

## Session 9: The Immunology of Organ Transplantation

### Overview:

Every year a significant number of patients require organ transplantation for survival. In order to reduce the chances of organ rejection, the recipient and donor immune systems need to match. If they don't match, the recipient immune system will recognize the donated organ as non-self. In this session, you will learn about the major types of transplantation, evaluate the immune cells involved in organ rejection, and understand the basics of transplantation tolerance (long-term graft acceptance without the long-term use of immunosuppressants). In order to achieve tolerance, a new transplantation protocol that induces mixed chimerism in patients has been developed. This protocol results in a patient's immune system resembling both the donor and the recipient, and allows for transplanting organs between donors and recipients that aren't an immunological "match".

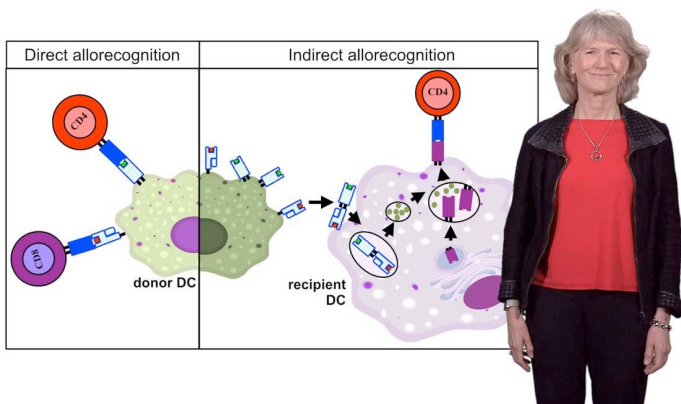
### First video:

Title: Introduction to Transplantation: The Immune Response to Allo- and Xenotransplantation

Speaker: Megan Sykes

Time: 32:19

Concepts: Allo- and Xenotransplantation, immune cells involved in transplantation rejection, HLA matching, acute & chronic rejection, direct & indirect T Cell response, immunosuppressive drugs, and hematopoietic cell transplantation

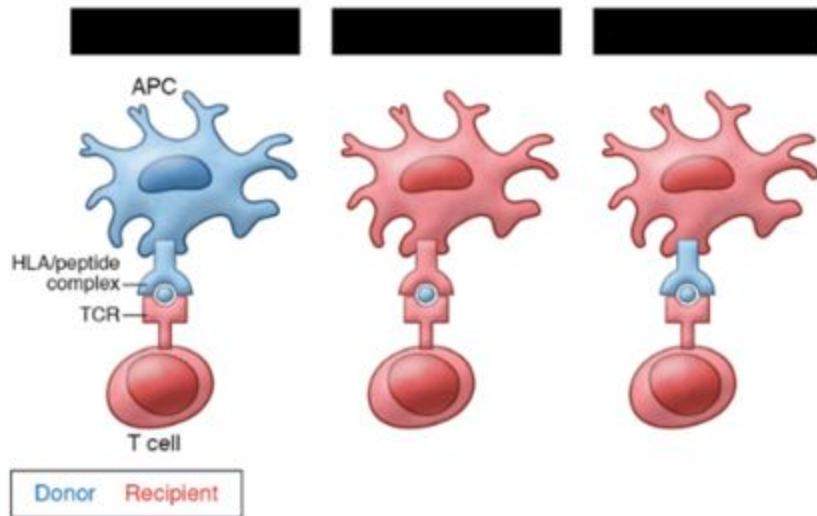


### Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
  - a. Syngeneic transplantation has the lowest rate of rejection because the immune system of the host views the donor organ as self.
  - b. Having similar HLA alleles correlates with poor transplantation rate. Donor dendritic cells can present antigen to recipient T cells, which can trigger an immune response that leads to organ rejection.
  - c. T cells are the key players in graft-versus-host disease.
  - d. The term alloantigen describes antigens that vary between individuals of different species.
  - e. The T cell is a central player in most instances of organ & tissue rejection.

- f. The innate immune system is not involved in rejection of transplanted tissues.
  - g. Acute and hyperacute rejection differ largely in terms of when anti-transplant antibodies are synthesized relative to transplantation.
2. Which of the following are factors that increase transplantation rejection? (Select all that apply)
- a. The donor and host have different blood types.
  - b. The donor was recently pregnant.
  - c. The recipient has antibodies against donor proteins.
  - d. The donor had a bad viral infection to which the host is susceptible.
  - e. None of the above.
3. The human leukocyte antigen (HLA) genes encode for the major histocompatibility complex (MHC) proteins in humans, and these genes are highly polymorphic. Two unrelated individuals have low chances of having identical HLA alleles.
- a. Why have we evolved to have such variability in the HLA locus? Briefly explain.
  - b. For the process of organ transplantation, it is important to consider if the donor and recipient are an "HLA match"; meaning, the HLA alleles of the patient are identical to the HLA alleles of the donor. Briefly explain why this is important.
4. Cyclosporin A is an immunosuppressant drug that is used for patients who need to have kidney transplants.
- a. Why do immunosuppressant drugs need to be given to patients undergoing organ transplantation? Briefly explain.
  - b. Why is organ rejection less likely if the organ is donated by a close relative (mother or brother) and completely eliminated if the organ were to be donated by an identical twin? Briefly explain.
  - c. Cyclosporin A is known to bind to proteins in T cells and inhibit interleukin-2 (a cytokine) release. How does this mode of action reduce the response of the immune system? Briefly explain.

5. Shown below are three different mechanisms by which the immune system is able to distinguish its own tissue from those of another (allorecognition) in organ transplantation.
  - a. Add the appropriate name to each mechanism.



DeWolf S & Sykes M (2017) J Clin Invest

- b. What characterizes each mechanism? Briefly outline the cells involved.
  
6. Compare and contrast the following sets of ideas or terms.
  - a. The use of immunosuppressive drugs and induction of immune tolerance in preventing transplant rejections.
  
