A Diverse Representation of the Immune Processes in Autoimmunity

Bowie High School

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I. Science Background

Content Knowledge

When the action of the immune system is in response to self-antigens and causes damage to one's organs, the result is an autoimmune disease[1]. The concept associated with the project allows students to be exposed to different aspects of autoimmunity, such as clinical (can be in the form of photographs or video of disease phenotype) and histological analysis of disease development, biomarkers for diagnosis, and key cells involved in inflammation. An autoimmune disease results when the immune system mistakenly attacks the body's own tissues. Generally, when the immune system is working properly, T lymphocytes, which are specific types of white blood cells, help kill tumor cells and can control immune responses. The process of self-tolerance ensures that the immune system does not mount a response to self-antigens that make up the tissues of the body[2]. However, this process is not perfect. Most healthy humans have self-reactive T cells in their mature repertoire as well as natural autoantibodies, but generally these are kept in check by mechanisms of peripheral tolerance. Central tolerance either deletes potentially self-reactive T cells or anergizes them ('anergy' is an immunological term meaning that T cells may still be around but are rendered non-functional; they do not cause any harm unless these are 'awakened' by an infection or another type of inflammation.)

The causes of autoimmune diseases involve several factors. For example, pathogens may activate the immune system by mimicking autoantigens, resulting in damage to healthy cells. By using models that focus on biomarkers like autoantigens, students are able to look at some of the various characteristics of autoimmune diseases to better understand disease detection and development.

Students will be introduced to histological samples and will be able to identify the varied level of disease progression of a collagen-induced arthritic model compared to the control animals. They will visualize cartilage and bone damage in the affected joints.

The students will be introduced to autoimmunity and will understand how the antibodies and cells of the immune system that could harm "self" tissues fail to be suppressed or deleted. Normally these immune cells are kept in check by suppression when they are in the periphery, or deleted during development in the thymus (T cells) or bone marrow (B cells). In some people, however, self-reactive cells may escape deletion and thereby make it to the periphery where subsequent activation can trigger autoimmunity. When self-attacks occur, immune cells are not able to properly discriminate between self-vs. non-self and diseases such as psoriasis, rheumatoid arthritis, lupus and multiple sclerosis can arise.

Some of the laboratory techniques the students will be exposed to will include conditions that mimic initiation of a cytokine storm. Various testing conditions that will take place in the lab will show examples of induced stress, such as T cell regulation and heat-shock proteins (stress can be induced in cells by various metabolic or inflammatory stimuli, like heating "fever", hypoxia, starvation (withholding glucose or certain amino acids), and inflammation. Stress of different types can lead to the induction of T cell activation or an expression of group of proteins called stress proteins, originally called heat-shock proteins because they were first identified when cells were heated. Later, it was found that many types of stress can induce the same proteins). Some of the stress proteins can be immunogenic and induce T cell response, which can be either good (protective) or bad (pathogenic), depending on the antigen, tissue, and dose.[3] In the class the students will test antibody-antigen interaction simulations using ELISA (enzyme-linked immunoabsorbant assay). They will also utilize histological aspects of immunology where they identify key features of autoimmune reaction/damage slides. The module will conclude with students completing group projects on autoimmune disorders including: rheumatoid arthritis, diabetes mellitus, Graves' disease, psoriasis, multiple sclerosis, and lupus.

REFERENCES:

1. Tortura, Funke, Case. "Ch.19. Disorders Associated with the Immune System." Microbiology: An Introduction. Custom edition for Prince George County Public Schools. (2013). Pearson Edition.

2. Anderson, D., Salm, S., Allen, D. "Ch.17 "Immunological Disorders". Nester's Microbiology: An Human Perspective. 8th edition. (2016)

3. Rajaiah R. and K.D. Moudgil. Heat-shock protein can promote as well as regulate autoimmunity. *Autoimmune. Rev.* (2009) 8: 388.

II. Student Outcomes

A. Science concepts covered in the module

-Innate and adaptive immunity

-Antibody-mediated immunity

-Primary and secondary antibodies

-Cell-mediated autoimmune reactions

-Importance of diagnostic immunology and use of ELISA

B. Science Standards

Next Generation Science Standards (NGSS)

HS-ETS1-2: Design a solution to a complex real world problem by breaking it down into smaller, more manageable problems that can be solved through design.

