Session 7: Autoimmunity and Allergy

Overview:
Allergy and autoimmunity occur when immune cells are activated by non-pathogenic antigens. In the case of allergies, the immune system recognizes innocuous non-self-antigens (e.g. proteins in peanuts), while in autoimmunity the immune system attacks cells expressing self-antigens. This session explores the molecular underpinnings of allergy and autoimmunity. Mast cells and IgE antibodies take center stage in the allergic response, while rogue T or B cells are the primary actors in autoimmunity.

First video:
Title: Allergies and the Immune System
Speaker: Avery August
Time: 15:55

Concepts: IgE, mast cell and basophil response to IgE, and allergic response

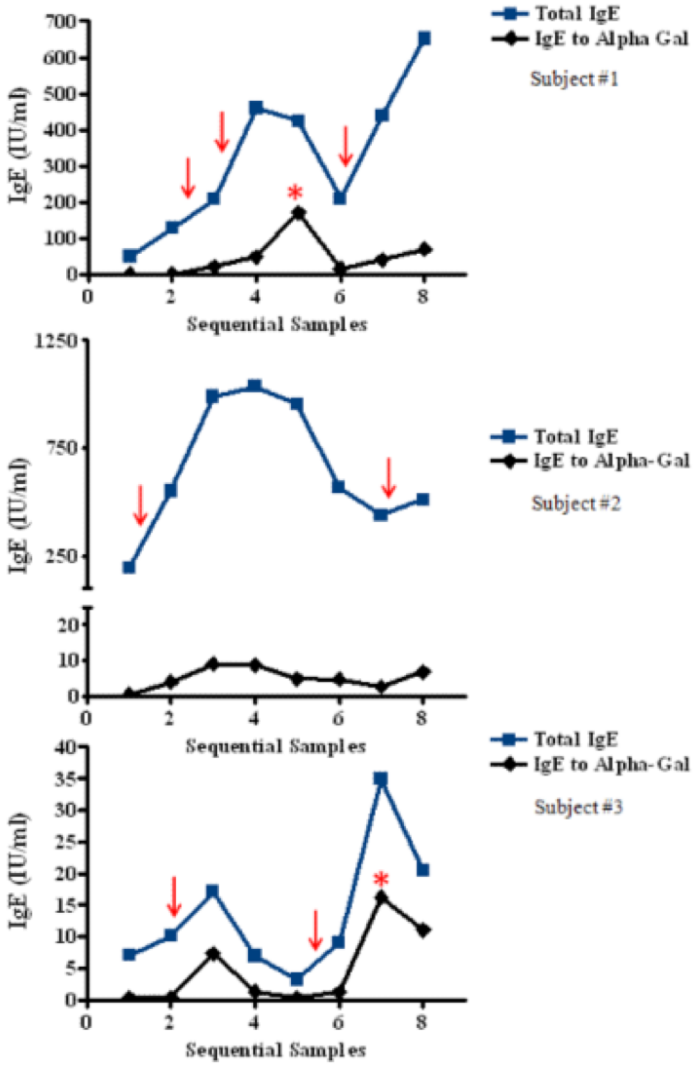
Questions for First Video:

1. Which of the following components is involved in an allergic response? (Select all that apply)
    a. IgK antibodies
    b. IgE antibodies
    c. Release of histamine by mast cells
    d. Increase in intracellular calcium in dendritic cells
    e. B cells

2. State whether the following statements are true or false. If false, explain why.
    a. For the body to mount an allergic reaction against antigen A, both T and B cells must have failed negative selection.
    b. IL-4 is crucial for the production of IgE.
    c. Both innate and adaptive immune components are responsible for allergy.
    d. It is typical to observe an allergic response on a first exposure, even if the exposure is small.

3. A tick bite can cause all sorts of diseases. It was recently observed that a number of patients developed allergy to red meat weeks after a bite by the lone star tick.
    a. Briefly explain the role of IgE in allergic reactions.

    b. Scientists followed the IgE response (total (blue) & alpha-gal-specific IgE (black)) of three individuals that were bitten multiple times by the lone star tick (red arrow). All subjects didn't experience allergies to red meat prior to the bites, but after the bites, subjects 1 and 3 experienced an allergic reaction to beef. What would you conclude from these results? Briefly explain.

