

Session 5: B Cells Development, Selection, and Function

Overview:

The body needs to generate a broad diversity of antibodies in order to recognize a vast number of pathogens, while it prevents recognition of the self. This process is secured by safety checkpoints introduced during B cell differentiation. This session showcases the generation of antibody diversity (via V, D, J recombination), and the mechanisms that ensure the proper function of B cells (pre-BCR signaling, and tolerance). In addition, generation of B cell diversity is compared with the generation of diversity in T cell receptors during T cell development.

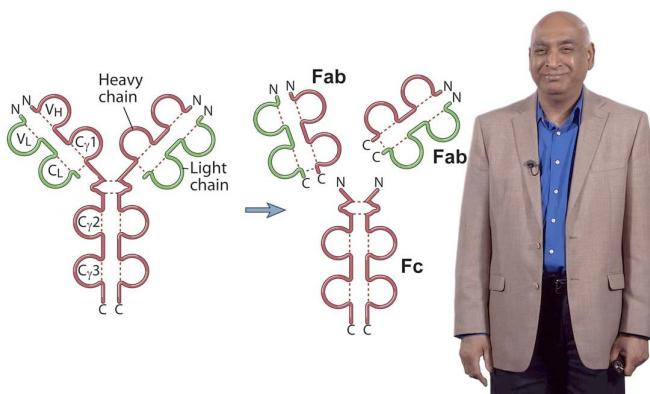
First video:

Title: Early B Cell Development: A Look at the Defining Questions in Immunology

Speaker: Shiv Pillai

Time: 37:30

Concepts: Historical perspective of the study of immunology, antigens/antibodies, side chain hypothesis, clonal selection hypothesis, antibody structure & function, generation of antibody diversity via VDJ recombination, and tolerance



Questions for First Video:

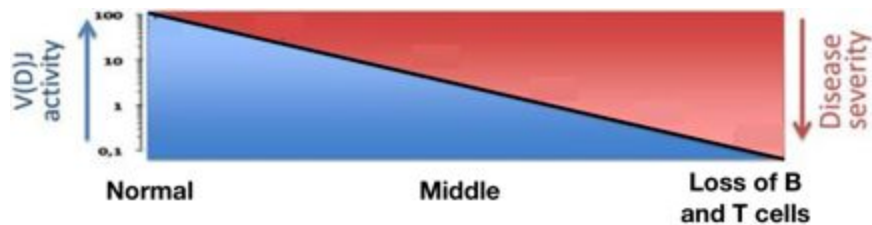
1. B cells undergo _____, where a specific cell will divide in response to its associated antibody recognizing an antigen.
2. The extraordinary diversity of antibodies results in part from
 - a. the action of monoclonal antibodies.
 - b. the splicing of protein molecules.
 - c. the action of cytotoxic T cells.
 - d. the rearrangement of genes.
 - e. their remarkable nonspecificity.

- b. How does a pre-B cell know whether rearrangement of the first locus has been successful?
- c. [Extension/Discussion] During the process of receptor editing, only the light chain is edited. Predict why this is the case.

13. Would you expect B cells isolated from a mature B cell tumor to express the same or different immunoglobulins? Why?

14. Why did the immune system evolve to allow self-reactive immature B cells to edit their BCRs, rather than simply kill them?

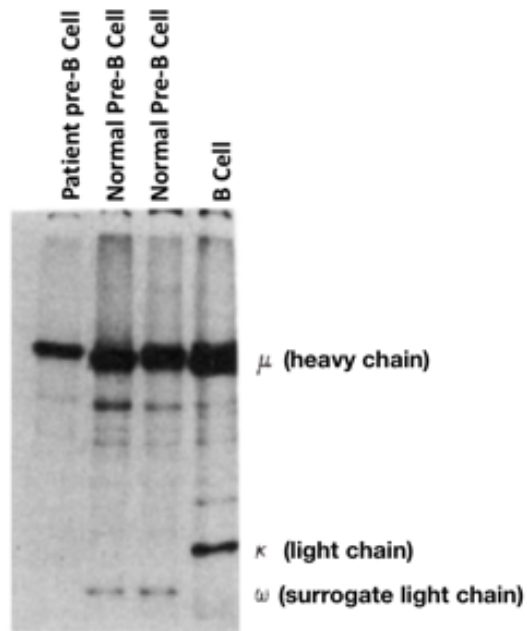
15. Shown below is a graph that represents the effect of defects in VDJ recombination in the severity of several diseases. Where in this graph (close to WT, middle or loss of T and B cells) would you expect to find a patient that has the following mutations:



