that the outcomes are a product of problems with central tolerance, peripheral tolerance, or neither.
 - a. An individual has CD4+ T cells that recognize a self-antigen.
Both. If individuals have T cells that recognize self-antigens it's likely that both mechanisms (central and peripheral tolerance) are compromised.
 - b. An individual doesn't have either CD4+ or CD8+ cells.
Central tolerance. T cells are double positive during development in the thymus (before they go to the periphery). It's possible that central tolerance is killing all T cells.

6. What would be the consequence for central and/or peripheral tolerance if an individual has a mutation that increases expression of peripheral protein A only in the thymus? Briefly explain.
Aside from being able to eliminate more efficiently T cells that recognize peripheral protein A, this individual should be completely healthy with no problem with either type of tolerance.
7. Provide a hypothesis that explains why T cells that contain TCRs with very strong affinity to the MHC get targeted for clonal deletion.
Too strong affinity to MHC may allow the TCR to bind the complex even when the peptide presented by the complex isn't a complete match. T cells could also get activated by binding to the MHC without the presence of an antigen. This will result in a production of T cells that could randomly get activated and cause autoimmune disease.
8. State whether the following statements are true or false. If false, explain why.
- T cell central tolerance is established in the thymus. **(True)**
 - Deletion, inactivation and diversion are unique processes for establishing tolerance in the periphery. **(False - deletion, inactivation and diversion are common to both central and peripheral tolerance)**
 - All suppressor cells are T cells. **(False - there are thought to be suppressor B cells and other types of leukocytes)**
 - Double-negative thymocytes have completed T-cell receptor gene rearrangement. **(False - gene rearrangement is initiated but not completed at the double negative stage)**
 - At the time of positive selection, T cell precursors express both CD4 and CD8. **(True)**
9. Compare and contrast two ways an individual could develop Type-1 diabetes. Briefly mention the cells involved and explain how they could lead to the development of Type-1 diabetes.
Defect in Clonal deletion in T cells – Individuals could have decreased expression of insulin in the thymus. This reduces negative selection of T cells that are capable of binding to insulin-derived peptides. Having these self-reactive cells in the body can induce targeting of insulin-producing cells for degradation.
- Treg deficiency – Decreased expression of Foxp3 could lead to a decrease in Treg numbers. Tregs normally inhibit self-reactive T cells in the periphery.**
- (Acceptable answer: Defect in B cell negative selection. A B cell with antibodies that recognize insulin-derived peptides could escape tolerance, the process by which B cells with self-reactive antibodies go through receptor editing).**
10. Polymorphisms in HLA-DQ, a component of the MHC II complex, have been linked to different autoimmune diseases, including rheumatoid arthritis and diabetes.
- Both positive and negative selection in the thymus contribute to form the repertoire of mature T cells in the periphery. Shown below is a representation of the thymic selection that occurs in individuals with HLA-DQ alleles resistant to disease (DQ0602) versus Type-1 diabetes susceptibility alleles (DQ0302). Briefly explain how the selection process differs in these individuals and how this could contribute to disease susceptibility.
Individuals with DQ0602 will be able to negatively select in the thymus T cells with high affinity to self-peptides, so that no autoreactive T cells would be present in peripheral blood and the likelihood of developing diabetes would be reduced. In

contrast, subjects who carry susceptibility alleles, such as HLA-DQ*0302, will negatively select autoreactive T cells less efficiently. These autoreactive cells will egress from the thymus and be present (even in small numbers) among peripheral T cells.

- b. Shown below is a molecular simulation of an insulin-derived peptide (green) bound to two genetic variants of HLA-DQ. Shown in yellow are the amino acids that are different between the Type 1 diabetes susceptible allele versus the Type-1 resistant allele. Given that there are no major conformational differences between these two variants, briefly explain what could be a contributor to Type-1 diabetes.
Most polymorphisms are found within the peptide-binding groove, suggesting that differences in peptide binding contribute to the mechanism of their association with T1D.
- c. A study investigated the frequency of aspartate at position β -57 in HLA-DQ (shown below). What would you conclude from these results? Briefly explain.
These results show the beneficial implication of having aspartate at position β 57 to prevent Type-1 diabetes. Not having aspartate correlates with disease.
- d. A closer look at position β 57 (shown below; V= Valine; D = Aspartate) may explain disease susceptibility. Does this result help to explain your conclusions in question (c)? Briefly explain.
Yes. Asp is a negatively charged amino acid. This diagram shows that β 57D (Asp) is close to an oxygen (red) in the insulin peptide. Having Asp at this position decreases binding of the insulin peptide by repulsion of two negative forces in close proximity.
11. Why, evolutionarily, might it make sense that women prefer the smell of men with different MHC alleles? Briefly explain.
Progeny will have higher variability, which will allow them to combat infection (pathogens). This is due to the greater number of different alleles coding for MHC proteins and therefore better chances of recognizing different types of pathogens (antigens) compared to offspring from parents with similar MHC complexes. The evolutionary advantage of offspring with a higher variability in MHC complexes means that they would be most likely to survive sudden outbreaks of diseases or other exposure to pathogens.
12. Compare and contrast T cell central and peripheral tolerance.
Central tolerance occurs in the thymus with developing T cells, while peripheral tolerance occurs in peripheral tissues with mature T cells. Central tolerance is obtained primarily three ways: deletion, inactivation and diversion, while peripheral tolerance is attained through more diverse mechanisms. Both ensure that T cells will not attack self tissues.

