

Xenografts — Alternative Transplants

Secrets of the Sequence Video Series on the Life Sciences • Grades 9 — 12

Teaching materials developed by VCU Life Sciences

V i r g i n i a C o m m o n w e a l t h U n i v e r s i t y

Classroom Tested Lesson

Video Description

“Secrets of the Sequence,” Show 113, Episode 2

“Xenografts – Alternative Transplants” approximately 8 minutes viewing time

How realistic is the idea that pigs may eventually make up the void in organs needed for transplants in humans? Will genetically modifying the pig by inducing tolerance in patients be enough to make the grafts possible, or will such challenges prove to be insurmountable?

Ward Television

Producer: Paul Gasek

Associate Producer: Julie James

Featuring: Dr. David Sachs, Massachusetts General Hospital, Julie Greenstein, Ph.D., President and CEO of Immerge Biotherapeutics, Inc

Lesson Author; Reviewers: Catherine Dahl; Dick Rezba, Selvi Sriranganathan

Trial Testing Teachers: Shelley Mitchell, Charlotte Smith

National and State Science Standards of Learning

National Science Education Standards Connection

Content Standard A: Science as Inquiry

As a result of their activities in grades 9-12, all students should develop

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard F: Science in Personal and Social Perspectives.

As a result of their activities in grades 9-12, all students should develop understanding of

- Personal and community health
- Natural and human-induced hazards

Selected State Science Standards Connections

Use <http://www.eduhound.com> (click on “Standards by State”) or a search engine to access additional state science standards.

Virginia

- BIO.1 The student will plan and conduct investigations in which
- observations of living organisms are recorded in the lab and in the field
 - hypotheses are formulated based on direct observations and information from scientific literature
 - a scientific viewpoint is constructed and defended (the nature of science)
- BIO.5 The student will investigate and understand life functions of Archaeobacteria, Eubacteria, protists, fungi, plants, and animals including humans. Key concepts include
- how their structure and functions vary between and within the kingdoms
comparison of their metabolic activities
 - human health issues, human anatomy, body systems, and life functions
- BIO.6 The student will investigate and understand common mechanisms of inheritance and protein synthesis. Key concepts include:
- exploration of the impact of DNA technologies.

Indiana

Principles of Biology

Students work with the concepts, principles, and theories that enable them to understand the living environment. They recognize that living organisms are made of cells or cell products that consist of the same components as all other matter, involve the same kinds of transformations of energy, and move using the same kinds of basic forces. Students investigate, through laboratories and fieldwork, how living things function and how they interact with one another and their environment.

Developmental and Organismal Biology

B.1.15

Understand and explain that, in biological systems, structure and function must be considered together.

B.1.18

Explain that the regulatory and behavioral responses of an organism to external stimuli occur in order to maintain both short and long-term equilibrium.

B.1.20

Recognize that and describe how the human immune system is designed to protect against microscopic organisms and foreign substances that enter from outside the body and against some cancer cells that arise within.

Genetics

B.1.23

Understand that and describe how inserting, deleting or substituting DNA segments can alter a gene. Recognize that an altered gene may be passed on to every cell that develops from it, and that the resulting features may help, harm, or have little or no effect on the offspring's success in its environment.

Evolution

B.1.35

Explain that the degree of kinship between organisms or species can be estimated from the similarity of their DNA sequences, which often closely matches their classification based on anatomical similarities. Know that amino acid similarities also provide clues to this kinship.

Overview

Approximately 80,000 people are currently on waiting lists for donor organs and 16 people are dying every day because they cannot survive the wait. Researchers are therefore looking for new sources of replacement organs. They believe they have found it in miniature pigs, which have a surprisingly similar physiology to humans. Even with human to human organ transplants (*allografts*) there is concern about the recipient immune system rejecting the new tissue because there is not an exact DNA match. Obviously this concern about rejection is even stronger when a pig organ is used (a *xenograft* – a transplant between species). To minimize one of the challenges associated with transplants, doctors try to match the size of the donor and recipient so the organs will be of similar size. This necessitates the use of miniature pigs that top out at around 300 lbs. as opposed to normal pigs that weigh up to 700 lbs and produce organs too large for humans. Another challenge associated with xenografts is that animals carry different viruses than humans and it could prove dangerous to introduce those viruses into the human body. One such virus has been identified as “PERV” (*porcine endogenous retro virus*) and scientists are working to ensure that the risks associated with this virus do not outweigh the benefits of increasing the organ supply.