  - b. Direct and indirect allorecognition

c. Major and minor histocompatibility antigens

d. Chronic and acute rejection

7. Explain why anti-HLA immune responses are stronger than other types of immune responses

8. Fill in the missing fields in the following table to compare and contrast the major types of allorecognition.

	Indirect allorecognition	Direct allorecognition	Semi-direct allorecognition
Source of antigen-presenting cell			
Source of MHC			
Size of MHC: whole or fragment?			

9. Explain why direct allorecognition of MHC II is unable to be the major cause of chronic rejection.

10. Would a person who previously received an HLA-incompatible blood transfusion be more or less likely to accept an organ transplant with the same HLA allotype? Briefly explain.

11. Predict why T cell depletion prior to bone marrow transplantation can increase likelihood of rejection.

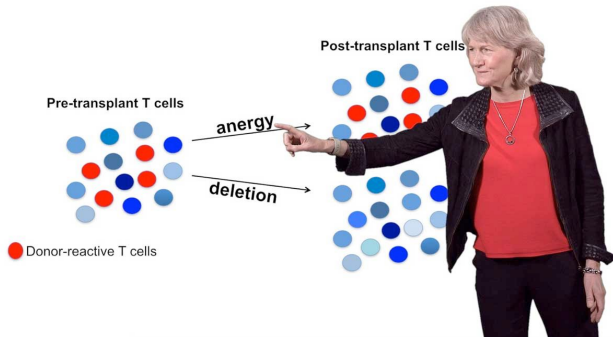
## Second video:

Title: Taming and Tracking the Human Alloresponse

Speaker: Megan Sykes

Time: 37:40

Concepts: Transplantation tolerance, and mixed chimerism



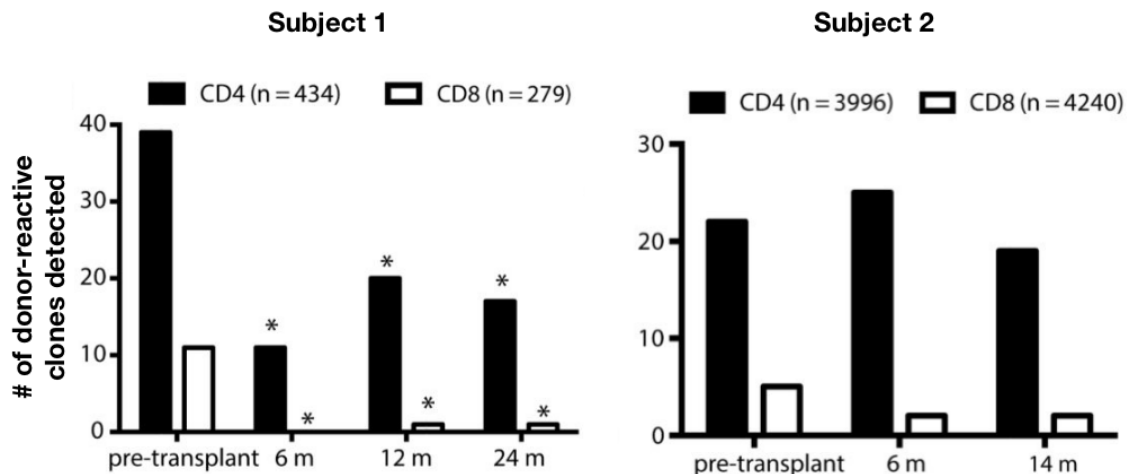
## Questions for Second Video:

1. Which of the following is (are) important factor(s) in the mixed chimerism organ transplantation protocol? (Select all that apply)
  - a. Dendritic cell depletion.
  - b. Blood injection of donor T-cells prior to organ transplantation.
  - c. Deletion of donor B cells.
  - d. Introduction of donor macrophages.
  - e. None of the above.
2. Jeremy is a candidate for a kidney transplant. For the following conditions, determine if you would consider enrolling Jeremy in a mixed chimerism trial. Briefly explain.
  - a. Jeremy doesn't have the same HLA allele as the donor.
  - b. Jeremy has antibodies against donor antigens prior to transplantation.
  - c. The donor organ has antigen presenting cells (APCs) that have the capacity to present antigen to Jeremy's T cells.

3. Amy, one of the first patients to receive mixed chimerism treatment for kidney transplantation, received a kidney from her father. Prior to being enrolled in this clinical trial, she received a kidney from her mom using the conventional method and rejected the organ.
  - a. Define the term “mixed chimerism”.
  
  - b. What advantages over traditional transplantation methods does mixed chimerism provide that convinced Amy to enroll in this clinical trial? Briefly explain.
  
  - c. What factors did the doctors look at to determine that Amy’s mom and then her dad were a “good match” for each type of transplant? Briefly explain.
  
4. The protocol used to induce mixed chimerism in humans has gone through a few iterations.
  - a. What is the importance of each of the following strategies for the mixed chimerism protocol? Be sure to outline the target cell(s).
    - i. Local irradiation to the thymus:
  
  
    - ii. Treatment with rituximab, a B cell depleting agent:
  
  - b. Why were patients treated with immunosuppressant drugs for a short period of time after organ transplantation?
  
  
  - c. What marker found in the patients confirmed that mixed chimerism was achieved?
  
  
  - d. Why were the researchers excited to observe an increase in regulatory T cells (Tregs) post organ transplantation? Briefly explain.

- e. What was found at the molecular level when researchers compared T cells from a patient that rejected the organ versus patients that did not?

5. Shown below are the results of a study in which two patients received a combined kidney and bone marrow transplantation to achieve mixed chimerism. Researchers measured the numbers of donor-reactive T cell clones in each recipient before and after the treatment. Can you predict the outcome of the organ transplantation (rejection versus non-rejection) for each patient? Briefly explain.