13. In the immune system, getting conditions “just right” is important for fighting infection while avoiding allergy or autoimmunity.
- Both too little and too much binding (affinity) can be deadly for a T cell. Explain the developmental stages that lead to these two types of death.
If a double positive T cell does not have its TCR activated, it will not be positively selected and will be cleared. Later in development, if a single-positive T cell binds pMHC too strongly, it will be negatively selected and will be cleared.
 - Depending on context, both too many and too few Tregs can be a bad thing. Give an example of each and explain how the wrong number of Tregs can contribute to disease.
Too few Tregs in the context of autoimmunity can help to promote immune attack of self antigen. Too many Tregs in the context of cancer or infection can inhibit immune attack of tumor cells or pathogens.
14. Describe each of the following T cell developmental stages in terms of their TCR gene rearrangement and checkpoint status (i.e., positive or negative selection).
- CD4-/CD8- T cells.
TCR gene rearrangement begins at the double negative stage. Have not completed any checkpoints.
 - CD4+/CD8+ T cells.
TCR gene rearrangement is completed at the double positive stage. Double positive cells undergo positive selection.
 - CD4+ or CD8+ T cells.
These cells express a rearranged TCR and either CD4 or CD8. Undergo negative selection.
15. Some T cells escape clonal deletion in the thymus.
- What is one mechanistic reason that enables a T cell to avoid clonal deletion? Briefly explain.
Antigen concentration too low in the thymus during negative selection; antigen absent from the thymus during negative selection; antigen seen with inadequate affinity during negative selection.
 - Why would it be beneficial for some T cells to escape clonal deletion? Briefly explain.
This allows the T cell repertoire to be sufficiently broad enough to recognize a range of pathogens. If all T cells that interacted with self antigen on any level were deleted, then there likely wouldn't be enough of a repertoire left to confer immunity.
16. Tregs express high levels of the receptor for IL-2, a pro-inflammatory cytokine. How does this expression relate to their effector function?
The high expression level of the IL-2 receptor on Tregs serves as a “sponge” for IL-2, so that it won't bind to IL-2 receptors on other types of effector cells and activate inflammation.

17. Four patients with a T-cell mediated autoimmune disorder of the upper digestive tract were evaluated and compared to four individuals without the disorder. The patients' immune cells attack cells that express a protein known as salivary protein 1 (SP1). It was determined that the patients had altered expression of SP1 in a certain tissue, as indicated below. GAD67 is a tissue-specific housekeeping protein and is shown as an endogenous control.
- What is the difference in expression of SP1 between patients and controls?
There is no expression of salivary protein 1 in this tissue in patients.
 - Which tissue - bone marrow, thymus, lymph nodes, esophageal epithelium - was evaluated in the figure above? Choose only one.
Thymus.
 - Explain the reasoning for your answer.
The tissue evaluated in this figure must be thymus, because that is where negative selection of T cells occurs. If SP1 is not expressed in the thymus during negative selection, then T cells that recognize SP1 will not be deleted and can lead to autoimmunity in the periphery.
18. Not all self-antigens are presented in the thymus during negative selection (for example, antigens from the eye). Therefore, some mature T cells are potentially self-reactive. How does the body prevent autoimmunity in these instances?
Some sites in the body, such as the eye, are known as "MHC II negative" or "immunologically privileged". Typically, this is accomplished by reducing leukocyte infiltration and/or MHC II expression by antigen presenting cells in those tissues.

Questions for Second Video:

- For the following T cells, provide two characteristics that defines the lineage.
 - Treg:
During differentiation, these T cells have a more reactive TCR compared to T helper cells. These are CD4+ cells that express Foxp3.
 - T_H17:
These are CD4+ cells express ROR γ t. Involved in autoimmune inflammation.
 - Cytotoxic T cells:
These are CD8 positive cells, and they are activated by MHC I presenting cells. They're effector cells that will release cytotoxic cytokines.
- State whether the following statements are true or false. If false, explain why.
 - Once a T cell is differentiated into a T helper cell, it can no longer become a Treg cell.
(False – T helper cells can be induced to become iTreg in IL-2, RA, and TGF β - dependent pathways)
 - T helper cells become activated when their TCR binds peptide:MHC II complexes.
(False. It also requires signaling through CD80/86 and cytokine stimulation)
 - ROR γ t is exclusively expressed in T_H17 cells. **(False. It's also express in other T lymphocyte lineages, e.g. iTreg).**
 - Depletion of ROR γ t protects against experimental autoimmune encephalomyelitis (EAE) due to the decrease in production of T_H17 cells. **(True.)**