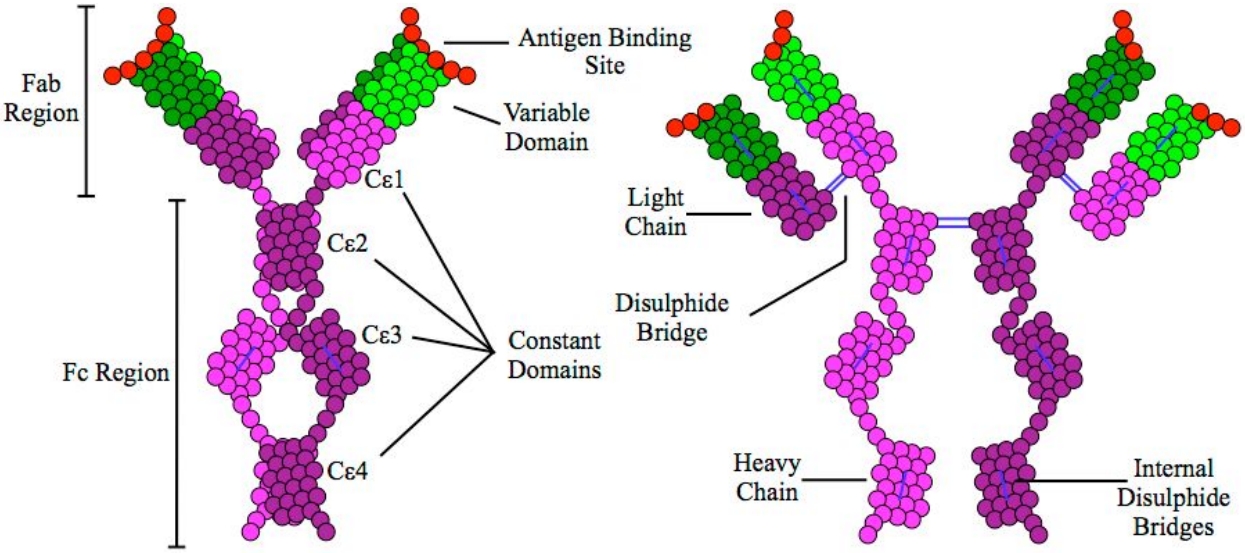


Commins. SP, et al. (2011) J Allergy Clin Immunol

c. Alpha-gal is a carbohydrate that is present in most mammals with the exception of some apes including humans. Would you expect a bear that was bitten by a lone star tick to experience similar meat allergies? Briefly explain.

d. You want to further study the tick-induced meat allergy in an animal model, like mice. Briefly explain what would be the first step to start this project.

4. Xolair is an antibody-based drug that is used to treat asthma. Xolair binds to the Fc portion of IgE (see structure below).



a. What could be a disadvantage of Xolair binding the constant (Fc) region of IgE? Briefly explain.

b. Why didn't scientists design Xolair to bind the antigen binding site? What is the advantage of binding the constant (Fc) region? Briefly explain.

c. Prolonged use of Xolair can cause cancer. Briefly explain why.

5. Researchers have studied the use of small but incremental daily ingestion of peanuts, a treatment known as oral immunotherapy, in the treatment of peanut allergy.
   a. How could oral immunotherapy help with the allergic reaction? Briefly explain.

   b. Shown below is a summary of the results of various clinical trials that studied the use of oral immunotherapy to treat peanut allergy. Briefly summarize what happens to peanut-specific IgE. Can this result explain the success of the trial?

Table 1. Clinical studies using different dosing protocols for oral immunotherapy (OIT) of peanut allergy and the observed immunological changes.

OIT agent	Success subjects	Dosing protocol	Immunological change	References
Peanut flour	16 of 19	*Escalation:* 0.1–6mg, doubles every 30 min *Build-up:* 75 mg (25–33%) *Maintenance*: 4g, every 2 weeks, 44 weeks *Challenge:* 5g	↓SPT ↑ IL-5 and IL-13, peanut-specific IgG_4. ↑ then ↓ peanut-specific IgE ↑$FoxP3^{hi}$: $FoxP3^{intermediate}$ CD4 + CD25 + T cells	[44]
Peanut flour	27 of 29	*Escalation:* 0.1–50mg, doubles every 30min *Build-up:* increased 25mg every 2 weeks until 300 mg *Maintenance:* 1800 mg daily *Challenge:* 3.9 g	↑ IL-10, IL-5, IFN-γ and TNF-α over 6–12 months ↓ Peanut-specific IgE by 12–18 months ↑ IgG4 Peanut-specific FoxP3 T cells ↑ 12 months then ↓	[42]
Whole crushed roasted peanut	14 of 23	*Escalation:* 0.0075–0.5 g doubles every 30 min[a] *Build-up:* 1 g on third day *Challenge:* 4g	↑ Peanut-specific IgG4, ↓ IL-5, IL-4 and IL-2	[43]
Peanut flour	39 of 49	*Escalation:* 2, 5, 12.5, 25, 50, 100, 200, 400 and 800 mg *Maintenance:* 800 mg daily, 26 weeks Challenge: 1400mg	Small ↓ in median SPT weal diameter ↑ Peanut-specific IgE increase after 24 weeks OIT in the active group	[46]
Peanut flour	19 of 22 (6 weeks) 18 of 22 (30 weeks)	*Escalation:* 1, 5, 25, 50, 75 and 100 mg[a] *Build-up*: 0.5, 1, 2, 5, 12, 25, 50, 100, 200, 400 and 800 mg for 2 weeks *Maintenance:* 800 mg daily, 30 weeks *Challenge:* 2.6 or 6.6g (6 or 30 weeks)	↓ SPT at 6 ug/mL after 30 weeks ↑ Peanut-specific IgE then ↓at 30 weeks ↓ Serum Ara h 2-IgE when challenged after 30 weeks	[45]
Peanut powder	15 of 22	*Escalation:* 33, 66, 133, 199 and 364 mg, every 30 min[a] *Up-dosing:* 16–795mg (9–55%) *Maintenance:* 795 mg daily, 3 months *Challenge:* 795 mg	↑ Median Ara h2-specific IgE at first month but ↓significantly at >6 months ↑ Peanut-specific IgG4 levels and Ara h 2-specific IgG4 after 1 month	[77]
Lactobacillus rhamnosus CGMCC1.3724	23 of 31	*Escalating:* 2×10^{10} CFU (freeze-dried powder) daily together with peanut OIT daily for 18 months *Build-up:* 2g every 2 weeks, 8 months *Maintenance:* 2g every 2 weeks, 10 months *Challenge:* 4g	↓ Peanut-specific IgE levels ↑ Peanut-specific IgG4 levels	[73]

[a]DBPCFC = Double-blind placebo-controlled food challenge.