Morris H, et al. (2015) Science Translational Medicine

6. Why would myeloablation prior to bone marrow transplantation reduce the odds of immune tolerance?
7. There are three classes of drug used to suppress acute transplant rejection: corticosteroids, cytotoxic drugs, and T-cell activation inhibitors.
- What aspects of immunity do you predict each acts on? Be as specific as possible.

b. What do you predict are the side effects of each class?

8. Researchers evaluated a novel strategy to induce transplant tolerance in humans by simultaneous transplantation of HLA-mismatched kidney and bone marrow. (Reference: Kawai, Am J Transplantation, 2014)

a. A summary of each of the patients in the study and their response to treatment are below.

**Table 1:** Subject profiles and results

	Age	Sex	Original disease	Time discontinued immunosuppression (months)	Graft survival	Current pathology	Immunosuppression
ITN036							
6	35	M	Alport's disease	8	>5.0 years	No rejection	None
7	37	F	Reflux uropathy	8	>4.8 years	No rejection	None
9	22	F	MPGN type 1	8	>4.4 years	No rejection	None
10	26	M	Alport's disease	8	3 years	Post-ACR	NA (retransplanted)

ACR = acute cellular rejection; NA = not applicable

i. Do you consider this strategy successful? Why or why not?

ii. What information would you need to strengthen your stance?



- b. The researchers sought to understand why some patients responded favorably to the transplant and others didn't. They performed a mixed-lymphocyte reaction (MLR) experiment, which measures anti-donor responses by CD4+/CD8+ effector T cells. The results of the MLR study are summarized in the table below.

**Table 2: Summary of MLR in ITN036 patients**

	MLR
<b>Pt6</b>	
preTx	R
3 months	-
6 months	R
9 months	DSH <sup>3</sup>
12 months	R
18 months	DSH
<b>Pt7</b>	
preTx	DSN
3 months	-
6 months	GN
12 months	GN
18 months	DSN
36 months	DSN
<b>Pt9</b>	
preTx	DSH
2 months	R
3 months	R
6 months	R
9 months	R
12 months	DSH
18 months	DSH
<b>Pt10</b>	
preTx	DSH
4 months	GN
6 months	DSH
9 months	DSN
12 months	DSN

R = equally responsive to donor and third party

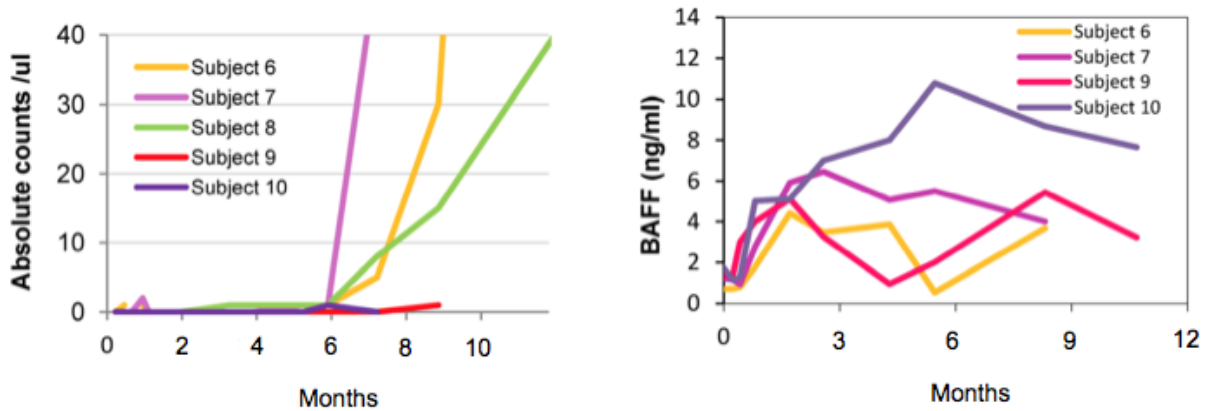
GN = global nonresponsiveness; no response to either donor or third party

DSH = donor-specific hyporesponsiveness; response to donor lower than response to third party

DSN = donor-specific nonresponsiveness; nonresponsive to donor but responsive to third party

- i. What do these data indicate about the role of effector T cells in transplant acceptance or rejection?
  
- ii. Name at least one limitation in the data above, as it relates to our understanding of transplant rejection.

- c. Finally, the researchers sought to evaluate the role of B cells in transplant acceptance or rejection. They measured absolute counts of mature B cells (left panel) as well as B cell activity (right panel) in each of the patients over several months (Ignore data for Subject 8 in your analysis).



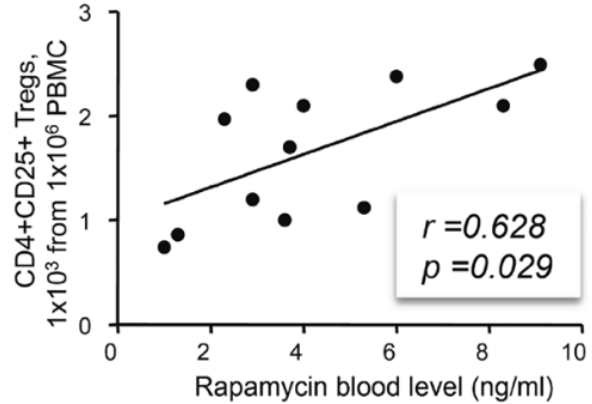
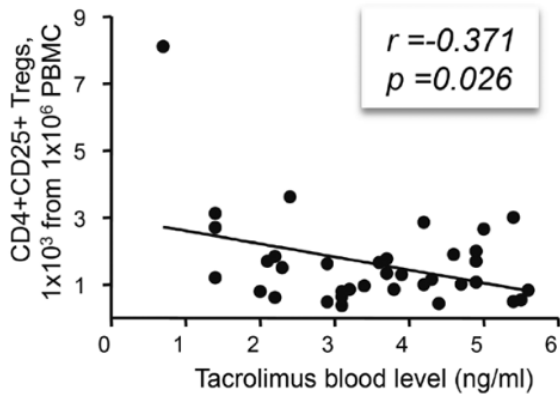
- i. Do absolute numbers of B cells appear to correspond with transplant acceptance or rejection? Briefly explain.
  