- e. T cells in the periphery must be replenished by cells from secondary lymphoid organs. **(False - Peripheral T cells are self-replenishing)**
 - f. Interleukins are comprised of more than one subunit. **(True)**
 - g. All interleukin receptors signal through STAT3. **(False - they typically signal through STAT proteins, but not always STAT3)**
 - h. Naive T cells encounter antigen in the periphery. **(False - naive T cells encounter antigen in secondary lymphoid tissues)**
3. What would be the consequence of a mutation that inhibits the degradation of Ski, a protein that recruits HDAC to the ROR γ t locus? Briefly explain.
Increased recruitment of HDAC to the ROR γ t locus will turn off ROR γ t expression via deacetylation of histones. Because ROR γ t expression is off, T helper cells will not be able to differentiate into Th17 cells. This mutation will decrease the likelihood of autoimmunity produced by T_H17 cells.
 4. Compare and contrast the two types of T_H17 cells (homeostatic and inflammatory Th17 cells).
Both cells express ROR γ t and produce IL-17 and IL-22. The homeostatic/non-pathogenic T_H17 cells are differentiated in the absence of IL-23, induced by microbiota, and function to protect barrier surfaces. Unlike pathogenic Th17 cells, they secrete IL-10, an anti-inflammatory cytokine. Pathogenic Th17 cells are induced under inflammatory conditions via IL-23 signaling. Unlike homeostatic Th17 cells, pathogenic Th17 cells produce inflammatory cytokines such as IFN- γ and are involved in autoimmunity.
 5. IL-23 is important for Th17-induced autoimmune disease.
 - a. Explain why an antibody that binds to IL-23 would be a good therapeutic approach.
IL-23 induces pathogenic Th17 cells. Inhibition of IL-23 with an antibody would decrease pathogenic Th17, and therefore decrease autoimmune diseases.
 - b. What would be a limitation of using IL-23 as a drug target? Briefly explain.
IL-23 could have other targets, aside from activating the differentiation of Th into Th17. These other targets could cause secondary effects upon treatment with an IL-23 antibody.
 6. Why is it advantageous for the immune system to have T cell activation and clonal expansion take place in the secondary lymphoid tissues versus in the periphery?
This helps the immune system compartmentalize immune activation and keep it under stronger control. It also helps to concentrate activators and effectors in one specific area.
 7. ROR γ t is a transcription factor that is important in thymocyte survival, T cell differentiation, and lymphoid organ development. Suggest two ways that a precursor cell “knows” which specific cell type to become when it expresses ROR γ t.
A cell could integrate external signals from cytokines, or it could simultaneously express additional transcription factors that help to direct its differentiation.

8. Researchers sought to identify which cytokines are necessary for mediating a type of experimental autoimmunity (abbreviated as EAE) in mice. To address their question, they attempted to induce the autoimmune phenotype in mice strains lacking a range of cytokine subunits, as shown below. They measured the progression of the autoimmune phenotype over several days after induction.
- Which cytokine subunit(s) are necessary for mediating the EAE phenotype?
p19 and p40.
 - Which cytokine subunit(s) are not necessary for mediating the EAE phenotype?
p35.
 - How would you explain the phenotypic difference between p19^{+/-} and p19^{-/-} mice?
There appears to be a dosage effect, where one allele of p19 is sufficient to mediate the EAE phenotype.
 - Based on the data, what do you think is the role of p35 in this phenotype?
It appears that p35 is part of a cytokine that inhibits EAE. When p35 is deleted in mice, the EAE phenotype is more pronounced.
 - How would you test your prediction in d)?
You could overexpress p35 or the antiinflammatory cytokine that p35 is a part of after EAE is initiated. If the prediction is correct, then the EAE phenotype should be inhibited.
9. Th1 and Th2 cell differentiation each rely on a single cytokine, while differentiation into Th17 and Tregs relies on signals from several cytokines. Predict why this is the case.
The activity of Th17 and Treg cells must be tightly regulated, so a naive T cell must integrate multiple signals before it commits to become a Th17 or Treg. In contrast, Th1 and Th2 cells are less regulated, perhaps because they play lesser roles in the development of autoimmunity.
10. Summarize the role of each of the following sites in terms of T cell development, activation, or function. Use one sentence for each site.
- Thymus.
Site of T cell development, including positive and negative selection.
 - Secondary lymphoid organs (i.e., lymph nodes).
Site of mature T cell activation by dendritic cells.
 - Periphery (i.e., skin).
Site of activated T cell function as well as T cell replenishment.