Testing: A sample related multiple choice item from State Standardized Exams

The significance of the major histocompatibility complex (MHC) in the immune response:

- A. Serves to minimize autoimmunity or “self-reactivity” of the immune system
- B. Serves to present fragments of antigens to T cells
- C. Used by helper T cells to regulate the expansion of antibody producing B cells
- D. All of the above *

Source: The Biology Project (<http://www.biology.arizona.edu/immunology/tutorials/immunology/main.html>)

Video Preparation

Preview the video and make note of the locations at which you will later pause the video for discussion.

Before Viewing

1. Ask: “What is the main reason why researchers want to find ways to safely use animals as donors for organ transplants to humans?”
There are people dying every day because there are not enough human donor organs available. Many people are unwilling to become organ donors due to some of the following misconceptions/qualms about organ donation:
 - *it's too morbid to discuss with family members*
 - *doctors let donors die to collect organs*
 - *donors can't have open-casket funerals*
 - *it costs money to donate*
 - *family does not know wishes of the deceased*

Note: You may want to mention the case of “Nicholas”, an American boy tourist who was accidentally shot and died in Italy a few years ago. Most Italians were shocked when his parents donated all his organs. However, follow-up articles on the lives that were subsequently saved because of the parent's gift resulted in sharply increased Italian donor registrations.

Courtesy of Virginia Commonwealth University • Richmond, Virginia • www.vcu.edu/lifesci
Made possible through the generous support of the National Academy of Sciences & the Pfizer Foundation
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2. Review human leukocyte antigens (HLAs), often called cell marker proteins, and their function as "ID" tags for cells.
 - *The proteins encoded by HLA genes are the cell-membrane [proteins](#) that are (effectively) unique to that person*
 - *HLA types are inherited*
 - *The [immune system](#) uses the HLAs to differentiate between self cells and non-self cells*
 - *Any cell displaying that person's HLA type belongs to that person*
 - *Any cell displaying some other HLA type is "not-self" but is an invader.*

During Viewing

1. **START** the video
2. **PAUSE** the video (1.46 minutes into the video) after the computer voice says, "...it likes its own kind."

Review and write the definitions of the following words on the board:

Xenograft – tissue transplant between species (from xenos meaning stranger/guest)

Allograft –transplant between genetically different members of the same species

Autograft –graft from one part of a human to another part of the same human

3. **PAUSE** the video (4:12 minutes into the video) after Dr. Sachs describes the two ways in which researchers are working towards a genetic middle-ground between pigs and humans.

Confirm that students understand the two methods by which researchers are combating the major barrier to xenotransplantation - rejection.

- a. *Modify the donor (pig) through genetic engineering in order to change the pigs' antigens to produce less of an immune response in the recipient.*
- b. *Modify the recipient (human) to create increased tolerance to pig organs.*

Note: A trial test teacher commented that her students asked about the possibility of genetically engineering pigs to match specific recipients' cell markers to further reduce rejection. The answer is a bit advanced for the high school level and beyond the scope of this lesson but the topic is certainly of interest if time allows. The treatment would be as follows:

- Isolate recipient DNA and clone his or her specific HLA genes into a vector
- Ideally this vector would simultaneously insert the recipient's HLA genes while deleting the donor's
- Expose a pig zygote to this targeted vector
- Assume the HLA genes stably integrate into an active region of the donor's DNA and are appropriately regulated
- Assume the genetically modified pig zygote can be implanted into a surrogate sow and is born alive and well
- Recipient would still have to wait for the pig to mature before the organ could be harvested. (most patients in need of an organ transplant can't wait years for a pig to grow up so alternative is to have a pig donor created years in advance and harvest organs as needed)
- Leads to a discussion on ethical considerations of human benefit versus animal rights.