- ii. Does B cell activity appear to correspond with transplant acceptance or rejection? Briefly explain.
  
- iii. In a separate experiment, no donor-specific antibody (DSA) was detected in any of the four patients for three years following transplantation. What does this indicate about the role of B cells in transplant acceptance or rejection?
  
- iv. How would you test your hypothesis in a mouse model of this transplantation strategy?

- v. Do you predict that B or T cells are more likely to be involved in transplant rejection? Explain your prediction.

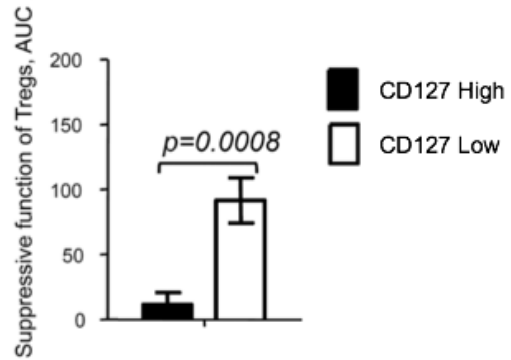
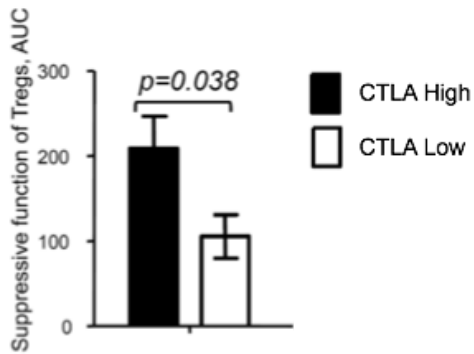
9. Modulation of effector T cell function is one strategy for inducing transplant tolerance. Researchers evaluated the effects of two different drugs on Treg populations in pediatric liver and kidney transplant recipients. (Reference: Akimova, Am J Transplantation, 2012)

- a. Why would modulation of Treg abundance or function in transplant recipients be a viable treatment strategy?

- b. Based on the data below, which drug (Tacrolimus or Rapamycin) appears to be promising for promoting organ transplant tolerance? Briefly explain. (PBMC = peripheral blood mononuclear cell)



- c. Based on the data below, which surface protein would serve as a better biomarker for a clinical study of the drug you chose above? Briefly explain.



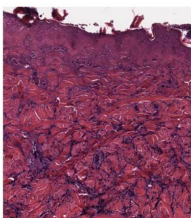
**Optional video:**

Title: Xenotransplantation

Speaker: Megan Sykes

Time: 31:32

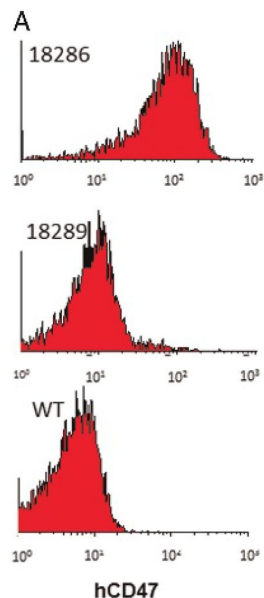
Concepts: Xenotransplantation,  $\alpha$ 1,3-Gal, genetically engineered pigs for organ transplants, xenograft rejection, tolerance induction, mixed chimerism, CD47 signaling/macrophages in xenotransplantation



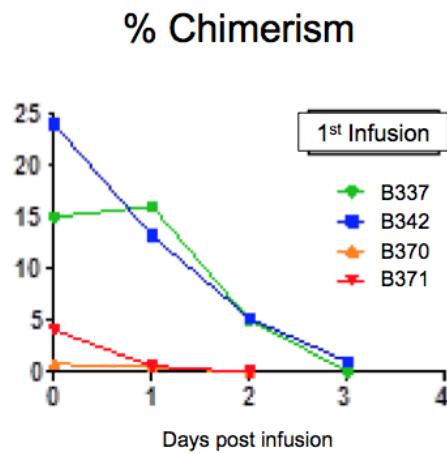
**Questions for Optional Video:**

1. Compare and contrast xenotransplantation and allotransplantation. Briefly describe the benefits and difficulties found in each form of organ transplantation.
  
  
  
  
  
  
  
  
  
  
2. Like the adaptive immune system, the innate immune system plays a role in organ rejection in primates.
  - a. Which innate immune cell(s) is/are involved in the rejection of an organ during xenotransplantation?
  
  
  
  
  
  
  - b. What induces the innate response in the recipient? Briefly explain.
  
  
  
  
  
  
  
  
  
  
3. Explain why reactivity to  $\alpha 1,3\text{Gal}$  antigens is a common barrier to xenotransplantation in humans.
  
  
  
  
  
  
  
  
  
  
4. Strategies for overcoming immune barriers to xenotransplantation include immunosuppression, genetic engineering, and establishment of tolerance.
  - a. Name a pro and con for each strategy.

- b. Adding and deleting genes in either the recipient or the donor can promote xenotransplantation. Give an example of each (addition or deletion of genes) and explain their mechanism.
5. In the thymokidney transplantation approach, where do the thymic cells come from? Explain how this approach can overcome obstacles to xenotransplantation.
6. Immune responses to foreign MHC are relatively stronger than immune responses to other types of antigens. Yet, knocking out the MHC genes in pigs would not be sufficient to prevent rejection of transplanted tissue. Briefly explain.
7. Researchers wanted to determine whether transgenic expression of human CD47 in donor tissues could increase hematopoietic xenotransplantation success. (Reference: Tena, Transplantation 2017)
- CD47 is a ubiquitously expressed cell surface protein in humans. To which cells does it send an inhibitory signal?
  - Why would researchers predict that exogenous expression of CD47 on donor tissue would reduce xenotransplant rejection?
  - The researchers created two different hCD47 transgenic pigs, #18286 and #18289. They evaluated the surface expression of hCD47 on leukocytes from each transgenic pig and a wild-type control using FACS.