Joo Chan C, et al. (2019) Int Rev Immunol

c. Researchers also studied how IgG4 changes with this treatment as it is thought that IgG4 competes with IgE. Are the IgG4 results supportive of this treatment? Briefly explain.

d. Studies also show that months of oral immunotherapy increases peanut-specific IgA in the gut. How could this prevent the body from mounting an allergic response against peanuts? Briefly explain.

6. Would you expect someone with hyperactive IL-4 production to have an increased or decreased risk of allergy? Explain your reasoning.

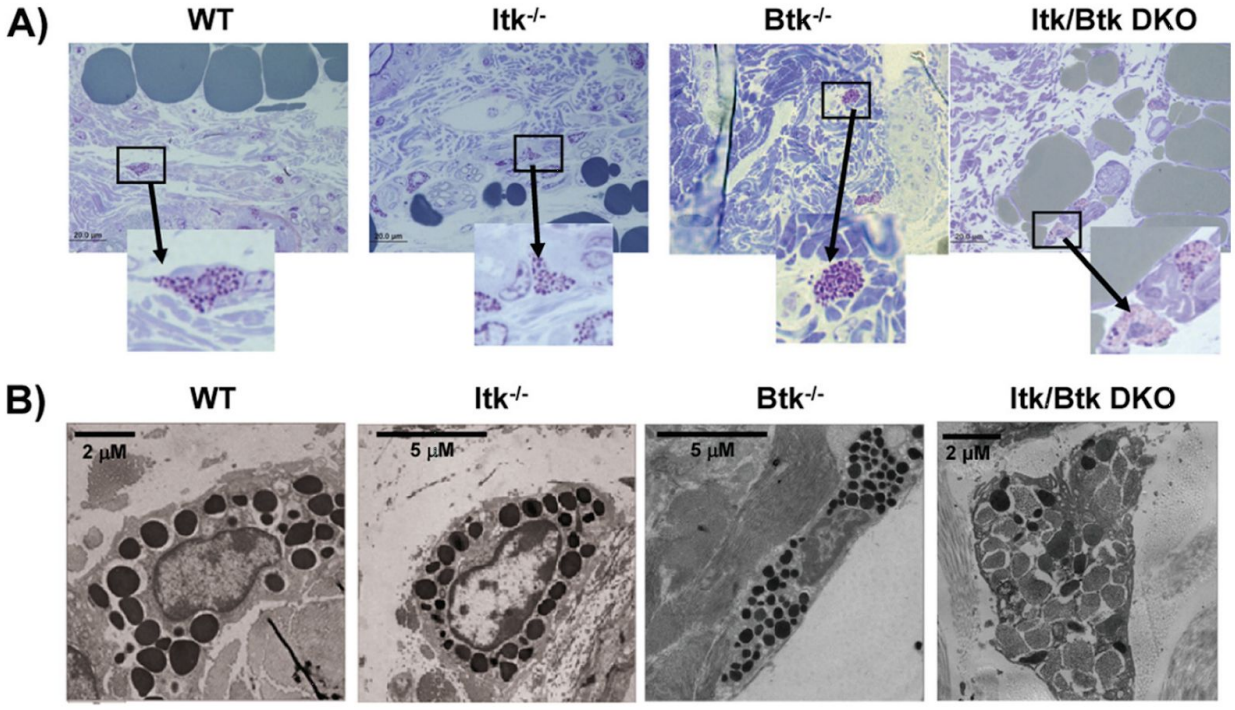
7. Histamine increases vascular permeability, while proteases restructure the extracellular matrix. Why have basophils and mast cells evolved to secrete these compounds?

8. The drug chromolyn prevents mast cell degranulation, although its exact mechanism is unclear. Predict one way that chromolyn might function. You do not need to state specific protein or molecule names in your response.

9. Barry has a mutation that renders the Fc portion of his IgE molecules unable to interact with the FcE receptors (FcεRI) on mast cells and basophils.
    a. What would be the effect of this mutation on Barry's normal immune responses?

    b. What would be the effect of this mutation on Barry's allergy responses?

10. The kinases Itk and Btk are expressed and activated in mast cells following FcεRI (IgE Fc receptor) stimulation. To determine the specific roles these kinases play in FcεRI signaling and mast cell function, researchers generated Itk and Btk double knock-out (DKO) mice and compared them to wild-type, Itk-/-, and Btk -/- mice. Below are histological analyses of granule density in mast cells. In (A), blue/purple identifies filled granules, while pink identifies empty granules. Individual cells are encircled by a black box and blown up for clarity. (B) represents transmission electron microscope images of skin mast cells from each strain. The dark circles represent filled granules. (From Iyer et al. JBC 2011)



a. Qualitatively, what differences do you detect between the mast cell granules of the different mouse strains?