Adapted from Lee et al. JACI, 2013

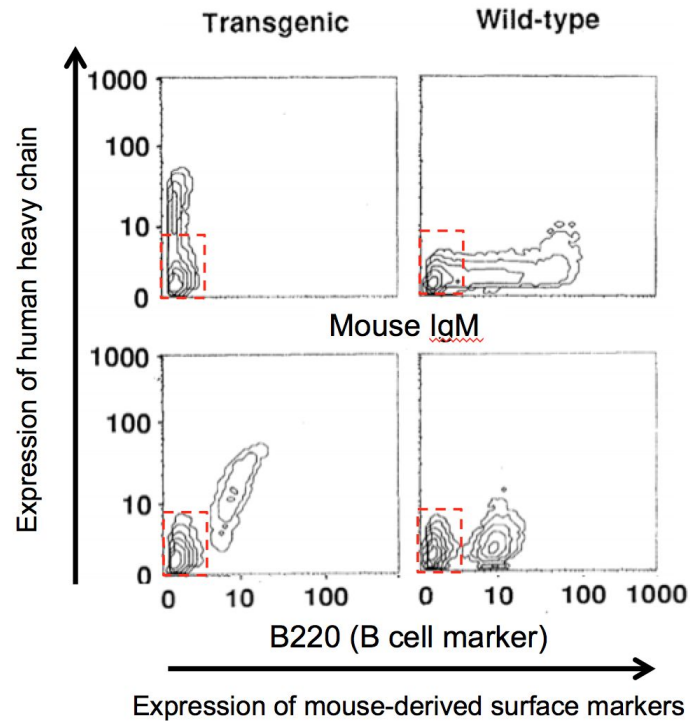
- a. A mutation where an allelic heptamer in a VDJ junction of an immunoglobulin gene is switched from the optimal consensus sequence.

- b. You run an experiment where you analyze the integrity of the pre-BCR in this patient and you found the following results (see data below). What would you conclude from this experiment? Briefly explain.



5. Staying “in frame” is important for successful V,D,J recombination.
- Describe one process during VDJ recombination that can shift the reading frame of an immunoglobulin gene.
 - What happens to the immunoglobulin protein if the reading frame is shifted?

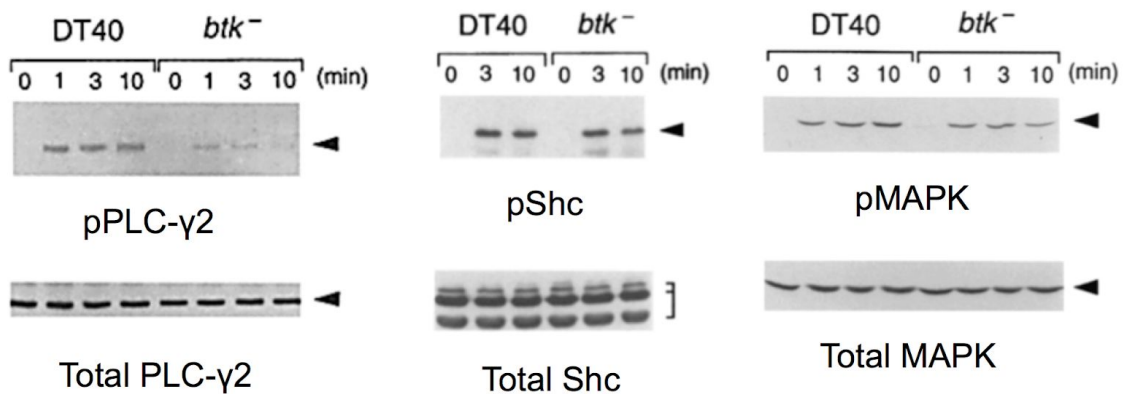
6. Researchers generated transgenic mice carrying a membrane-bound version of the human immunoglobulin M heavy-chain gene. Blood cells isolated from adult wild-type and transgenic mice were analyzed using flow cytometry, a method to evaluate expression of surface markers on cells. The results are shown below. Populations inside the red boxes indicate cells that are not positive for markers on either axis. (Reference: [Nussenzweig, Science 1987](#))



- In which animals is human IgM heavy chain expressed? Not expressed?
 - In which animals is mouse IgM expressed? Not expressed?
 - Do these data support the hypothesis of allelic exclusion? Why or why not?
7. During B cell development, B cells go through allelic exclusion.
- Why is allelic exclusion an important step in B cell development? Briefly explain.
 - How is allelic exclusion activated during B cell development? Briefly explain.

c. How is allelic exclusion related to the clonal selection hypothesis?