Maryland College and Career-Ready Standards (MDCCRS)
ELA/Literacy RST. 11-12.1: Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes

C. **Course Placement**

This unit was planned for an advanced high school science semester elective Microbiology class and is also used as a prerequisite course for the semester advanced science elective Anatomy and Physiology course. When studying the academic content of science, lesson plans are developed using the five E model which includes: engage, explore, explain, elaborate, and evaluate

1) Content—what the student needs to learn or how the student will get access to the information; → will be infused in the **Explanation, Engagement and Elaboration section**
2) Process—activities in which the student engages in order to make sense of or master the content→ **Engagement**
3) Products— culminating projects that ask the student to rehearse, apply, and extend what he or she has learned in a unit→**Evaluation**

D. **Science Practices**[1]

Science Practice 1: The student will demonstrate the ability to observe safe procedures when conducting an investigation.

Science Practice 2: The student will demonstrate the ability to carry out scientific investigations effectively and appropriately employ the instruments, systems of measurements, and materials of science

Science Practice 3: The student will demonstrate the ability to access and process information from readings, diagrams, investigations, or oral communications

Science Practice 4: The student will demonstrate the ability to formulate questions that lead to a testable hypothesis which demonstrate logical connections between scientific concepts and the design of an investigation

Science Practice 5: The student will demonstrate the ability to design experimental approaches that answer scientific questions.

Science Practice 6: The student will demonstrate the ability to use mathematical processes (measuring, calculating, etc.) when conducting investigations, analyzing information, and displaying information

Science Practice 7: The student will demonstrate the ability to conclude, justify, and support that data analysis is a vital aspect of the process of scientific inquiry and communication

Science Practice 8: The student will demonstrate the ability to apply mathematical processes to solve problems.

Science Practice 9: The student will demonstrate the ability to carry out effective scientific investigations, analyze data, communicate results, and apply results to explain phenomena occurring outside the laboratory

Science Practice 10: The student will demonstrate the ability to use appropriate communication methods, both written and oral, to present the processes and results of scientific investigations.

1. Adapted from Skills and Processes 2015-16 HCPSS Secondary Science Standards

E. **Relevance**

Students in the course have an innate desire to become future scientists, medical professionals such as researchers, nurses, and other professions in the allied health field. The course normally touches on immunity very briefly as far as lymphatics, which represents the Segway to look at epidemiology in the anatomy and physiology course. With the extension of the in-depth module focused on autoimmunity, students have a direct correlation of how the body can self-sabotage.

III. Learning Objectives

Students will understand the principle of diluting concentrated solutions.

Students will demonstrate correct micropipette use.

Students will know how to prepare solutions.

Students will recognize that the body has two main lines of defense against injury and infection (non-specific immunity and specific immunity).

Students will be able to understand the importance of diagnostic immunology.

Students will be able to identify that autoimmunity is a condition in which cells of the specific immune response attack healthy tissues.

Students will recognize that stress proteins can be immunogenic and induce a T cell response, which can be either good (protective) or bad (pathogenic).

Students will be able to graphically depict scientific data and differentiate between independent and dependent variables.

IV. Time Requirements

The class meets every other day for 90 minutes. This is a two week unit implementation for the module.

Lesson 1	Lecture on Adaptive Immunity Antibody- antigen Activity
Lesson 2	Assays, Micropipettes, and Dilution rations
Lesson 3	Microscopy and Histological Examination
Lesson 4	Importance of Diagnostic Immunology and the Use of ELISA
Lesson 5	Immunity Flip Flop and the Imperfect Storm
Lesson 6	Modeling of the Joints: An Activity of Cells Involved in Rheumatoid Arthritis
Lesson 7	**College and Career Readiness Aptitude Science Practice**: Analysis of Data tables and Graphs (protein quantification of VEGF induced signaling in mice models of multiple sclerosis)
Lesson 8	The Great Debaters: Autoimmunity and Vaccinations
Lesson 9	PowerPoint Activity: Autoimmune Disorder and Oral Defense

V. Advance Preparation, Materials, and Equipment

Lesson 1: The Adaptive Immune Response: All About Antigens and Antibodies

- LCD projector
- internet access to show video and lecture on adaptive immunity
- worksheets for antibody antigen activity