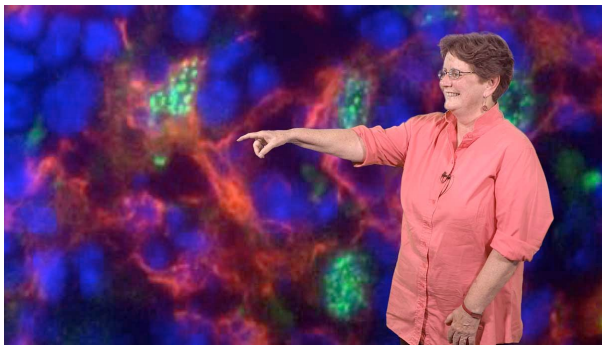
b. Based on these data, is Itk (alone) necessary for mast cell granule integrity? Is Btk (alone) necessary for mast cell granule integrity?

c. Do you predict that Itk and Btk signal in the same or different pathways in mast cells? Explain your reasoning.

d. Suggest an experiment to test your hypothesis.

Second video:
Title: Transcription Factor Aire Orchestrates T Cell Tolerance
Speaker: Diane Mathis
Time: 37:23

Concepts: APS-1 Syndrome, AIRE protein role in T cell tolerance, AIRE expression/localization, and importance of Tregs for APS-1



Questions for Second Video:

1. State whether the following statements are true or false. If false, explain why.
    a. AIRE is primarily in the bone marrow.
    b. Mutations in AIRE can reduce the capacity of an individual to fight pathogens. (
    c. Aire activity contributes to both central and peripheral tolerance.
    d. Aire functions as a classic transcription factor.
    e. Aire is expressed by pre-T cells in the thymus.

2. AIRE is localized in the medulla, particularly in medullary epithelial cells (MECs).
    a. What is unique about MEC gene expression?

    b. Briefly explain what would be the overall effect of deleting these cells during early development.

    c. Compare and contrast the two mechanisms by which AIRE aids immune development.

3. Predict why endocrine tissues, such as the pancreas and thyroid, are frequently targets of autoimmunity.

4. AIRE deficient mice have circulating autoantibodies. Describe how deletion of AIRE, which is a thymic gene, can lead to this phenotype.

5. Ovalbumin (OVA) is a protein that is unique to chicken eggs. Why was a non-murine protein chosen as the self-antigen to study in the AIRE mouse studies?

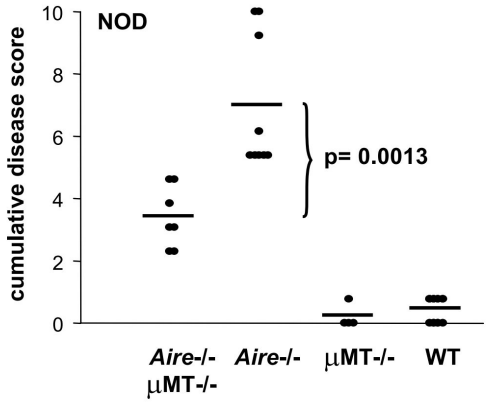
6. mRNA transcription is not binary (i.e., genes are not simply “on” or “off”).
    a. Describe what is meant by the term “stalled” or “paused” transcription.

    b. When is RNA pol II paused during transcription? You do not have to name specific molecules in your answer.

    c. Describe one mechanism by which pausing of RNA pol II is reversed. You do not have to name specific molecules in your answer.

    d. Where does AIRE fit into this picture?

7. Researchers sought to determine the contribution of B cells to the autoimmune phenotype of Aire-deficient mice. To address their question, they generated mice that were deficient in both *Aire* and *μMT*, a gene necessary for B cell development. They then tracked the development of nonobese diabetes (NOD), a hallmark of autoimmunity, in mice from four genotypes. Data are summarized below. (Reference: Gavanescu PNAS 2008)



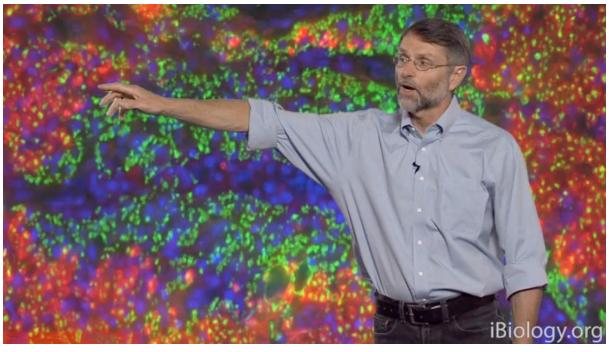
a. Based on the data above, are B cells responsible for the development of NOD in Aire-deficient mice?

b. Why or why not?

c. In the paper from which this figure is derived, the authors make the claim that “B cells are responsible for lethal immunopathology in NOD.” What evidence is needed to support this claim?

Optional video:
Title: Discovery and Development of Tysabri (Natalizumab) for the Treatment of Multiple Sclerosis: Immune Cell Migration to the Central Nervous System
Speaker: Ted Yednock
Time: 34:53

Concepts: Introduction to multiple sclerosis (MS), EAE mouse model to study MS, α4β1-Integrin, Anti-α4β1-Integrin experiments before & post MS manifestation, and development of Tysabri (Natalizumab)