8. A group of researchers sought to identify the pathway through which Bruton's tyrosine kinase (Btk) signals from the B cell receptor (BCR). To address their question, they generated a B cell line deficient in Btk (*btk*⁻) and compared the intracellular response of these cells to that of wild-type cells (DT40) following BCR stimulation. The figures below represent Western blot analyses of cell lysates taken at different timepoints following BCR stimulation. The total protein (bottom panels) and phosphorylation levels (top panels) of three proteins (PLC-γ2, Shc, MAPK) were measured. (Reference: [Takata & Kurosaki, JEM 1996](#))



a. Which signaling protein(s) are dependent on Btk expression for phosphorylation? Which are not? Explain your reasoning.

b. Assuming that all three proteins assayed (PLC-γ2, Shc, MAPK) are involved in signaling from the BCR, state whether each is likely “upstream” or “downstream” of Btk in the signaling pathway.

c. For those protein(s) that are NOT downstream of Btk signaling, suggest an experiment to confirm whether they are upstream of Btk (i.e., necessary for its activation). Be sure to state how you would interpret your results.

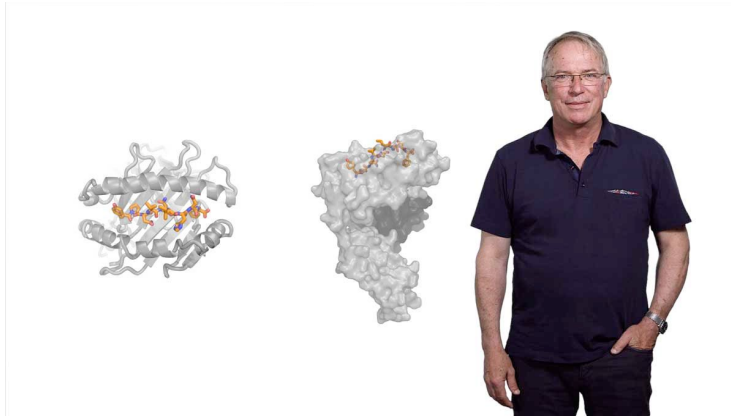
Second video:

Title: Immunology: The Basics of Antibody Diversity

Speaker: Hidde Ploegh

Time: 18:21

Concepts: B cell development, class-switch recombination, T cell receptor recombination, MHC complex assembly, and pathogen disruption of T-cell mediated immunity

**Questions for Second Video:**

1. State whether the following statements are true or false. If false, explain why.
 - a. B cells are positively selected to either generate membrane-bound antibodies or secrete antibodies.
 - b. Interaction with T helper cells is necessary to induce class switch recombination in B cells.

2. Briefly describe the main function of class switch recombination.

3. For each of the following processes, indicate whether they are regulated genomically, post-transcriptionally, or post-translationally. Briefly describe each process in 1-2 sentences.
 - a. Generation of BCR and TCR diversity.

 - b. Generation of different immunoglobulin isotypes.

 - c. Generation of membrane-bound vs. secreted immunoglobulin.

9. Upon infection by the poxvirus, host cells activate a signaling pathway that inhibits translation.

- a. Why would the host cell evolve to block translation, even though this is a crucial process for cell survival?

- b. The poxvirus evolved to produce a protein that will bind protein A in the host to stop the translation inhibition. Studies have shown high genetic variability in the host protein A as well as the viral protein. Briefly explain this phenomenon.