- i. Based on the data above, which clone do you predict will be a better donor?
  - ii. Explain your choice.
- d. The researchers then transplanted hematopoietic stems cells (HSCs) from the transgenic pigs into recipient primates and measured the extent of chimerism between the donor and host immune systems. Two of the recipients received HSCs from pig #18286 and two received HSCs from pig #18289.



- i. Based on these data, do primate immune cells respond to hCD47?
- ii. Which transgenic pig, #18286 or #18289, do you predict was the donor for primates #B370 and #B371?

# Answers for Session 9:

## Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
  - a. Syngeneic transplantation has the lowest rate of rejection because the immune system of the host views the donor organ as self. **(True)**
  - b. Having similar HLA alleles correlates with poor transplantation rate. **(False - it correlates with high transplantation rate)**
  - c. Donor dendritic cells can present antigen to recipient T cells, which can trigger an immune response that leads to organ rejection. **(True)**
  - d. T cells are the key players in graft-versus-host disease. **(True)**
  - e. The term alloantigen describes antigens that vary between individuals of different species. **(False - alloantigens are polymorphic antigens between individuals of the same species)**
  - f. The T cell is a central player in most instances of organ & tissue rejection. **(True)**
  - g. The innate immune system is not involved in rejection of transplanted tissues. **(False - NK cells are often involved in hematopoietic stem cell transplant issues)**
  - h. Acute and hyperacute rejection differ largely in terms of when anti-transplant antibodies are synthesized relative to transplantation. **(True)**
  
2. Which of the following are factors that increase transplantation rejection? (Select all that apply)
  - a. **The donor and host have different blood types.**
  - b. The donor was recently pregnant.
  - c. **The recipient has antibodies against donor proteins.**
  - d. The donor had a bad viral infection to which the host is susceptible.
  - e. None of the above.
  
3. The human leukocyte antigen (HLA) genes encode for the major histocompatibility complex (MHC) proteins in humans, and these genes are highly polymorphic. Two unrelated individuals have low chances of having identical HLA alleles.
  - a. Why have we evolved to have such variability in the HLA locus? Briefly explain.  
**Variations in HLA can lead to variations in MHC ability to bind & present antigen. Different populations co-evolved with different pathogens and therefore evolved to have their own set of HLA alleles.**
  
  - b. For the process of organ transplantation, it is important to consider if the donor and recipient are an "HLA match"; meaning, the HLA alleles of the patient are identical to the HLA alleles of the donor. Briefly explain why this is important.  
**Because our immune system is "trained" to not react to self-antigens, human T cells (and B cells) can react to non-self HLA proteins. In other words, the recipient immune system can detect non-identical HLA as non-self and start killing the newly transplanted organ. By having a donor with identical HLA proteins, it decreases the chances of organ rejection.**



4. Cyclosporin A is an immunosuppressant drug that is used for patients who need to have kidney transplants.

a. Why do immunosuppressant drugs need to be given to patients undergoing organ transplantation? Briefly explain.

**Organ transplantation introduces proteins (and other compounds) that can be recognized as foreign (non-self), which will trigger an immune response that will attack the transplanted organ. The immunosuppressant drugs are given to suppress this response and increase the life of the transplanted organ.**

b. Why is organ rejection less likely if the organ is donated by a close relative (mother or brother) and completely eliminated if the organ were to be donated by an identical twin? Briefly explain.

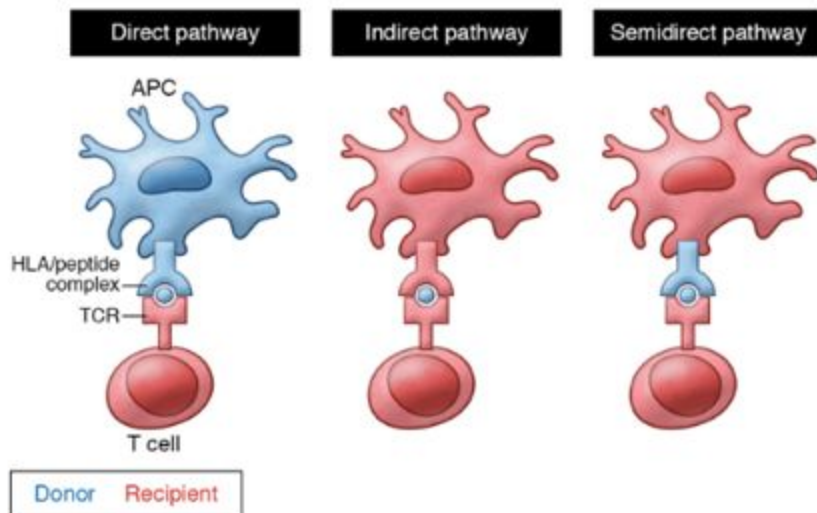
**Members from the same family have a higher chance of sharing the same MHC complex alleles (identical twins will have exactly the same MHC proteins) and therefore less chances of getting a donor that will cause a reaction by the immune system.**

c. Cyclosporin A is known to bind to proteins in T cells and inhibit interleukin-2 (a cytokine) release. How does this mode of action reduce the response of the immune system? Briefly explain.