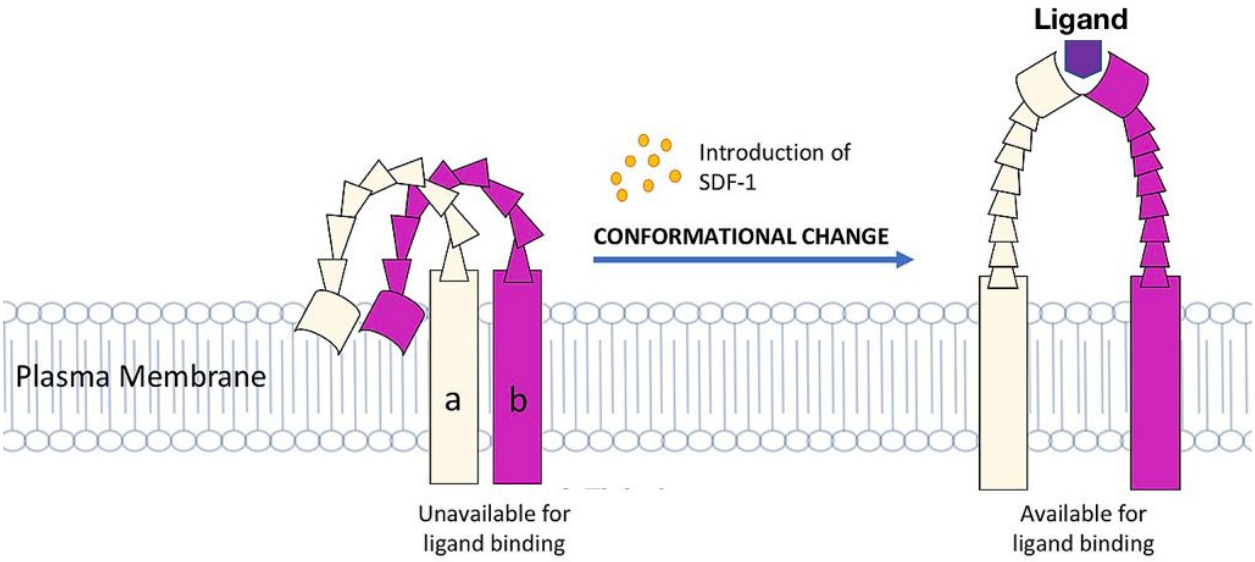


Questions for Optional Video:

1. Briefly describe the Experimental Autoimmune Encephalomyelitis (EAE) model to study multiple sclerosis (MS).

2. α4β1 integrin is the drug target of Natalizumab for the treatment of multiple sclerosis (MS).
    a. Briefly explain the involvement of α4β1 integrin in the development of MS.

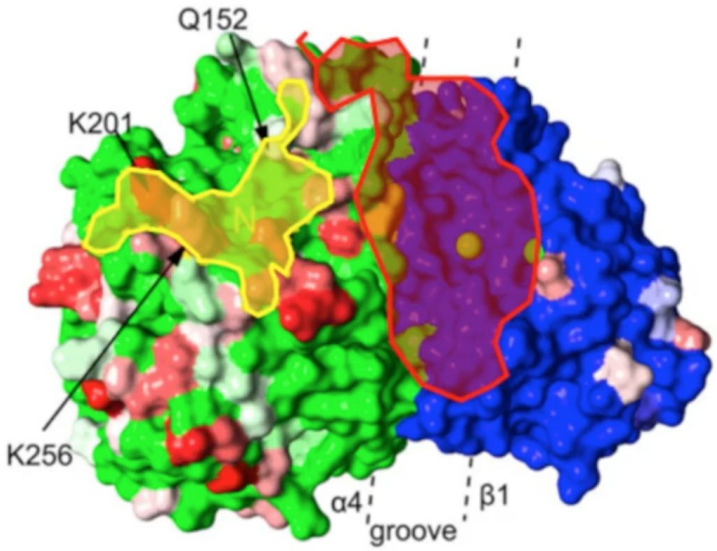
    b. α4β1 is activated by a conformational change facilitated by the chemokine SDF-1. Would you consider SDF-1 as a possible drug target for the treatment of MS (see image below)? Briefly explain.



    c. A secondary effect of using the antibody Natalizumab is flu-like symptoms. Why?

3. Why were the researchers in Yednock's group disappointed to learn that α4 integrin is expressed on a range of leukocytes?

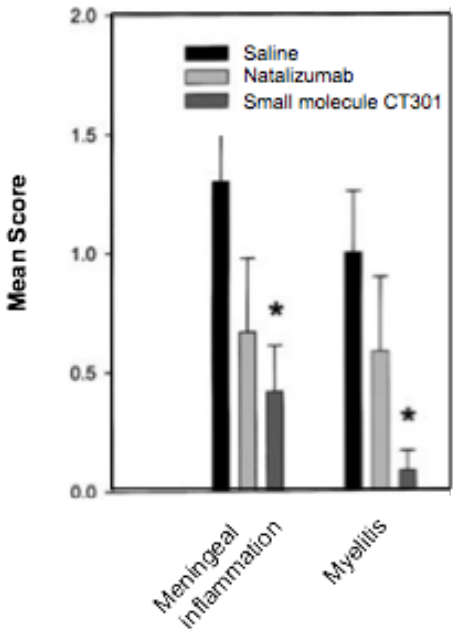
4. In the image below, the binding sites of Natalizumab (Tysabri, yellow outline) and VCAM (red outline) are shown on α4β1 integrin. α4 integrin is represented in green; β1 integrin is represented in blue. (Reference: Yu et al., JBC 2013)



   a. How can Natalizumab (Tysabri) inhibit α4β1 integrin binding to its ligand VCAM, if the antibody and VCAM bind different locations on the integrin?

   b. If you were to design a small molecule inhibitor of α4β1 integrin for MS therapy, would you design it to bind to the same or to a different location as Natalizumab? Explain your reasoning.

c. Researchers sought to compare the effects of Natalizumab (Tysabri) with a small molecule inhibitor of α4β1 integrin-VCAM binding in a mouse model of EAE. They quantified the pathological changes in the spinal cord for two markers of disease, meningeal inflammation and myelitis. Mice were treated with either saline, Natalizumab, or the small molecule CT301 following EAE induction.



i. How do the efficacies of Natalizumab and CT301 for treating EAE compare?

ii. Predict why the researchers likely observed this difference.