Lesson 2 and 3: Assays, Micropipettes, and Dilution rations

- Immunological and Histological Lab Practices
- Item: SKU PLA10 "Waverly LitePette Pipettor, adjustable volume 0.5-10µl * 7 (one for each lab table) source→ SoCal Biomed lab equipment and supplies cost $75 * 7= 525
- Item: KT10 10µl (short) KaliTips, Racked/Sterile, 960/pk
  96 tips/box x 10 boxes = 960/pack source→ SoCal Biomed lab equipment and supplies cost $25
- Item: #211145 Practice Pipetting Stations Kit source→ Carolina Company cost $47 (enough for 10 lab stations)
- Wells (for practice loading of agarose gels)
- Microcentrifuge tubes and practice loading dye
- Item # 311974 Histology slides: Introductory Histology Microscope Slide Set (25 slides of major tissue types)

Possible sources for access to expensive equipment:

- Contact local university/community college and request loaning of equipment if they have a science outreach program (*i.e. Maryland Loaner Lab through Towson University)*
- Micropipette, Student, 20 µL Item # 214610 source→ Carolina company cost $29.95* 7= 209.65
- Item: # 555346043 Food coloring: blue, green red, yellow from local grocery store source-> Walmart cost $4* 7= 28 (lab stations can share the food coloring or the colors can be diluted to make several sets for each station)
- Item # 311974 Histology slides: Introductory Histology Microscope Slide Set
  - 25 slides of major tissue types (students focus on the spleen, mammalian bone and the skeletal muscle)
  - possible to order only the spleen, mammalian and skeletal muscle as cost alternative.

Lesson 4: Diagnostic Immunology and use of ELISA

- Materials and equipment
- Teacher prep time: 40 min to set up 7 lab stations for 35 minutes experiments

- ELISA simulation kit Carolina Products (Item # 211248) https://www.science.purdue.edu/science-express/labs/labs/ELISA_simulation_kit.pdf[1]
    - Included in the kit positive control: -6 mL* negative control,-6 mL* Patient A sample-6 mL* Patient B sample -6 mL* Patient C sample, -6 mL* Patient D sample -6 mL* Patient E sample -6 mL* Patient F sample, -6 mL* simulated antigen, 30 mL simulated secondary antibody, 30 mL simulated chromogen, 30 mL 16 microtiter plates 180 plastic pipets
- Store samples A–F and the positive and negative controls in a freezer (approximately -20°C)
- 2006 Carolina Biological Company
- Computer access http://media.hhmi.org/biointeractive/vlabs/immunology/index.html?_ga=2.198629743.1190452864.1530895030-853823094.1530293813

Lesson 5: Autoimmunity Flip Flop and the Imperfect Storm

- Flip Flop Student worksheet
- Sepsis and the imperfect storm article and analysis

Lesson 6: Modeling of the Joints: An Activity of cells involved in rheumatoid arthritis

- Computer access for websites https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967437/
- Cut and paste handouts

Lesson 7: College and Career Readiness Aptitude Science Practice

- Scientific article on autoimmunity

Lesson 8: The Great Debaters: Immunity and vaccinations

- Rubric and assigned special interest group

Lesson 9: PowerPoint Activity and Oral Defenses

- Rubric and assigned group project

VI. Student Prior Knowledge and Skills

- **Expected prior content knowledge**
    - Students should already have a background in innate immunity. They should be familiar in the first line of defense, which includes the skin and mucosal membranes. They should review cells of the innate immune system including: neutrophils, basophils, mast cells, natural killer cells and eosinophils.

- **Expected prior technical skills**
  - Lab safety (how to analyze a graph and data)
- **Possible preconceptions:**
  - Reinforcement of the importance of the innate immunity system in correlation with the adaptive immune system is paramount. Autoimmune diseases result from complex interactions among different immune cell types, including both T and B lymphocytes and professional antigen-presenting cells, such as macrophages and dendritic cells[1]
  - Cells such as dendritic cells and macrophages are important immune cells, but students may fail to realize they play a role in autoimmunity as well as they can cause an immunological response such as synovial tissue damage when an infections autoantigen is in the synovial membrane.