10. Pathogens have evolved different strategies to prevent detection by the host.

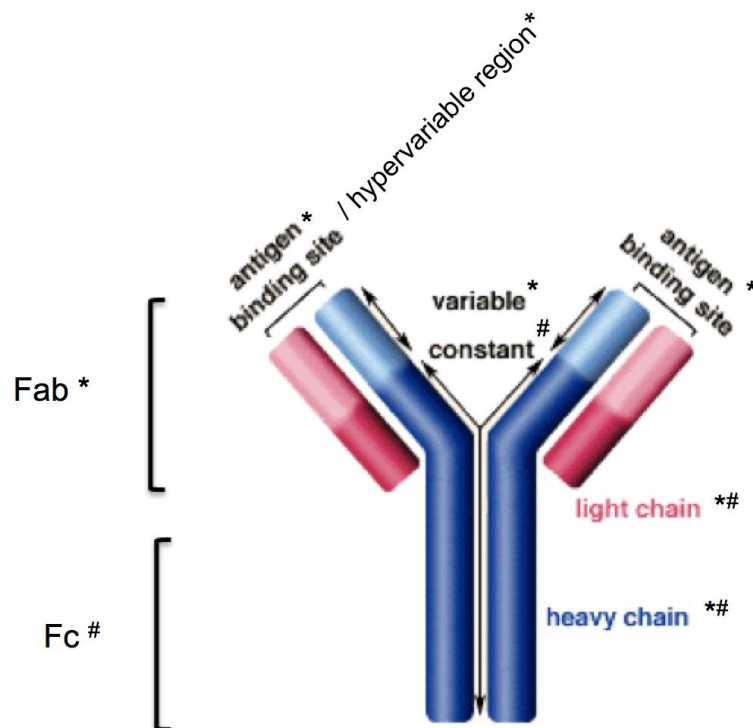
- a. Briefly describe a strategy used by pathogens to prevent activation of the immune system.

- b. The human papilloma virus (HPV) has evolved several proteins that inhibit antigen presentation by MHC. Researchers found that HPV is more likely to develop resistance to drugs that inhibit these proteins than drugs that inhibit viral replication. Explain why this is the case.

Answers for Session 5:

Questions for First Video:

1. B cells undergo _____, where a specific cell will divide in response to its associated antibody recognizing an antigen. **Clonal Expansion**
2. The extraordinary diversity of antibodies results in part from
 - a. the action of monoclonal antibodies.
 - b. the splicing of protein molecules.
 - c. the action of cytotoxic T cells.
 - d. the rearrangement of genes.**
 - e. their remarkable nonspecificity.
3. According to the clonal selection theory,
 - a. an antibody changes its shape to match the antigen it meets.
 - b. all B cells within an individual are identical.
 - c. an individual animal contains many types of B cells, each producing an antibody with a single antigen specificity.**
 - d. each B cell produces many types of antibodies.
 - e. many clones of anti-self lymphocytes appear in the bloodstream.
4. The structure of an antibody is critical for its function.
 - a. Using the following terms, draw and label the structure of a typical IgG antibody molecule: Heavy chain (H chain), light chain (L chain), variable region, constant region, Fab, Fc, antigen-binding site, hypervariable region. Use a * to identify terms that are important for antigen recognition, and a # to identify terms that are important for effector function.



- b. Antibodies have several effector functions in the body.
 - i. List two of these functions.
Neutralization of microbes/toxins; opsonization and phagocytosis of microbes; antibody-dependent cellular toxicity; complement activation
 - ii. How does the immune system differentiate between the different effector roles?
The immune system can differentiate between these roles because there are a number of different constant regions that can be expressed with the same antigen-binding site. The varying structures of the constant region determine their function.
- 5. You vaccinate a patient against tetanus. Which statement regarding the patient's antibody-mediated immunity to tetanus is accurate?
 - a. Every B cell in her body produces antibodies against C. tetani.
 - b. Every cell in her immune system produces antibodies against C. tetani.
 - c. **The B cells with antibodies that recognize C. tetani are stimulated to divide, producing plasma cells that make antibodies, and memory cells that “remember” C. tetani.**
 - d. The B cell with the appropriate antibody undergoes genomic rearrangement in order to produce other cells that produce the same antibody.
- 6. What is the purpose of getting a “flu shot” every year?
 - a. It lets your body get used to having the flu so that you won't feel as sick when you actually do get the flu.
 - b. **It exposes your immune system to antigens present on the flu virus so anti-flu antibodies can be made by plasma cells.**
 - c. It exposes your immune system to antibodies present on the flu virus, and antigens can be made by the immune system.
 - d. It exposes your immune system to the virus and causes gene recombination in memory B-cells.
- 7. State whether the following statements are true or false. If false, explain why.
 - a. Fab fragment is described as the tail of the antibody. **(False – it is the region that contains the antigen binding site)**
 - b. Ehrlich's Side Chain Hypothesis states that prior to interaction with a pathogen, vertebrates already have proteins that would bind to antigens produced by the pathogen. **(True)**
 - c. Immunoglobulin genes have three highly variable regions that define the antigen binding site. **(True)**
 - d. Upon binding, antigens induce a conformational change in antibodies to make them more specific for an antigen. **(False – Induced hypothesis is incorrect. Vertebrates already have antibodies that interact with antigens.)**
 - e. An enzyme adds nucleotides at the VDJ junctions increasing antibody diversity. **(True)**
 - f. B cells that have left the bone marrow will have already gone through VDJ recombination and pre-BCR checkpoint positive selection, and these B cells will produce antibodies that don't react to self-antigens. **(True)**