4. RESUME the video and play to the end.

After Viewing

1. Review the most important similarities between pigs and man. Ensure that the students understand why miniature swine are used as organ donors to humans, as opposed to regular pigs.
 - Heart rate
 - Blood Pressure
 - Serum Chemistry

Note: As with human donors, it is important to match the weight of the donor to the recipient in order to ensure that the sizes of the organs are similar. Normal adult swine can weigh over 1,000 lbs. and their organs are too large to be used in humans. Miniature swine are better matched to humans in that their maximum weight is approximately 300 lbs.

2. The “key” safety risk in xenotransplantation is infecting a human with a virus hidden in pig DNA. One such virus has been identified as PERV – Porcine Endogenous Retrovirus. Use a search engine, such as Google or Yahoo, to update yourself on the status of research about this virus and what scientists are doing to counter the risk. See “Additional Resources,” attached.
3. You may wish to expand upon the video’s mention of thymus cells and review the role of the thymus in recognizing invasive cells and how the immune system responds to these cells. In addition, though it is not mentioned in the video, you may want to discuss “chimerism” (a chimera is an organism containing tissues from two or more organisms) involving bone marrow cells that is discussed in the Student Handout: Background Information on Xenotransplant Rejection.
4. This video and lesson explores the use of xenografts to replace diseased tissue and organs in humans. A number of other videos in the *Secrets of the Sequence Series* examine alternative methods that also hold promise in meeting the growing demand for replacement organs. One in particular is the “The Cloning Conflict – Parkinson’s Disease”. View that video and compare and contrast the featured method of replacing tissues with that outlined in “Xenografts”.
5. Conduct the Student Activity: Xenotransplantation Rejection.

Note: A trial test teacher commented that not all biology classes will cover the immune system in such detail and therefore a good alternative to the Student Activity would be to have the students:

- Explain what it means to “genetically engineer” an organism.
- Use E. coli as an example and research how insulin can be produced (see <http://www.stanforddaily.com/tempo?page=content&id=279&repository=0001> article)
- Research another product such as golden rice or Roundup-resistant soybeans to discover how other “GM” discoveries benefit humans.

OR

Conduct an Internet activity on blood-typing that emphasizes immunity reaction.

See <http://gslc.genetics.utah.edu/teachers/tindex/overview.cfm?id=96>

Teacher Notes for the Student Activity: Xenotransplantation Rejection

In this activity students are introduced to the series of events that occur during the rejection of a xenograft, such as when an organ from a baboon or pig is transplanted into a human. Students are given the task of assigning titles and ordering a set of slides for a presentation on xenotransplant rejection. If desired, one group of students can be selected to give the presentation.

Objectives

- Understand the nature of species-specific antigens.
- Recognize the difference between *hyperacute rejection* through the complement system and *acute rejection* through cell-mediated immunity.
- Place the events involved in hyperacute rejection of a xenotransplanted organ in the proper order.
- Understand the impact of different xenograft anti-rejection strategies

Materials

- Student Handout 1: Background Information on Xenograft Rejection
- Student Handout 2: Xenograft Rejection - Directions and Captions
- Student Handout 3: Slide Diagrams **in color** (Black and white copies of the slides on Student Handout 3 do not show the details well enough for this activity.)
- Presentation Answer Key
- Scissors (if student groups cut their own captions and slides)

Preparation

Make sufficient copies of Handout 1 for each student and one copy of Handout 2 and one *reusable color copy* of Handout 3 for *each group*. To save time you may wish to pre-cut the 'captions' and 'slides' on Handouts 2 and 3 for each group with a paper cutter. Arrange to save the color copies of the slides to re-use in other classes. You may also wish to number the slides on the back and put letters on the backs of the slides.