5. Explain why targeting α4β1 integrin has been shown to be effective for treating a number of disease models, including multiple sclerosis, viral inflammation, and CNS graft rejection.

Answers for Session 7:

Questions for First Video:

1. Which of the following components is involved in an allergic response? (Select all that apply)
    a. IgK antibodies
    b. IgE antibodies
    c. Release of histamine by mast cells
    d. Increase in intracellular calcium in dendritic cells
    e. B cells

2. State whether the following statements are true or false. If false, explain why.
    a. For the body to mount an allergic reaction against antigen A, both T and B cells must have failed negative selection. **(False. Negative selection deletes T and B cells that are reactive against self-antigens, not allergens)**
    b. IL-4 is crucial for the production of IgE. **(True)**
    c. Both innate and adaptive immune components are responsible for allergy. (**True**)
    d. It is typical to observe an allergic response on a first exposure, even if the exposure is small. (**False - an allergic response isn't observed until the second exposure**)

3. A tick bite can cause all sorts of diseases. It was recently observed that a number of patients developed allergy to red meat weeks after a bite by the lone star tick.
    a. Briefly explain the role of IgE in allergic reactions.
    Mast cells and basophils contain receptors that recognize IgE. When IgE binds to these cells and interacts with the allergen, it causes these cells to release granules that are responsible for the allergic response (e.g. inflammation).

    b. Scientists followed the IgE response (total (blue) & alpha-gal-specific IgE (black)) of three individuals that were bitten multiple times by the lone star tick (red arrow). All subjects didn't experience allergies to red meat prior to the bites, but after the bites, subjects 1 and 3 experienced an allergic reaction to beef. What would you conclude from these results? Briefly explain.
    IgE against alpha-gal may be associated with the induced allergic reaction against beef after a tick bite. IgE to alpha-gal peaked after multiple bites in Subjects 1 and 3, but not in Subject 2.

    c. Alpha-gal is a carbohydrate that is present in most mammals with the exception of some apes including humans. Would you expect a bear that was bitten by a lone star tick to experience similar meat allergies? Briefly explain.
    No. Given that alpha-gal exists in bears, their immune system (B and T cells) will be trained to not produce antibodies against this carbohydrate (negative selection).

d. You want to further study the tick-induced meat allergy in an animal model, like mice. Briefly explain what would be the first step to start this project.
Because mouse has alpha-gal, they will not react the same way to tick bites as humans do. You would need to develop a strain of mice that lack alpha-gal (i.e., gene knockout) before you can do further experiments.

4. Xolair is an antibody-based drug that is used to treat asthma. Xolair binds to the Fc portion of IgE (see structure below).
    a. What could be a disadvantage of Xolair binding the constant (Fc) region of IgE? Briefly explain.
    It's non-specific. It binds to all IgE which can reduces total count of IgE and increase secondary effects of this drug (e.g. flu-like symptoms).

    b. Why didn't scientists design Xolair to bind the antigen binding site? What is the advantage of binding the constant (Fc) region? Briefly explain.
    Targeting the antigen binding site would be too specific - it would only eliminate IgEs that target a specific allergen, and asthma could be caused by multiple allergens. Targeting the Fc region targets all IgE and allows Xolair to have a broader effect.

    c. Prolonged use of Xolair can cause cancer. Briefly explain why.
    IgE is important for proper immune response, including detection of cancer cells. Reducing the total number and/or function of IgE with Xolair can decrease immune surveillance against cancer cells.

5. Researchers have studied the use of small but incremental daily ingestion of peanuts, a treatment known as oral immunotherapy, in the treatment of peanut allergy.
    a. How could oral immunotherapy help with the allergic reaction? Briefly explain.
    Researchers are expecting a desensitization of the immune system. The immune system has mechanisms to maintain homeostasis. If the immune system is constantly exposed to an allergen, the body could activate a negative feedback loop that will decrease the immune response upon allergen interaction (desensitization).

    b. Shown below is a summary of the results of various clinical trials that studied the use of oral immunotherapy to treat peanut allergy. Briefly summarize what happens to peanut-specific IgE. Can this result explain the success of the trial?
    Yes. Most of these studies show an initial increase followed by a decrease in the peanut-specific IgE after a few months of treatment with oral immunotherapy.

    c. Researchers also studied how IgG4 changes with this treatment as it is thought that IgG4 competes with IgE. Are the IgG4 results supportive of this treatment? Briefly explain.
    Yes. These studies observed an increase in IgG4 upon treatment which should decrease the activity of IgE.

d. Studies also show that months of oral immunotherapy increases peanut-specific IgA in the gut. How could this prevent the body from mounting an allergic response against peanuts? Briefly explain.
Increasing IgA in the gut will capture peanut allergens in the gut and will prevent its release in the blood. This will decrease the interaction between allergen and possible remaining IgEs.

6. Would you expect someone with hyperactive IL-4 production to have an increased or decreased risk of allergy? Explain your reasoning.
Increased, as IL-4 is necessary for IgE production.