8. The immune system is able to generate antibodies that recognize 10^{14} antigens, even though the genome has only a few immunoglobulin genes.
- List two ways that diversity is generated during recombination of immunoglobulin genes.
Different combinations of V/D/J segments, junctional diversity attained through differential overhangs & TdT nucleotide additions.
 - How would inhibition of TdT activity affect the B cell repertoire? Be specific in your answer.
Inhibition of TdT would lead to a reduction in the diversity of the B cell repertoire, namely through loss of junctional diversity through addition of N nucleotides. However, the B cell repertoire would likely still be diverse because at least two other mechanisms (VDJ recom & differential overhangs at hairpins) would remain.
9. Several checkpoints enhance the development of functional B cells.
- What is the importance of the pre-BCR checkpoint? Briefly explain
pre-BCR checkpoint positively selects for clones that have successfully rearranged the antibody heavy chain. VDJ recombination can introduce errors that would result in a non-functional antibody. This step ensures that mature B cells have functional heavy chains.
 - Briefly explain how the pre-BCR checkpoint works.
After VDJ recombination, the heavy chain associates with surrogate light chains to create the pre-BCR (pre-B Cell Receptor). This binding will constitutively activate the receptor whose signal is an indication of a functional heavy chain. B cells with a signaling pre-BCR will be positively selected. Any B cell without a functional pre-BCR will be eliminated.
 - Briefly describe one major checkpoint that occurs during B cell development after the pre-BCR checkpoint.
Negative selection of clones that recognize self-antigens. The antibodies in B cells that recognize self-antigens will undergo receptor editing, which will modify the sequence of the light chain such that it doesn't recognize self-antigens (mostly important in B cells). Alternatively, B cells that have antibodies that recognize self-antigens will be targeted for deletion (mostly important for T cells).

Alternative answer: Peripheral tolerance mediated by Treg cells, which target B cells that have self-antigen reactivity for degradation.
10. Compare and contrast the pre-B cell and the immature B cell checkpoints. Include:
- Their purpose/goal.
Overall, both ensure that the BCR expressed by the B cell can perform its appropriate function. For the pre-B cell checkpoint, the goal is to check whether VDJ recombination of the heavy chain has been successful and whether the resulting protein is in frame. For the immature B cell checkpoint, the goal is to identify any immature B cells with self-reactive BCRs.

- b. The structure of the BCR at each.
The structure of the BCR at the pre-B cell stage includes the recombined heavy chains with surrogate light chains; the structure of the BCR at the immature B cell checkpoint includes both recombined heavy and light chains.
- c. What happens to the developing B cell if it doesn't "pass".
If a cell doesn't pass the pre-B cell stage it is directed to die; if a cell doesn't pass the immature B cell checkpoint it is directed to repeat VDJ recombination of its light chain.
11. Variability in immune cell surface proteins allows for the recognition of different pathogens.
- a. Briefly describe how variation in MHC proteins and T and B cell receptors is created.
MHC proteins' variability is established by allelic variability in the human population. On the other hand, T and B cells' variability comes from V(D)J recombination.
- b. Explain whether all of the cells that express each receptor type will be identical or different within the same individual.
MHC proteins are identical on all cells of one individual, while B and T cell receptors can be different. T and B cell receptors are the same only when they are clones.
12. Immunoglobulin heavy and light chain genes are encoded at different genetic loci.
- a. Which locus is rearranged first?
The heavy chain locus.
- b. How does a pre-B cell know whether rearrangement of the first locus has been successful?
After recombination, the heavy chain assembles with the surrogate light chain into the pre-BCR, which is expressed intracellularly and constitutively signals to the cell. If the cell doesn't receive this positive signal, then it will not continue through B cell development and will be instructed to die.
- c. [Extension/Discussion] During the process of receptor editing, only the light chain is edited. Predict why this is the case.
Receptor editing likely evolved this way because it is unfavorable to re-enter the pre-B cell checkpoint.
13. Would you expect B cells isolated from a mature B cell tumor to express the same or different immunoglobulins? Why?
We would expect them to express the same Igs, as mature B cells have completed development, which includes recombination of their immunoglobulin genes and activation of allelic exclusion. Therefore, a mature B cell tumor (which is a clone of a single mature B cell) will have identical Igs.