Procedure

1. Provide students with Handout 1: *Background Information on Xenotransplant Rejection*
2. After students have read the handout, provide students time to ask questions to ensure they understand the material.
3. Review the meaning of "species-specific antigens" and discuss the significance of the genetic similarity of donor and recipient – the closer two organisms are genetically, the more likely a transplant will be successful. Also emphasize the role of the complement system in hyperacute rejection and the cytotoxic T cells in the acute response so students will accurately determine the order of events in the activity.
4. Divide students into groups and provide them with scissors and one copy of Handout 2: *Xenograft Rejection – Directions and Captions* and one color copy of Handout 3: Slide Diagrams (or a set of pre-cut slides and captions).
5. As a group, have students pair the captions with the slides and then put them in an order that makes sense for a presentation about xenotransplant rejection¹. It may be possible to create an order other than the one provided in the 'answer key', but a major deviation would likely result in a poorly organized talk.
6. If desired, select one group to present a short talk to the rest of the class using their ordering scheme.

¹Note: If students are confused, give them a hint with the first slide and caption. Also explain that there are two pictures of a diseased/dying liver but one is the result of hyperacute reaction and the other is the result of acute reaction.

Student Handout 1: Background Information on Xenotransplant Rejection

Introduction

The human immune system is our own “personal bodyguard.” Like any good defender, it must recognize “the enemy”— that wide range of viruses, bacteria, fungi, protozoa and other potential pathogens that we encounter every day. Our immune system is genetically programmed to recognize certain proteins on our cells (self) from the thousands of invading pathogens (nonself) trying to gain a foothold in our body. But how is the immune system able to differentiate between friend and foe?

Recognition of the Body’s Own Cells

Cells possess unique antigenic proteins on their membranes that are like fingerprints; no two people (except identical twins) have the same protein structures in their membranes. Our immune cells see these proteins as normal or “self”, but if our cells are transplanted into another person, they cause an immune reaction. T and B cells mount an attack in an attempt to rid the body of these foreign proteins.

The Recognition Problem in Xenotransplants

In the case of xenotransplants (transplanting organs across species), the genetic separation between donor and recipient is even greater. Membrane-bound proteins are less similar, and the rejection of the organ is stronger, faster, and more complex. In particular, xenotransplants trigger a response called *hyperacute rejection*, which can destroy a transplanted organ within just a few hours.

Hyperacute rejection

All mammals have a set of species-specific antigens on the surfaces of cells lining blood vessels. When antibodies circulating in human blood see antigens from another species, they quickly bind onto them and activate a part of the immune system known as the *complement system*. The complement system is an array of proteins that circulates in the blood that, when activated, bind together to form “membrane-attack complexes” that can poke large holes in cell membranes. The membrane-attack complexes then destroy the blood vessels supplying the organ with nutrients. Once hyperacute rejection begins, the organ usually doesn’t survive for more than a few hours.

Acute rejection

Even if hyperacute rejection is blocked, rejection continues at another level. The cellular or cell-mediated response involving T-cells comes into play. T-cells bind to foreign (“non-self”) antigens on xenograft cells and produce clones of cytotoxic (killer) T-cells. These killer cells, along with macrophages, attack the transplanted organ cell by cell, and can cause the transplanted organ to fail after weeks or months.

Blocking Rejection

Scientists are exploring several strategies to short-circuit the rejection mechanisms described above.

- They are genetically engineering pigs so that they don’t express the species-specific antigens that activate complement and hyperacute rejection.
- They give immunosuppressive drugs, medications to suppress the immune system, which do a fairly good job when used in human-to-human transplants. In xenotransplantation, these treatments must be used in such high doses that the transplant recipient is left open to infection.
- They induce bone marrow chimerism (a chimera is an organism containing tissues from two or more organisms). This involves replacing part of the recipient’s bone marrow with bone marrow from the organ donor. Immune cells from this new bone marrow will not recognize the xenograft as foreign.

None of these strategies is perfect, but scientists continue to seek ways to improve each of them. Xenotransplantation, once deemed impossible, is now viewed as a viable option in responding to the growing number of people who die while waiting for a compatible human organ transplant.

Student Handout 2: Xenograft Rejection – Directions and Captions

Your Challenge

Dr. Hanna Slip was on her way to your classroom to give a presentation on xenotransplant rejection and how scientists hope to avoid it. Unfortunately, a crisis intervened, and she had to cancel (flat tire, space aliens, whatever—you decide). Fortunately, she sent her presentation slides ahead. However, you dropped the box and now the slides are all out of order. Your job is to put Dr. Slip's slides back in the right order and give the presentation in her absence using the following captions and slides. Use Student Handout 1: Background Information on Xenotransplant Rejection as a guide.