**The release of cytokines is going to play a critical role in the proliferation and differentiation of cells within the immune system. If this drug reduces cytokine release, this should decrease the activation of the immune system. This will reduce the number of cytotoxic-T cells and B cells action in patients that use this drug.**

5. Shown below are three different mechanisms by which the immune system is able to distinguish its own tissue from those of another (allorecognition) in organ transplantation.

a. Add the appropriate name to each mechanism.



- b. What characterizes each mechanism? Briefly outline the cells involved.  
**Direct allorecognition – The host T cells (CD4 and CD8 positive cells) recognize donor peptide:MHC complex presented by donor dendritic cells.**

**Indirect-allorecognition- A recipient dendritic cell picks-up donor HLA protein and after processing it presents it as antigen to recipient T cells (CD4+).**

**Semi-direct allorecognition – Recipient dendritic cell picks up peptide:MHC complex from the donor and presents it to the recipient T cells (CD8 and CD4 positive cells).**

6. Compare and contrast the following sets of ideas or terms.
- The use of immunosuppressive drugs and induction of immune tolerance in preventing transplant rejections.  
**Immunosuppressive drugs modulate the effects of effector cells. Induction of immune tolerance modulates the maturation of effector cells. Immunosuppressive drugs prevent organ rejection by inhibition of immune system; Tolerance prevents rejection by training the immune system.**
  - Direct and indirect allorecognition  
**MHC molecules are either recognized directly (via donor DCs presenting to host T cells) or indirectly (via host DCs presenting donor-derived MHC antigens to host T cells)**
  - Major and minor histocompatibility antigens  
**Major histocompatibility antigens refer to polymorphisms in the HLA gene between individuals. These can induce a strong immune response. Minor histocompatibility antigens refer to proteins other than HLA/MHC that have polymorphisms between individuals and can lead to rejection.**
  - Chronic and acute rejection  
**Acute rejection occurs within days of transplantation and typically involves direct allorecognition. Chronic rejection can take place months to years after transplantation and involves indirect allorecognition.**
7. Explain why anti-HLA immune responses are stronger than other types of immune responses  
**Host T cells have been selected to weakly recognize self-MHC. However, during negative selection, T cells that recognize other MHC molecules strongly have not been weeded out. Therefore, they exist in the repertoire. Alternative answer: All cells in the tissue express MHC-1, which can lead to overall rejection if the immune system recognizes it as foreign.**

8. Fill in the missing fields in the following table to compare and contrast the major types of allorecognition.

	Indirect allorecognition	Direct allorecognition	Semi-direct allorecognition
Source of antigen-presenting cell	<b>Recipient</b>	<b>Donor</b>	<b>Recipient</b>
Source of MHC	<b>Donor</b>	<b>Donor</b>	<b>Donor</b>
Size of MHC: whole or fragment?	<b>Fragment</b>	<b>Whole</b>	<b>Whole</b>

9. Explain why direct allorecognition of MHC II is unable to be the major cause of chronic rejection. **Direct allorecognition relies on antigen presentation by resident cells in the donor tissue. MHC II-presenting APCs (such as dendritic cells) are replaced over time by the recipient APCs.**
10. Would a person who previously received an HLA-incompatible blood transfusion be more or less likely to accept an organ transplant with the same HLA allotype? Briefly explain. **They would be less likely. Their immune system would be primed to recognize and attack the donor tissue, leading to a hyperacute rejection (for example, the person will have developed antibodies against the donor tissue).**
11. Predict why T cell depletion prior to bone marrow transplantation can increase likelihood of rejection. **Regulatory T cells are likely necessary to maintain tolerance in the periphery.**

#### Questions for Second Video:

- Which of the following is (are) important factor(s) in the mixed chimerism organ transplantation protocol? (Select all that apply)
  - Dendritic cell depletion.
  - Blood injection of donor T-cells prior to organ transplantation.
  - Deletion of donor B cells.
  - Introduction of donor macrophages.
  - None of the above.**
- Jeremy is a candidate for a kidney transplant. For the following conditions, determine if you would consider enrolling Jeremy in a mixed chimerism trial. Briefly explain.
  - Jeremy doesn't have the same HLA allele as the donor. **Yes. Mixed chimerism should allow for the recipient to train its immune system to not reject the organ.**
  - Jeremy has antibodies against donor antigens prior to transplantation. **Yes. The mixed chimerism protocol accounts for this problem by using a drug that depletes the donor's B cells.**

- c. The donor organ has antigen presenting cells (APCs) that have the capacity to present antigen to Jeremy's T cells.  
**Yes. The increase in regulatory T cells (Tregs) that occurs during the mixed chimerism protocol should inhibit donor-reactive T cells.**
3. Amy, one of the first patients to receive mixed chimerism treatment for kidney transplantation, received a kidney from her father. Prior to being enrolled in this clinical trial, she received a kidney from her mom using the conventional method and rejected the organ.
  - a. Define the term "mixed chimerism".  
**Mixed chimerism is the coexistence of donor and recipient hematopoietic elements: the donor elements aren't eliminated by the host immune system, and the recipient elements aren't eliminated by the treatment.**
  - b. What advantages over traditional transplantation methods does mixed chimerism provide that convinced Amy to enroll in this clinical trial? Briefly explain.  
**If successful, the mixed chimerism treatment is a "cure", as it provides the patient with a new organ without the detrimental side effects of using immunosuppressant drugs for the rest of their lives. The patient also has an intact immune system that is capable of fighting diseases.**
  - c. What factors did the doctors look at to determine that Amy's mom and then her dad were a "good match" for each type of transplant? Briefly explain.  
**For the first transplantation, they had to have the same blood type and similar HLA alleles to prevent chronic organ rejection. For the second transplantation, this is not the case (HLA and blood type do not need to match).**
4. The protocol used to induce mixed chimerism in humans has gone through a few iterations.
  - a. What is the importance of each of the following strategies for the mixed chimerism protocol? Be sure to outline the target cell(s).
    - i. Local irradiation to the thymus:  
**Depletion of recipient T cells.**
    - ii. Treatment with rituximab, a B cell depleting agent:  
**Prevention of antibody-mediated rejection.**
  - b. Why were patients treated with immunosuppressant drugs for a short period of time after organ transplantation?  
**Patients were treated with immunosuppressant drugs for a short period after the organ transplantation to prevent rejection.**
  - c. What marker found in the patients confirmed that mixed chimerism was achieved?  
**Co-existence of donor and recipient lymphoid elements.**