References:

1Sanatamaria, Pere. Cytokines and Chemokines in Autoimmune Disease: An Overview. Madam Curie Bioscience Database. Landes Bioscience. 2000-2013

VII. Daily Unit Plans

Lesson 1: The adaptive Immune response: All about antigens and Antibodies

The adaptive Immune response	**All about antigens and Antibodies**
Content Objective(s): Students will be able to: describe the immune system and explain how it detects and attacks any foreign organism that enters the body. SWBAT: Students will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.	**KEY VOCABULARY:** antibody, antigen, immunoglobulin, epitope, spleen, lymphatic system, antigen presenting cells, T cells, B cells, epitope, Ig G, Ig A, Ig M, Ig E, Ig D,

LESSON SEQUENCE: 5 E'S OF SCIENCE

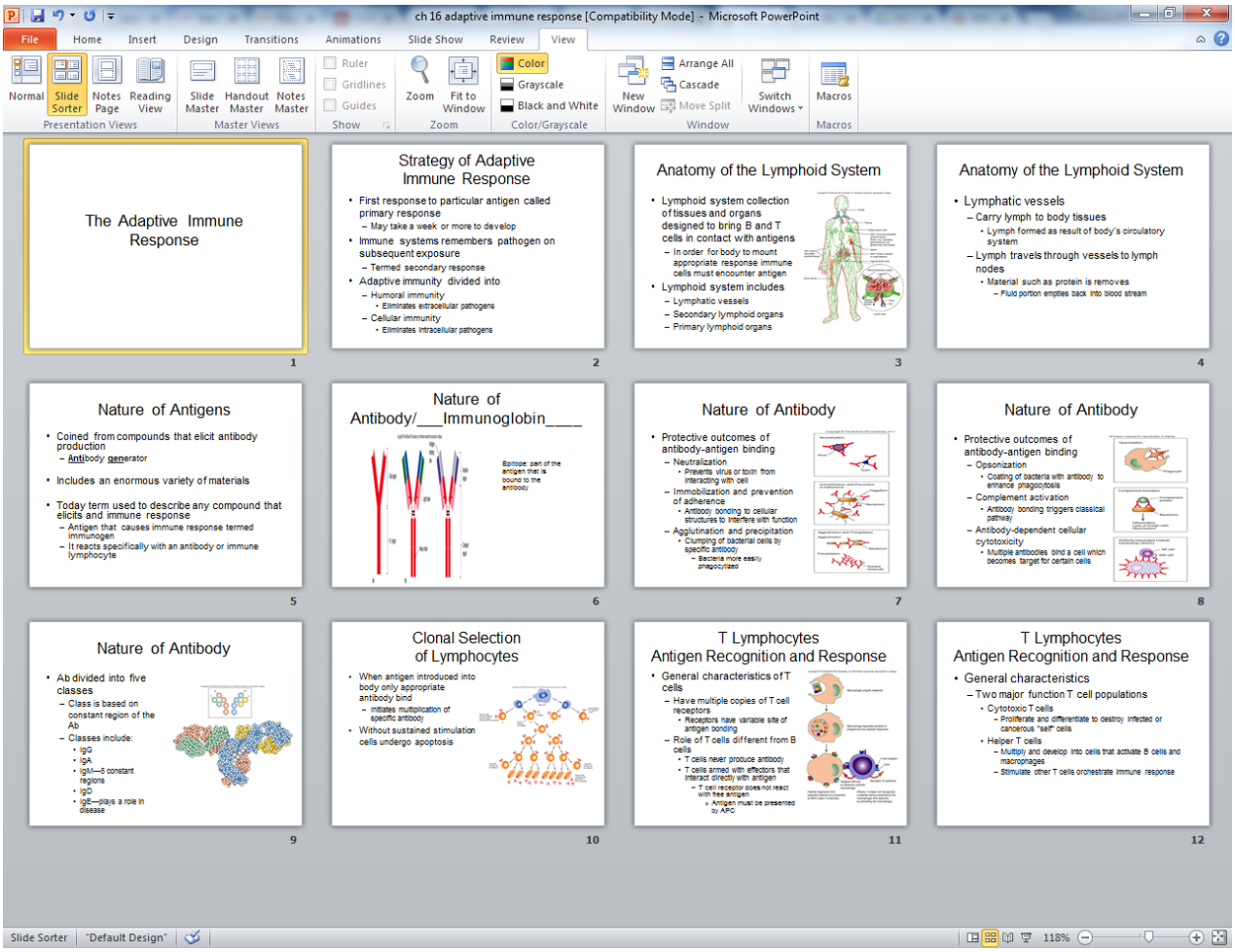
Engagement : video describes the Immune System and explains how it detects and attacks any foreign organism that enters the body https://www.youtube.com/watch?v=G4jobV6-bFA [1]

[1] Medical Research council. October 2014.