Procedure

A. Using scissors, cut out the individual slide captions and slides below.

1. Match the 12 captions below with the correct slides found on Student Handout 3: Slide Diagrams (*in color*). On your desk arrange the 12 slides with captions in an order that could be used in a presentation about xenotransplant rejection.
2. Give the presentation if selected by your teacher.

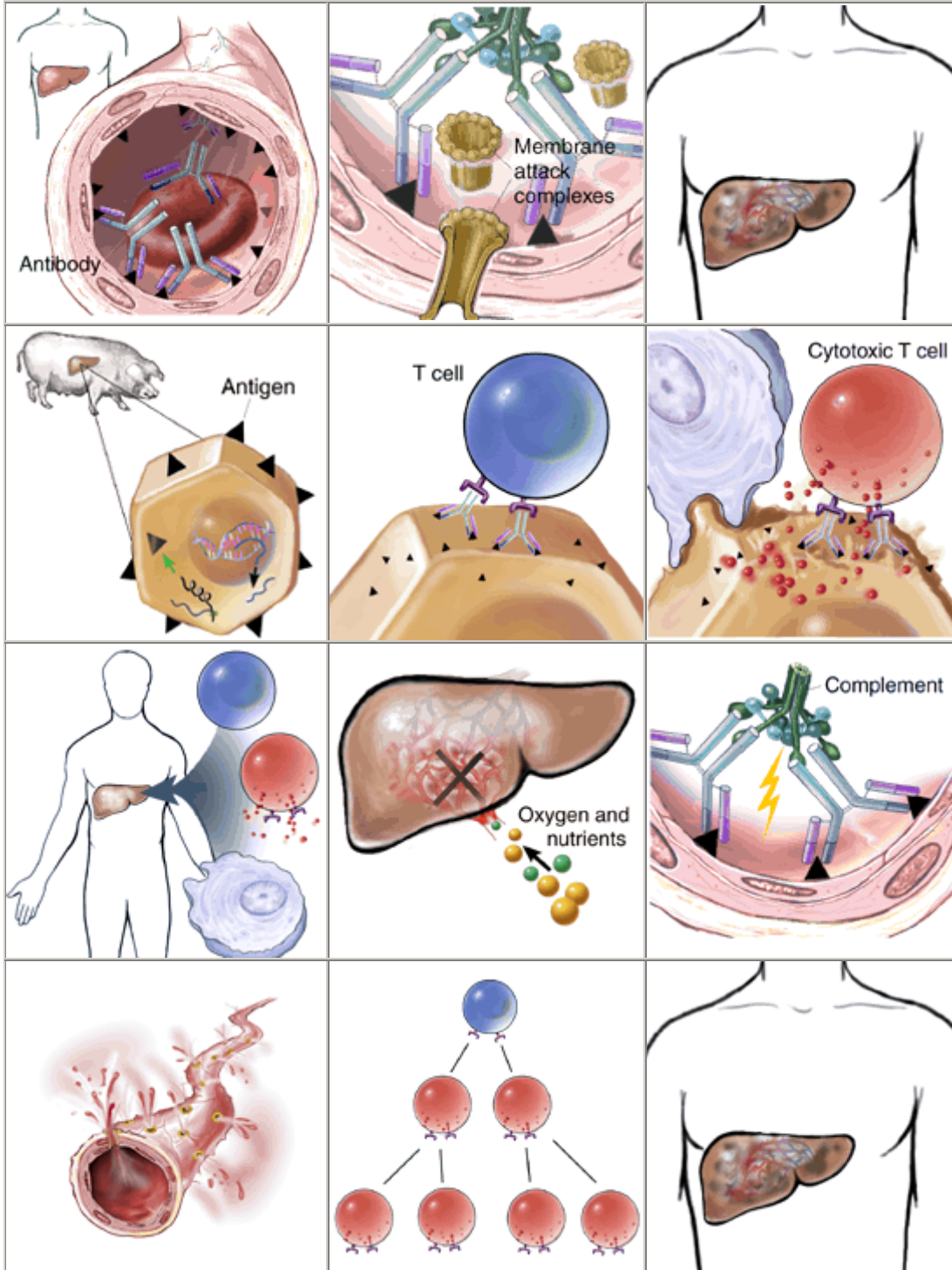
B. Use your sequence of slides to discuss where in the rejection sequence the various interventions would reduce the risk of xenotransplant rejection. Those interventions are genetic engineering of donor animals, immune-suppressing drugs, and induced bone marrow chimerism.