- d. Why were the researchers excited to observe an increase in regulatory T cells (Tregs) post organ transplantation? Briefly explain.  
**Tregs are responsible for the elimination of peripheral self-reactive T cells. An increase in Treg is a sign that donor-reactive T cells would be eliminated or inhibited.**
- e. What was found at the molecular level when researchers compared T cells from a patient that rejected the organ versus patients that did not?  
**They found that patients that rejected the organ after mixed chimerism treatment had a greater number of donor-reactive T cell clones.**
5. Shown below are the results of a study in which two patients received a combined kidney and bone marrow transplantation to achieve mixed chimerism. Researchers measured the numbers of donor-reactive T cell clones in each recipient before and after the treatment. Can you predict the outcome of the organ transplantation (rejection versus non-rejection) for each patient? Briefly explain.  
**Yes. It is likely that the transplantation for Subject 1 is successful (no rejection-mixed chimerism achieved) as the donor-reactive CD4+ T cells decrease with time. Subject 2 is more likely to reject the organ as it has a stable number of donor-reactive CD4+ cells following transplantation.**
6. Why would myeloablation prior to bone marrow transplantation reduce the odds of immune tolerance?  
**The host and donor immune systems would not be exposed to one another. This is a necessary step for building tolerance by the host.**
7. There are three classes of drug used to suppress acute transplant rejection: corticosteroids, cytotoxic drugs, and T-cell activation inhibitors.
- What aspects of immunity do you predict each acts on? Be as specific as possible.  
**Corticosteroids: reduce inflammation**  
**Cytotoxic drugs: inhibit clonal expansion of activated lymphocytes**  
**T-cell activation inhibitors: inhibit T cell activation**
  - What do you predict are the side effects of each class?  
**Corticosteroids: Innate immune suppression**  
**Cytotoxic drugs: Adaptive immune suppression; off target effects to GI system & hair follicles**  
**T-cell activation inhibitors: Suppression of T-cell mediated immunity**
8. Researchers evaluated a novel strategy to induce transplant tolerance in humans by simultaneous transplantation of HLA-mismatched kidney and bone marrow. (Reference: Kawai, Am J Transplantation, 2014)
- A summary of each of the patients in the study and their response to treatment are below.
    - Do you consider this strategy successful? Why or why not?  
**(Open to interpretation) At first pass, this strategy may be interpreted as successful (¾ recipients had 5+ years of graft survival without the need for immunosuppression).**

- ii. What information would you need to strengthen your stance?  
**Lots of possible answers: greater sample size; randomized controlled comparison to standard of care; increased length of follow-up studies.**
- b. The researchers sought to understand why some patients responded favorably to the transplant and others didn't. They performed a mixed-lymphocyte reaction (MLR) experiment, which measures anti-donor responses by CD4+/CD8+ effector T cells. The results of the MLR study are summarized in the table below.
  - i. What do these data indicate about the role of effector T cells in transplant acceptance or rejection?  
**It suggests that CD4/CD8 T cells are not involved in transplant rejection, as donor-specific non-responsiveness was detected in Patient 10 one year following the transplant.**
  - ii. Name at least one limitation in the data above, as it relates to our understanding of transplant rejection.  
**MLRs were only performed over a 12 month period for Patient 10, but this patient's transplant lasted 3 years. It is possible that anti-donor T cell reactivity was established between years 1 and 3. This represents a gap in the data.**
- c. Finally, the researchers sought to evaluate the role of B cells in transplant acceptance or rejection. They measured absolute counts of mature B cells (left panel) as well as B cell activity (right panel) in each of the patients over several months (Ignore data for Subject 8 in your analysis).
  - i. Do absolute numbers of B cells appear to correspond with transplant acceptance or rejection? Briefly explain.  
**No - B cell counts were low in both patients 9 and 10 over the period, and these patients had opposite responses to the transplantation.**
  - ii. Does B cell activity appear to correspond with transplant acceptance or rejection? Briefly explain.  
**Possibly. Patient 10 had the highest B cell activity over the period, and was the only patient out of the cohort that ultimately rejected the transplant. But statistics are not shown, and there is a gap in data between the first year and the third year, when patient 10's transplant was lost.**
  - iii. In a separate experiment, no donor-specific antibody (DSA) was detected in any of the four patients for three years following transplantation. What does this indicate about the role of B cells in transplant acceptance or rejection?  
**This information suggests that humoral immunity (antibody-mediated immunity) did not play a role in patient #10's rejection.**

- iv. How would you test your hypothesis in a mouse model of this transplantation strategy?

**You could devise a study in which you perform analogous transplantations (simultaneous transplantation of HLA-mismatched kidney and bone marrow) in mice depleted of B cells. If B cells are not necessary for transplant rejection, then you should expect to see no difference in the rejection rate between mice that do and don't have B cells.**

- v. Do you predict that B or T cells are more likely to be involved in transplant rejection? Explain your prediction.

**T cells are more likely than B cells to be involved in transplant rejection. Since donor-specific antibodies were not detected in any patient for three years following transplantation, this strongly suggests that humoral immunity did not play a role in Patient 10's transplant rejection. However, there are no data available to definitively rule out the role of T cell-mediated rejection between years 1 and 3.**

9. Modulation of effector T cell function is one strategy for inducing transplant tolerance. Researchers evaluated the effects of two different drugs on Treg populations in pediatric liver and kidney transplant recipients. (Reference: Akimova, Am J Transplantation, 2012)

- a. Why would modulation of Treg abundance or function in transplant recipients be a viable treatment strategy?