Students will recall key concepts from innate immunity and be introduced to adaptive immunity

-Explanation:

(lecture on adaptive immune response) https://sites.google.com/a/pgcps.org/ms-shaw-s-microbiology-class/



Exploration: Class switching Antibody activity.

Elaboration: Students work in lab groups to complete the lab activity, students can ask teacher for clarification

Evaluation

Matching: Part 1. Matching: Match the following terms on the left with their corresponding definitions on the right.

__E_ 1. Antibodies	A. foreign substances inducing an immune response
__F_ 2. T Cells	B. the part of an antigen molecule to which an antibody attaches itself
__G_ 3. B Cells	C. examples include interferon, interleukins, and growth factors, that are secreted by certain cells of the immune system and have an effect on other cells.
__B_ 4. Epitope	D. molecules that usually bind to larger molecules
__C_ 5. Cytokines	E. also known as immunoglobulins
__A_ 6. Antigens	F. plays a role in cell-mediated immunity
__D_ 7. Ligands	G. plays a role in humoral immunity

Also see assessments section → formative assessment immunity quiz

Exit ticket: Stop and think: Think of a way to differentiate between cell-mediated immunity and humoral immunity.

Class Switching Antibody activity

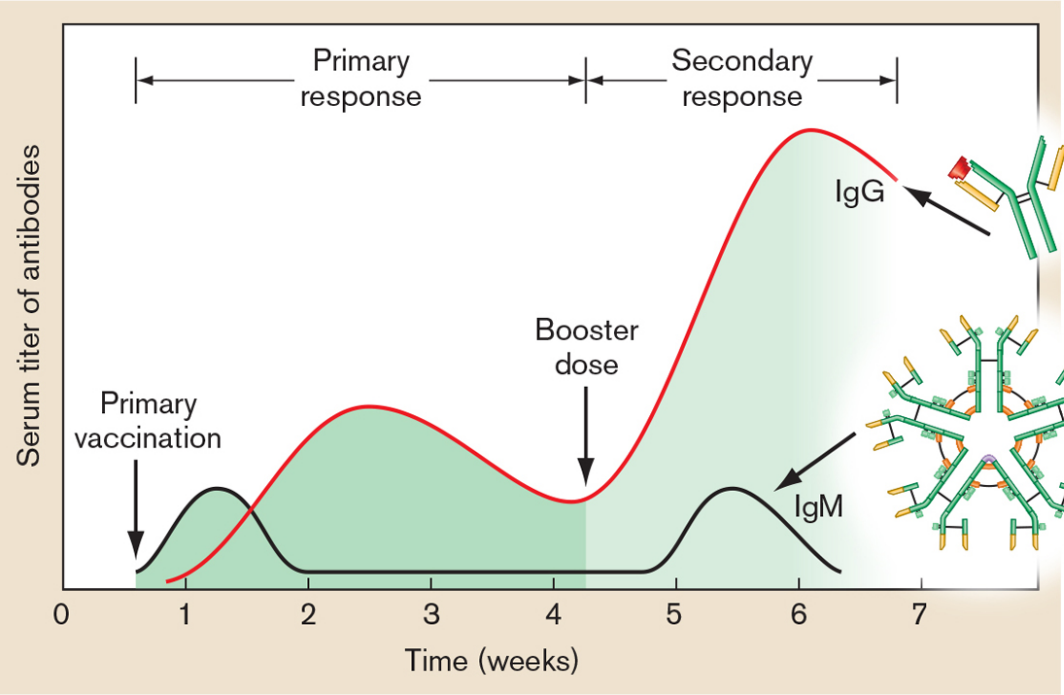
Part 1:

Class switch recombination is a biological mechanism that allows the class of antibody produced by an activated B cell to change during a process known as isotype or class switching

Directions: Read the following articles:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2707252/

https://www.focusforhealth.org/what-are-titers/

Part 2:



1. What is the independent variable?
2. What is the depndent variable?
3. Differentate between IgM and IgG
4. At the secondary response level, which antibody is more prevalent? Does this graph show evidence og class switching. Explian.
5. Extension: Suppose someone got a MMR shot in 1984, then enrolled in grad school in 2014, but tirters for MMR was low, what does this mean, what would this individual need to produce more antiobodes?