I. Captions

Match these captions with the appropriate slide diagrams that follow:

If organ somehow survives hyperacute rejection, cell-mediated rejection mechanisms still occur	Complement activation generates membrane attack complexes (protein complexes that poke holes in cell membranes)	Transport of nutrients and oxygen to the xenograft cut off
Antibodies in transplant recipient's blood bind to antigens on the cells lining the transplanted organ's capillaries.	Membrane attack complexes lyse (burst open) cells lining the capillaries of blood vessels in xenograft	T cells bind to foreign antigens on xenograft cells
Complement proteins attach to the bound antibodies, and activate	Activated T cells form clones of cytotoxic ("killer") T-cells	Organ dies due to lack of nutrients
Organ is damaged or dies due to cell-mediated immune attack	Pig cells display species-specific antigens on their surfaces	Cytotoxic T cells and macrophages attack xenograft cells

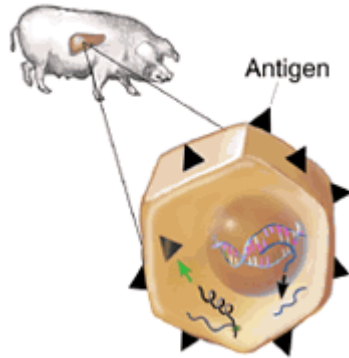
Student Handout 3: Slide Diagrams *in color* Match these slides with the appropriate caption.



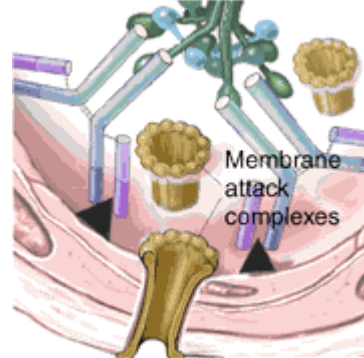
Source: **The Xenotransplant-Rejection Cascade**

Prepared by Fred Sculco, Morrison Chair in Science, Noble and Greenough School, Dedham, Mass.
http://science-education.nih.gov/newsnapshots/TOC_Xeno/index/Science_Activity_2/science_activity_2.html

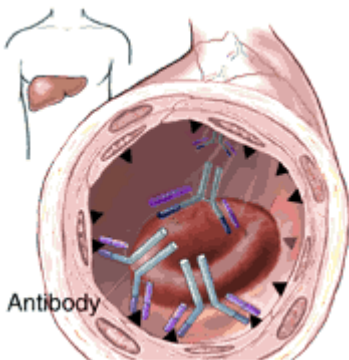
ANSWER KEY: CORRECT ORDER OF XENOGRAFT-REJECTION LECTURE



A. Pig cells display species-specific antigens on their surfaces.



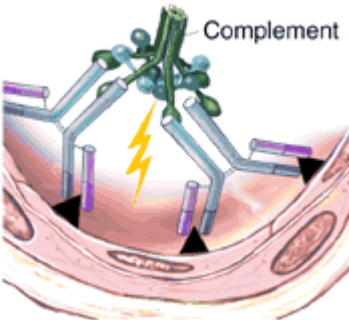
D. Complement activation generates membrane-attack complexes (protein complexes that poke holes in cell membranes).



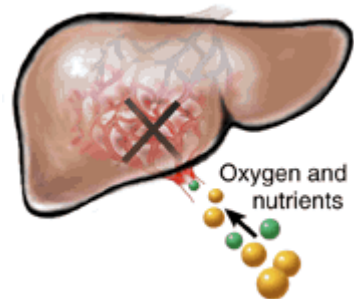
B. Antibodies in transplant recipient's blood bind to antigens on the cells lining the transplanted organ's capillaries.