14. Why did the immune system evolve to allow self-reactive immature B cells to edit their BCRs, rather than simply kill them?

This provides more “chances” to expand the host B cell repertoire. If cells were deleted on the first try, there would be fewer clones overall in the repertoire and therefore fewer antigens could be recognized overall.

Alternative interpretation: the body has invested sufficient energy into creating these cells. It would be wasteful to eliminate them on the first try.

15. Shown below is a graph that represents the effect of defects in VDJ recombination in the severity of several diseases. Where in this graph (close to WT, middle or loss of T and B cells) would you expect to find a patient that has the following mutations:

- a. A mutation where an allelic heptamer in a VDJ junction of an immunoglobulin gene is switched from the optimal consensus sequence.

Middle. You would expect a reduction of B cells and antibody production, but T cells should not be affected.

- b. A loss of function mutation in Rag2, a protein involved in VDJ recombination in both B and T cells.

Middle or severe. A mutation in Rag2 will decrease both T and B cell production and will have severe defects in the immune system of the patient. Inhibitory mutations of the RAG proteins would lead to inhibition of VDJ recombination in both B and T lymphocytes. The functionality of Rag2 will dictate the severity of the disease.

- c. A mutation in terminal deoxynucleotidyl transferase (tdt), a protein that adds nucleotides at the junctions during VDJ recombination.

Close to WT. Antibody and TCR diversity will be affected, but this patient should still have functional B and T cells.

- d. (Question corresponds to optional video) A patient that has a mutation in Btk.

Middle. You would expect to reduce B cell and antibody production, but T cells should not be affected.

Questions for Optional Video:

1. The ligand-independent activation of receptor (LIAR) hypothesis states that
 - a. B cells that bind to self-antigens are positively selected.
 - b. Upon ligand/antigen binding, the pre-BCR will produce a signal that will lead to B cell positive selection.
 - c. **Upon rearrangement of immunoglobulin heavy chain, constitutive signaling through the pre-BCR will trigger positive selection.**
 - d. Ligand-independent signaling through pre-BCR induces negative selection of B cells.
 - e. B cells contain light chain like proteins that bind to the heavy chain upon negative selection.

2. Briefly describe the two types of B cells (B1 and B2) found in vertebrates.
The liver produces one type of B cell (B1 cells) which are self-renewing and have a generic function to deal with certain types of pathogens. The other type of B cell is developed in the bone marrow.

3. State the defining characteristics of each of the following B cell developmental stages, in terms of their immunoglobulin genes and protein expression.
 - a. pro-B cell
Ig genes not recombined. No BCR expression.

 - b. pre-B cell
Expression of pre-BCR (recombined heavy chain gene with surrogate light chains).

 - c. Immature B cell
Expression of BCR (recombined heavy and light chain genes)

 - d. Mature B cell
Expression of BCR (recombined heavy and light chain genes; not self-reactive).

 - e. Plasma cell
Expression of secreted antibodies (recombined heavy and light chain genes; not self-reactive).

4. You are studying a patient that has Severe Combined Immunodeficiency (SCID)-like symptoms. The patient has a normal number of T cells, but a deficiency in the B cell production.
 - a. Could a mutation in Rag2, a protein involved in VDJ recombination, explain this patient's outcome? Briefly explain.
No. Rag2 is involved in recombination of antibodies in B cells, but also TCR. A defect in Rag2 that affects the production of B cells should also result in defective T cells.