Lesson 2: Assays, Micropipettes, and Dilution ratios

Content Objective(s): SWBAT: understand the importance of micropipetting, assays and dilution ratios

LESSON SEQUENCE: 5 E'S OF SCIENCE

Engagement : Video describes the technique in using micropipetting https://www.youtube.com/watch?v=bex0itUMxmI

[1] Fisher Science Education, 2010.

Exploration: Assays, Micropipettes, and Dilution ratios student worksheet

Explanation: Clarify any misconceptions students may have on pipetting

Extension: Analysis from comprehension of the lab questions to grasp understanding of content→writing standard focus infused throughout Assays, Micropipettes, and Dilution ratios student worksheets

Evaluation: Exit slip activity 3-2-1 Activity

Assays, Micropipettes, and Dilution ratios

Lab group members' names: _____ _____ _____ _____

Lab table #:_____ Date: _____

Directions: Look at the image on how to use the micropipette. Then answer questions below

Micropipette

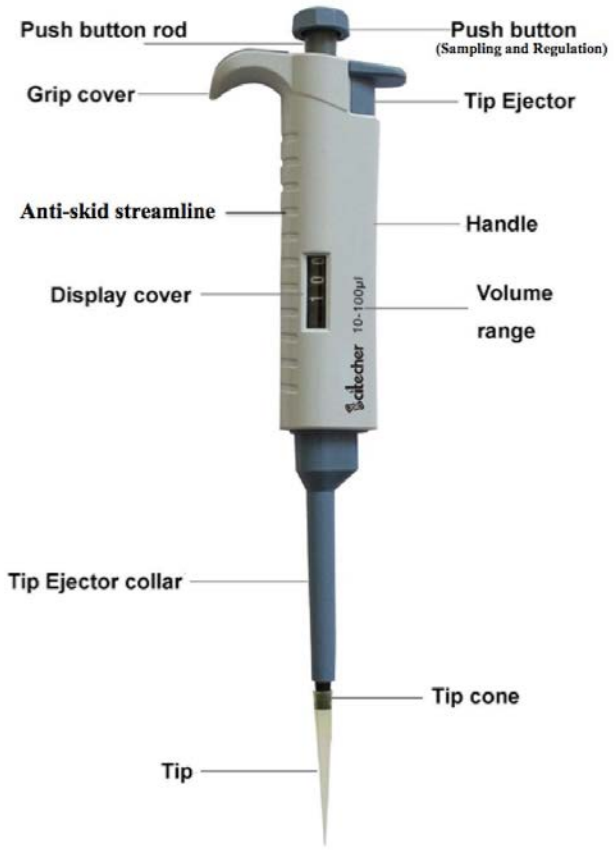


Image from Scitecher Adjustable Laboratory Micro Pipette Pipettor-Single-Channel-10-100ul

Station 1 – How to use Micropipette

1) What do you do with the push button and the volume range of the micropipette?

2) Why is it crucial to change pipette tips when working with various samples?

Key

1) What do you do with the push button and the volume range of the micropipette?

- *Adjust volume range to desired sample amount.*
- *Push down and tip is inserted on the tip cone area, and liquid is drawn up the tip with release.*

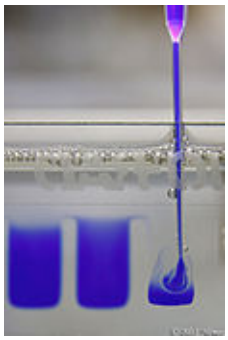
2) Why is it crucial to change pipette tips when working with various samples

- *To ensure samples that samples are not mixed with previous sample drawn up from tip.*

Station 2 – Micropipette Usage and Gel Loading

In the lab assays are keys to molecular biological techniques, and often times the samples sizes that scientists used are very small. With the use of the micropipette, scientists are able to use very small samples for analysis and quantification of substances. They work with microliters (µl) volumes.

- Find the box of pipette tips on your lab table. Make sure to replace the pipette tips with each usage. In the lab if pipette tips are not replaced with each sample, samples can get mixed up, and this can skew data
    - Gel electrophoresis is an important molecular technique that can be used to separate DNA and proteins.
- Use the practice pipetting kit to practice loading the sample in an agarose gel.