7. Histamine increases vascular permeability, while proteases restructure the extracellular matrix. Why have basophils and mast cells evolved to secrete these compounds?
They aid leukocyte infiltration to the site of infection to clear a pathogen.

8. The drug chromolyn prevents mast cell degranulation, although its exact mechanism is unclear. Predict one way that chromolyn might function. You do not need to state specific protein or molecule names in your response.
Possible prediction: chromolyn binds and sequesters membrane proteins that are necessary for granule docking and release.

9. Barry has a mutation that renders the Fc portion of his IgE molecules unable to interact with the FcE receptors (FcεRI) on mast cells and basophils.
    a. What would be the effect of this mutation on Barry's normal immune responses?
    Barry's immune responses would lack degranulation by mast cells and basophils. This would restrict his immunity, most notably through inhibition of leukocyte infiltration to his infected tissues.

    b. What would be the effect of this mutation on Barry's allergy responses?
    Barry would have little to no allergies, because his mast cells would be unable to be activated.

10. The kinases Itk and Btk are expressed and activated in mast cells following FcεRI (IgE Fc receptor) stimulation. To determine the specific roles these kinases play in FcεRI signaling and mast cell function, researchers generated Itk and Btk double knock-out (DKO) mice and compared them to wild-type, Itk-/-, and Btk -/- mice. Below are histological analyses of granule density in mast cells. In (A), blue/purple identifies filled granules, while pink identifies empty granules. Individual cells are encircled by a black box and blown up for clarity. (B) represents transmission electron microscope images of skin mast cells from each strain. The dark circles represent filled granules. (From Iyer et al. JBC 2011)
    a. Qualitatively, what differences do you detect between the mast cell granules of the different mouse strains?
    It appears that the DKO mice lack filled granules in their mast cells. The other strains (WT, Itk -/-, Btk -/-) have comparable, normal, granules.

    b. Based on these data, is Itk (alone) necessary for mast cell granule integrity? Is Btk (alone) necessary for mast cell granule integrity?
    Neither Itk or Btk appear to be necessary for mast cell granule integrity.

c. Do you predict that Itk and Btk signal in the same or different pathways in mast cells? Explain your reasoning.
It is most likely that Itk and Btk signal in parallel pathways that are able to compensate for one another. That is why deletion of each individually doesn't affect granule formation, but deletion of both does.

d. Suggest an experiment to test your hypothesis.
Itk pathway should be activated in Btk -/- mast cells, and Btk pathway should be activated in Itk -/- mast cells.

Another way to confirm this would be to identify signaling molecules directly upstream or downstream of each kinase, then do a similar experiment as shown here (knock out each individually, then create a DKO with these two new genes). The results should be the same.

Questions for Second Video:

1. State whether the following statements are true or false. If false, explain why.
    a. AIRE is primarily in the bone marrow. (**False - it's expressed in the thymus.)**
    b. Mutations in AIRE can reduce the capacity of an individual to fight pathogens. (**False - AIRE only affects clonal deletion and training of T cells; it doesn't affect the overall immunological response to pathogens.)**
    c. Aire activity contributes to both central and peripheral tolerance. (**True**)
    d. Aire functions as a classic transcription factor. (**False - AIRE does not bind DNA strongly. Instead, it helps to relieve stalled transcription**)
    e. Aire is expressed by pre-T cells in the thymus. (**False - AIRE is expressed by medullary epithelial cells in the thymus**)

2. AIRE is localized in the medulla, particularly in medullary epithelial cells (MECs).
    a. What is unique about MEC gene expression?
    These cells are capable of generating transcripts found in fully differentiated tissues throughout the body (e.g. muscle, pancreas, etc.).

    b. Briefly explain what would be the overall effect of deleting these cells during early development.
    The proteins produced by these cells are used in the "training" of T cells (clonal deletion). Deletion of these cells will lead to an increase in autoimmune diseases.

    c. Compare and contrast the two mechanisms by which AIRE aids immune development.
    First mechanism: Involved in the transcriptional regulation of thousands of genes in the thymus, which is crucial for the elimination of self-reactive T cells. This mechanism is important throughout the life of the individual.

    Second mechanism: Important for the development of a specific subset of Tregs within the first 10 days. Tregs aids the elimination of peripheral autoreactive T cells.

3. Predict why endocrine tissues, such as the pancreas and thyroid, are frequently targets of autoimmunity.
   Secretory tissues such as those in the endocrine system express and secrete large amounts of distinctive proteins. This makes them an easier target for autoreactive immune components.

4. AIRE deficient mice have circulating autoantibodies. Describe how deletion of AIRE, which is a thymic gene, can lead to this phenotype.
   When AIRE is not expressed in the thymus, CD4 cells that recognize self antigen are not deleted. These cells can then enter secondary lymphoid tissues where they aid the activation of naive B cells that produce antibodies against the self antigen.

5. Ovalbumin (OVA) is a protein that is unique to chicken eggs. Why was a non-murine protein chosen as the self-antigen to study in the AIRE mouse studies?
   By creating a neo-self-antigen, the researchers could study the development of T cells that were specific to an antigen that would not have redundancy in the mouse. That way, their experimental readout (pancreatitis) would be very clear and they wouldn't observe off-target autoimmunity.