 - b. You run an experiment where you analyze the integrity of the pre-BCR in this patient and you found the following results (see data below). What would you conclude from this experiment? Briefly explain.
The pre-BCR of this patient doesn't associate with the surrogate light chain, because the surrogate light chain is not expressed. Immunodeficiency and decreased B cell production is caused by impaired signaling through the pre-BCR (Binding to surrogate light chain is critical for pre-BCR signaling and proper B cell development).

5. Staying "in frame" is important for successful V,D,J recombination.
 - a. Describe one process during VDJ recombination that can shift the reading frame of an immunoglobulin gene.
During VDJ recombination, the TdT enzyme can increase junctional diversity by adding nucleotides between the V-D-J segments. If new nucleotides are not added in multiples of three, then the reading frame of the mRNA is shifted.

- b. What happens to the immunoglobulin protein if the reading frame is shifted?
If the reading frame of the gene is shifted, during translation entirely different amino acids will be added to the peptide chain. This will result in a protein that is very different from the intended Ig protein, and it will likely have impaired function.
6. Researchers generated transgenic mice carrying a membrane-bound version of the human immunoglobulin M heavy-chain gene. Blood cells isolated from adult wild-type and transgenic mice were analyzed using flow cytometry, a method to evaluate expression of surface markers on cells. The results are shown below. Populations inside the red boxes indicate cells that are not positive for markers on either axis. (Reference: [Nussenzweig, Science 1987](#))
- a. In which animals is human IgM heavy chain expressed? Not expressed?
Expressed in transgenic; not expressed in wild-type.
- b. In which animals is mouse IgM expressed? Not expressed?
Expressed in wild-type; not expressed in transgenic.
- c. Do these data support the hypothesis of allelic exclusion? Why or why not?
Yes. There are no cells that are positive for both the human and mouse IgM; therefore, this supports allelic exclusion.
7. During B cell development, B cells go through allelic exclusion.
- a. Why is allelic exclusion an important step in B cell development? Briefly explain.
It ensures that a particular B cell will produce only ONE type of antibody.
- b. How is allelic exclusion activated during B cell development? Briefly explain.
Upon heavy chain rearrangement, the pre-BCR (heavy chain bound to a surrogate light chain) is formed, which through ligand-independent signaling will activate a signaling cascade via bruton tyrosine kinase (Btk) activation that will inhibit recombination of the second allele and mediate allelic exclusion.
- c. How is allelic exclusion related to the clonal selection hypothesis?
The process of allelic exclusion ensures that each B cell will only express one copy of each immunoglobulin gene. That way, the B cell and its progeny will consistently express the same, specific BCR.
8. A group of researchers sought to identify the pathway through which Bruton's tyrosine kinase (Btk) signals from the B cell receptor (BCR). To address their question, they generated a B cell line deficient in Btk (*btk*⁻) and compared the intracellular response of these cells to that of wild-type cells (DT40) following BCR stimulation. The figures below represent Western blot analyses of cell lysates taken at different timepoints following BCR stimulation. The total protein (bottom panels) and phosphorylation levels (top panels) of three proteins (PLC- γ 2, Shc, MAPK) were measured. (Reference: [Takata & Kurosaki, JEM 1996](#))
- a. Which signaling protein(s) are dependent on Btk expression for phosphorylation? Which are not? Explain your reasoning.
PLC- γ 2 is dependent on Btk for phosphorylation, while Shc and MAPK are not. In Btk deficient mice, only phosphorylation of PLC- γ 2 is inhibited following BCR stimulation. Phosphorylation of the other two proteins is the same in wild-type and KO cells following BCR stimulation.

- b. Assuming that all three proteins assayed (PLC- γ 2, Shc, MAPK) are involved in signaling from the BCR, state whether each is likely “upstream” or “downstream” of Btk in the signaling pathway.

PLC- γ 2 is likely downstream of Btk, while Shc and MAPK are likely upstream of Btk.

- c. For those protein(s) that are NOT downstream of Btk signaling, suggest an experiment to confirm whether they are upstream of Btk (i.e., necessary for its activation). Be sure to state how you would interpret your results.

Could generate B cells that are deficient in Shc or MAPK. Following BCR stimulation, could assay for markers of Btk activation (such as phosphorylation). If Btk activation is impaired in the absence of either of these proteins following BCR stimulation, this would indicate that they signal upstream of Btk.