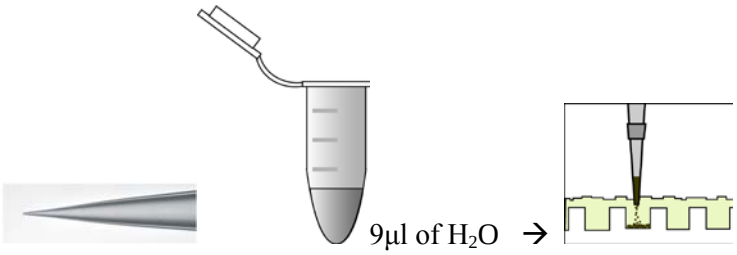
Questions: Why do you have to be careful with the micropipette tips when you enter the practice loading dye in the gel wells?

Answer: To ensure proper sample is not mixed with another sample

Station 3 – Dilution Ratios and Fluorescence

1. Find the 4 samples of food coloring (red, blue yellow, green).

Scientists have to ensure when taking samples that the desired concentration is achieved. For each of the colors draw out 9µl of distilled water, and put into microfuge tube, then using the same tip, draw out 1 µl the food coloring then use different pipette tips again for the remaining colors. Examine the contents in the microfuge tube. Then draw up 1 µl of the total volume and dispense in the practice gel sample wells.



+ 1 µl of food coloring (Note: Use different tip for each color.)

This can represent 4 various samples used when conducting an assay. An assay is based on the targets being measured in a lab. In the field of immunology assays can be used for cell counting. Assays can also be used to add a fluorescence, or luminescence to be able to visualize the sample. Notice the 4 samples: blue, red, green and yellow, these colors represent tools used in helping scientist to identify the use of an antibody-based method to detect a specific protein in a sample based on color.

2. Now practice with dilution ratios below and fill out the table below.

Volume calculations for 1X solutions

Fluorescence label	Amount of solute 10X dye (µl)	Amount of solvent Distilled H_2O (µl)	Total volume (µl)
Red	23		230
Blue	56		560
Yellow	400		4000
Green	125		1250

Key

Fluorescence label	Amount of solute 10X dye (µl)	Amount of solvent (distilled H_2O) µl	Total volume µl
Red	23	207	230
Blue	56	504	560
Yellow	400	3600	4000
Green	125	1125	1250

Exit Slip: 3-2-1 Activity

3-2-1 Activity

3 Things You Learned	2 Important Details	1 Question You Still Have
1 **2** **3**	**1** **2**	**1**

Lesson 3: Microscopy and Histological Examination

Content Objective(s): SWBAT

- understand the importance of using microscopy
- differentiate between chronic and acute inflammation
- analyze inflammation and synovitis of an arthritic rat model utilizing histology

LESSON SEQUENCE: 5 E'S OF SCIENCE

Engagement : video of histopathology of the joint- rheumatoid arthritis
https://www.youtube.com/watch?v=ZcTi28aBY1s

[1] Washington Deceit, 2007.

Exploration: Microscopy and Histological Examination student worksheet

Explanation: Prior knowledge is connected to current findings. Students develop explanations for their observations.

Elaboration: Students need to connect what they have learned to something concrete in their understanding of the world around them

Evaluation: Exit slip activity and self-assessment and group assessment of lab techniques → (assessment section)

Lesson 3: Microscopy and Histological Examination

Look through the microscope on your lab table; carefully draw the three histology samples:

Spleen, mammalian bone, and skeletal muscle.

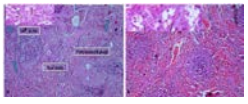
Spleen Mammalian bone Skeletal Muscle

Spleen Mammalian bone Skeletal Muscle

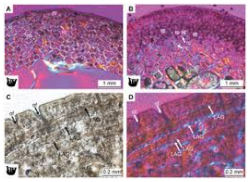
Total Magnification _____ Total Magnification _____ Total Magnification _____

Note: Remember ocular* objective when writing the total magnification.

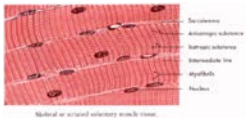
Key: Some images will vary based on objective students use to view slides.