**Tregs help to induce and maintain peripheral tolerance.**

- b. Based on the data below, which drug (Tacrolimus or Rapamycin) appears to be promising for promoting organ transplant tolerance? Briefly explain. (PBMC = peripheral blood mononuclear cell)

**Rapamycin is a more promising drug, as it increases levels of Tregs in the peripheral blood.**

- c. Based on the data below, which surface protein would serve as a better biomarker for a clinical study of the drug you chose above? Briefly explain.

**[Note: Either could be used as a biomarker; this question is open-ended] Some students may argue that CTLA would serve as a better biomarker, as it is detected at higher levels on Tregs that have greater suppressive function overall. Some students may argue that CD127 is a better biomarker, as high CD127 expression is inversely correlated with suppressive function of Tregs. Also, the difference in Treg function between CD127 high vs CD127 low cells is more significant than the difference between CTLA high and CTLA low cells.**

### Questions for Optional Video:

1. Compare and contrast xenotransplantation and allotransplantation. Briefly describe the benefits and difficulties found in each form of organ transplantation.
  - **Allotransplantation uses organs from the same species, while in xenotransplantation the donor and recipient belong to different species.**
  - **Because different species have different repertoires of proteins, recipients have more donor-reactive T cells in xenotransplantation than in allotransplantation. In allotransplantation the main factor considered for rejection is the HLA gene.**
  - **There are more organs available for xenotransplantation.**
  - **Xenotransplantation has more religious concerns.**
  - **Xenotransplantation has higher genetic modification potential.**
  - **NK cells are a big component in transplant rejection in xenotransplantation, but are not as important for allotransplantation rejection.**
  
2. Like the adaptive immune system, the innate immune system plays a role in organ rejection in primates.
  - a. Which innate immune cell(s) is/are involved in the rejection of an organ during xenotransplantation?  
**Macrophages and NK cells.**
  
  - b. What induces the innate response in the recipient? Briefly explain.  
**Primate cells express a membrane protein (CD47) that binds a receptor on macrophages (SIRP $\alpha$ ) to inhibit them from acting on self-cells. When non-primate cells are transplanted into primates, macrophages will be activated against them because these cells lack CD47.**
  
3. Explain why reactivity to  $\alpha$ 1,3Gal antigens is a common barrier to xenotransplantation in humans.  
**Humans lack the enzyme that makes these modifications on proteins and lipids, therefore we have pre-existing antibodies against them. Most non-primate mammals have the enzyme to produce these modifications, therefore tissues from these animals would have the antigen.**
  
4. Strategies for overcoming immune barriers to xenotransplantation include immunosuppression, genetic engineering, and establishment of tolerance.
  - a. Name a pro and con for each strategy.  
**Immunosuppression - PROS: can be induced or modulated depending on need; can be combined with genetic engineering. CONS: chronic use can result in increased susceptibility to infection and disease**  
  
**Genetic engineering - PROS: wouldn't require daily interventions; can be very precise; can be combined with immunosuppression if necessary; multiple targets can be added or deleted simultaneously. CONS: can't be modulated (typically an all or nothing approach)**  
  
**Establishment of tolerance - PROS: Host immunity wouldn't be impaired. CONS: many obstacles to tolerance because of B-cell, T-cell, NK-cell, and macrophage-mediated rejections**



- b. Adding and deleting genes in either the recipient or the donor can promote xenotransplantation. Give an example of each (addition or deletion of genes) and explain their mechanism.

**Deleting the Gal-T gene in donor animal could overcome  $\alpha$ 1,3Gal antibody-mediated rejection. Adding CD47 in donor animal could overcome macrophage-mediated rejection.**

5. In the thymokidney transplantation approach, where do the thymic cells come from? Explain how this approach can overcome obstacles to xenotransplantation.

**They come from the same donor as the kidney they are combined with. Simultaneous transplantation of donor kidney tissue and T cell precursors is thought to provide a level of immune chimerism that promotes xenotransplantation.**

6. Immune responses to foreign MHC are relatively stronger than immune responses to other types of antigens. Yet, knocking out the MHC genes in pigs would not be sufficient to prevent rejection of transplanted tissue. Briefly explain.

**There would be other pig-specific antigens that could be recognized by the recipient's immune system (such as  $\alpha$ 1,3Gal). Also macrophages could still attack the tissue because it lacks CD47.**

7. Researchers wanted to determine whether transgenic expression of human CD47 in donor tissues could increase hematopoietic xenotransplantation success. (Reference: Tena, Transplantation 2017)

- a. CD47 is a ubiquitously expressed cell surface protein in humans. To which cells does it send an inhibitory signal?

**Macrophages**

- b. Why would researchers predict that exogenous expression of CD47 on donor tissue would reduce xenotransplant rejection?

**Macrophages are known to engulf foreign cells. Cells that express CD47 are seen as "self" and are spared.**

- c. The researchers created two different hCD47 transgenic pigs, #18286 and #18289. They evaluated the surface expression of hCD47 on leukocytes from each transgenic pig and a wild-type control using FACS.

- i. Based on the data above, which clone do you predict will be a better donor?

**18286**

- ii. Explain your choice.

**Clone 18286 expresses hCD47 at much higher levels than the other clone or the control. Its cells will be more effective at sending stop signals to recipient macrophages.**

d. The researchers then transplanted hematopoietic stems cells (HSCs) from the transgenic pigs into recipient primates and measured the extent of chimerism between the donor and host immune systems. Two of the recipients received HSCs from pig #18286 and two received HSCs from pig #18289.

i. Based on these data, do primate immune cells respond to hCD47?  
**Yes.**

ii. Which transgenic pig, #18286 or #18289, do you predict was the donor for primates #B370 and #B371?  
**18289**