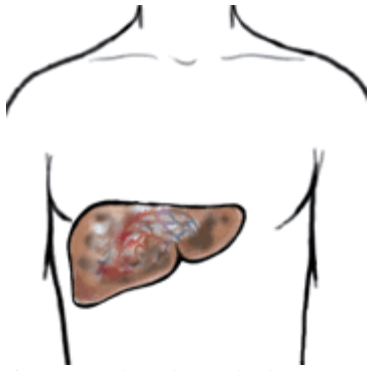
E. Membrane-attack complexes lyse (burst open) cells lining the capillaries of blood vessels in the xenograft.



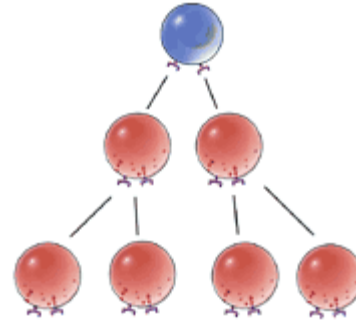
C. Complement proteins attach to the bound antibodies and activate.



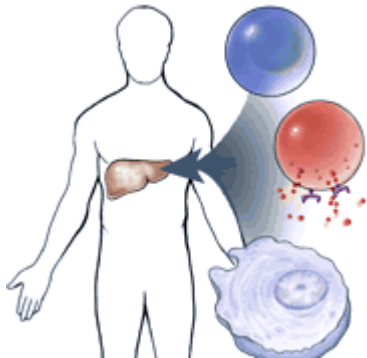
F. Destruction of blood vessels cuts off transport of nutrients and oxygen to the xenograft.



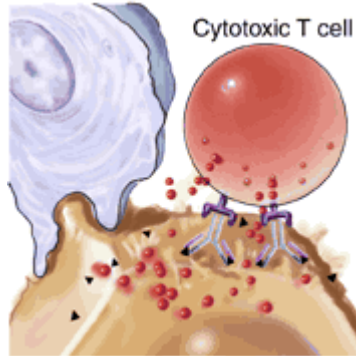
G. Organ dies due to lack of nutrients.



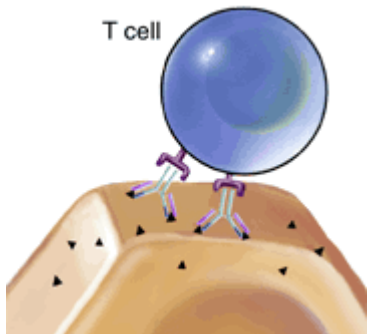
J. Activated T cells form clones of cytotoxic ("killer") T-cells.



H. If organ somehow survives hyperacute rejection, cell-mediated rejection mechanisms still occur.



K. Cytotoxic T cells and macrophages attack xenograft cells.



I. T cells bind to foreign antigens on xenograft cells.



L. Organ is damaged or dies due to cell-mediated immune attack.

Additional Resources

Because Web sites frequently change, some of these resources may no longer be available. Use a search engine and related key words to locate new Web sites.

Xenotransplantation.

http://science-education.nih.gov/newsnapshots/TOC_Xeno/toc_xeno.html

<http://www.transweb.org/qa/askarchive/answers9601/xenograft.html>

http://www.transweb.org/qa/qa_txp/faq_xeno.html

<http://www.animalliberationfront.com/Philosophy/Animal%20Testing/Xenografts%20and%20Animal%20Rights.html>

<http://www.lhsc.on.ca/transplant/xenograft.html>

Genetic Engineering Benefits

http://www.stanforddaily.com/tempo?page=content&id=279&repository=0001_article (insulin)

http://www.biotech-info.net/GR_tale.html (golden rice)

http://ipcm.wisc.edu/uw_weeds/extension/articles/suycontgly.htm (soybeans)

Genomic Revolution

http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml

This Web site of the government-funded Human Genome Project has links about genomics, the history of the project, and more.

Secrets of the Sequence Videos and Lessons

This video and 49 others with their accompanying lessons are available *at no charge* from www.vcu.edu/lifesci/sosq