6. mRNA transcription is not binary (i.e., genes are not simply "on" or "off").
   a. Describe what is meant by the term "stalled" or "paused" transcription.
      Transcription has been initiated, but RNA pol II is stalled on the DNA.

   b. When is RNA pol II paused during transcription? You do not have to name specific molecules in your answer.
      If the C-terminal tail of RNA pol II hasn't been appropriately phosphorylated and/or if certain regulatory proteins are bound to pol II, transcription is paused.

      Alternative answer: pausing happens during mRNA capping.

   c. Describe one mechanism by which pausing of RNA pol II is reversed. You do not have to name specific molecules in your answer.
      When kinases phosphorylate the C-terminal tail of pol II, or when other proteins come in and relieve binding of the regulatory proteins (for example by post translationally modifying them), the stall is lifted.

   d. Where does AIRE fit into this picture?
      Aire is part of a protein complex that helps to release pausing of RNA pol II through phosphorylation of the C-terminal tail of pol II and/or phosphorylation of regulatory proteins.

7. Researchers sought to determine the contribution of B cells to the autoimmune phenotype of Aire-deficient mice. To address their question, they generated mice that were deficient in both *Aire* and *μMT*, a gene necessary for B cell development. They then tracked the development of nonobese diabetes (NOD), a hallmark of autoimmunity, in mice from four genotypes. Data are summarized below. (Reference: Gavanescu PNAS 2008)
    a. Based on the data above, are B cells responsible for the development of NOD in Aire-deficient mice?
    No.

    b. Why or why not?
    Although incidence of NOD is decreased in DKO mice, it is not ablated. This suggests that other immune factors contribute to the disease, beyond B cells.

    c. In the paper from which this figure is derived, the authors make the claim that "B cells are responsible for lethal immunopathology in NOD." What evidence is needed to support this claim?
    A survival curve comparing Aire KO to Aire/μMT DKO mice is necessary to support this claim.

Questions for Optional Video:

1. Briefly describe the Experimental Autoimmune Encephalomyelitis (EAE) model to study multiple sclerosis (MS).
    EAE is an animal model of brain inflammation where immunization with spinal cord homogenate causes a very strong immune response by B and T cells against myelin. The immune cells infiltrate the central nervous system causing demyelination (depletion of the myelin-producing cells).

2. α4β1 integrin is the drug target of Natalizumab for the treatment of multiple sclerosis (MS).
    a. Briefly explain the involvement of α4β1 integrin in the development of MS.
    α4β1 is an integrin expressed on immune cells that aids their binding to inflamed blood vessels. α4β1 facilitates the transmission of immune cells to the central nervous system, which enhances the progression of MS.

    b. α4β1 is activated by a conformational change facilitated by the chemokine SDF-1. Would you consider SDF-1 as a possible drug target for the treatment of MS (see image below)? Briefly explain.
    Yes. One could also develop an antibody against SDF-1. If the chemokine is not present to cause the conformational change, α4β1 cannot be activated and the immune cells couldn't infiltrate the central nervous system to cause MS.

    c. A secondary effect of using the antibody Natalizumab is flu-like symptoms. Why?
    Presumably, decreasing the capacity of immune cells to infiltrate infected areas would reduce the ability to fight infection.

3. Why were the researchers in Yednock's group disappointed to learn that α4 integrin is expressed on a range of leukocytes?
   They were concerned that targeting α4 integrin would lead to off-target effects, since it was expressed so broadly.

4. In the image below, the binding sites of Natalizumab (Tysabri, yellow outline) and VCAM (red outline) are shown on α4β1 integrin. α4 integrin is represented in green; β1 integrin is represented in blue. (Reference: Yu et al., JBC 2013)
   a. How can Natalizumab (Tysabri) inhibit α4β1 integrin binding to its ligand VCAM, if the antibody and VCAM bind different locations on the integrin?
      Natalizumab binding to α4β1 integrin leads to steric hindrance of a VCAM domain. The resulting conformational change decreases VCAM's affinity for α4β1 integrin.

   b. If you were to design a small molecule inhibitor of α4β1 integrin for MS therapy, would you design it to bind to the same or to a different location as Natalizumab? Explain your reasoning.
      It would be best to design the small molecule to bind directly where VCAM interacts with α4β1 integrin. Because of their size, small molecules would likely be unable to affect the conformation of VCAM the way the relatively larger Natalizumab antibody does.

   c. Researchers sought to compare the effects of Natalizumab (Tysabri) with a small molecule inhibitor of α4β1 integrin-VCAM binding in a mouse model of EAE. They quantified the pathological changes in the spinal cord for two markers of disease, meningeal inflammation and myelitis. Mice were treated with either saline, Natalizumab, or the small molecule CT301 following EAE induction.
      i. How do the efficacies of Natalizumab and CT301 for treating EAE compare?
         CT301 is more effective than Natalizumab at stopping the progression of EAE.

      ii. Predict why the researchers likely observed this difference.
         It is likely that CT301 directly interferes with the binding of α4β1 integrin to VCAM. Direct inhibition of binding likely has a stronger effect than the indirect inhibition that Natalizumab confers. Alternatively, the affinity of CT301 for its target is higher than that of Natalizumab for its target. Another possibility could be that CT301 has better pharmacokinetics/ pharmacodynamics than Natalizumab.

5. Explain why targeting α4β1 integrin has been shown to be effective for treating a number of disease models, including multiple sclerosis, viral inflammation, and CNS graft rejection.
   Tysabri inhibits a fundamental biological process - immune cell infiltration - that is necessary for the pathologies associated with these various diseases.