Questions for Second Video:

1. State whether the following statements are true or false. If false, explain why.
 - a. B cells are positively selected to either generate membrane-bound antibodies or secrete antibodies. **(False - B cells generate both types of antibodies)**
 - b. Interaction with T helper cells is necessary to induce class switch recombination in B cells. **(True)**
2. Briefly describe the main function of class switch recombination.

This is the process by which a recombined VDJ cassette can be placed in juxtaposition to generate different types of antibodies (IgM, IgA, IgE, and IgG) with different effector functions.
3. For each of the following processes, indicate whether they are regulated genomically, post-transcriptionally, or post-translationally. Briefly describe each process in 1-2 sentences.
 - a. Generation of BCR and TCR diversity.

Genomically. Lymphocyte diversity is generated through recombination of the immunoglobulin and TCR gene variable regions.
 - b. Generation of different immunoglobulin isotypes.

Genomically. Class switch recombination follows VDJ recombination of the heavy and light chains. When a mature B cell is activated, the recombined VDJ cassette is placed upstream of the appropriate constant region.
 - c. Generation of membrane-bound vs. secreted immunoglobulin.

Post-transcriptionally. Alternative polyadenylation directs the expression of secreted or membrane-bound immunoglobulin.
 - d. Signaling downstream of BCR and/or TCR stimulation.

Post-translationally. Signaling from the BCR and TCR is mediated through phosphorylation cascades.

4. A group of researchers seek to make a vaccine using a carbohydrate-based antigen. For each of the immune effectors listed below, state whether this vaccine would be effective and explain why or why not.
- Immunoglobulin/antibody response.
This would be effective, as antibodies are able to recognize antigens derived from a range of macromolecules.
 - Helper T cell response.
This would not be effective, as the TCR can only recognize peptide antigens presented in MHC molecules.
5. How would treatment of an individual with anti-IL-4 antibodies affect their B cell activity? State which stage of mature B cell activation would specifically be affected.
Class switch recombination would be impaired, because it requires CD4 T cells, IL-4, and AID (activation-induced deaminase). This means that the switching of immunoglobulin isotypes would be inhibited following B cell activation and the effector function of mature B cells would be limited in this person.
6. Predict the effect of a mutation in Ig- α or Ig- β that renders them unable to localize to the plasma membrane.
This would inhibit BCR signaling from the plasma membrane, as Ig- α /Ig- β are necessary for intracellular signaling from the BCR. An individual with this mutation would likely have impaired B cell-mediated immunity.
7. Antibodies made in mice and rabbits have limited therapeutic potential in humans. Predict why this is the case.
This is likely because the constant regions of the antibodies, which determine their effector functions, are sufficiently different between rodents and humans that human immune cells cannot fully recognize and interact with them.
8. Linked recognition refers to the ability of B cells and helper T cells to recognize distinct epitopes of the same pathogen. Why is this advantageous?
It allows individual helper T cells to activate a range of mature B cells (rather than just 1:1), leading to broader pathogen specificity and greater overall efficacy of the immune response.
9. Upon infection by the poxvirus, host cells activate a signaling pathway that inhibits translation.
- Why would the host cell evolve to block translation, even though this is a crucial process for cell survival?
By inhibiting translation in an infected cell, the host contains infection by preventing new viruses from being made.
 - The poxvirus evolved to produce a protein that will bind protein A in the host to stop the translation inhibition. Studies have shown high genetic variability in the host protein A as well as the viral protein. Briefly explain this phenomenon.
Co-evolution with pathogens generate diversity in proteins involved in pathogen-host interactions. Evidence of an evolutionary arms race.

10. Pathogens have evolved different strategies to prevent detection by the host.

- a. Briefly describe a strategy used by pathogens to prevent activation of the immune system.

Some pathogens inhibit the adaptive immune system (e.g. inhibit MHC presentation) or the innate immune system (e.g. inhibition of histamine release).

- b. The human papilloma virus (HPV) has evolved several proteins that inhibit antigen presentation by MHC. Researchers found that HPV is more likely to develop resistance to drugs that inhibit these proteins than drugs that inhibit viral replication. Explain why this is the case.

These proteins aren't crucial for the survival of the virus and therefore can have higher mutation rates. Even if you were to find a good drug, high mutation rates will create drug-resistant strains.