Can vary based on field of views (40, 100 or 450X)



Can vary based on field of views (40, 100 or 450X)



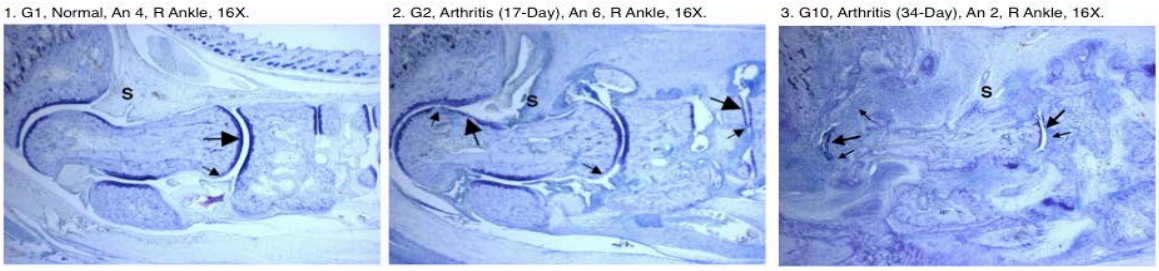
Can vary based on field of views (40, 100 or 450X)

STATION 5 – Histological Examination

Collagen induced arthritis is an autoimmune model studied in rats which serves as a model of rheumatoid arthritis in humans. Cartilage destruction is associated with immune complex deposition on articular surfaces. Bone resorption and periosteal proliferation, and moderate to marked synovitis and periarticular inflammation as the disease progresses to about the two week window.[123]

1. Bendele AM. Animal Models of Rheumatoid Arthritis. J Musculoskel Interact 2001; 4: 377– 385.
2. Bendele A, Chlipala L, Sennello R, Frazier J, Edwards C. Combination benefit of treatment with soluble TNF- RI and IL-1ra in rat models of arthritis. Arthritis Rheum 2000; 43:2648-2659.
3. Bendele AM, McComb J, Gould T, McAbee T, Sennello G, Chlipala E, Guy M. Animal models of arthritis: relevance to human disease. Toxicologic Pathol 1999; 27:134-142.

Photomicrographs
RTTC/G-1



1. Ankle of normal rat shows normal synovium (S) and normal cartilage (large arrow) with no pannus or bone destruction (small arrow).

2. Ankle of arthritic rat (with approximate mean score for the group) has marked synovitis (S) and periarticular inflammation with moderate cartilage damage (large arrows) and mild pannus and bone destruction (small arrows).

3. Ankle of arthritic rat (with approximate mean score for the group) has marked synovitis (S) and periarticular inflammation with severe cartilage damage (large arrows) and severe pannus and bone destruction (small arrows).

Image above from Sample data obtained from Bolderbiopath Inc.

Directions:

Fill in the data table by comparing and contrasting the three images above:

1. Normal rat

2. Mild stage progression

3. Longer onset progression

Make sure to talk about the synovial area, pannus/bone destruction or no evidence of this, and whether there is evidence of inflammation and/or cartilage damage.

Normal rat	**Mild stage progression**	**Longer onset progression**

Make sure to complete the lab evaluation sheet (see assessment section).

Key

Normal rat	**Mild stage progression**	**Longer onset progression**
normal synovium	synovitis	synovitis
no pannus or bone destruction	mild pannus or bone destruction	severe pannus or bone destruction
non-inflamatory	inflammation and cartilage damage	inflammation and severe cartilage damage

Exit slip activity questions:

1. Why do you think the two week window was used for the study?
2. If the study was only 3 days, do you think the data would be similar? Explain.

Lesson 4: Importance of Diagnostic Immunology and Use of ELISA

Importance of diagnostic immunology and use of ELISA
Content Objective(s): SWBAT: commercial uses of the ELISA (enzyme linked immunosorbent assay) SWBAT: Use a test to measure whether a specific antibody is associated with an illness (lupus) can be found in a patient's blood. Key concept: antibody + antigen = color change

LESSON SEQUENCE: 5 E'S OF SCIENCE

Engagement

(video on ELISA) https://www.youtube.com/watch?v=zR_xlV5v_f4

Explanation:

Antibody + antigen = color change

With a partner, discuss the benefits of having an assay that is able to analyze 96 reactions at a time in microtiter dishes and how this has changed the advancement of molecular medicine in research.

Exploration: ELISA Lab and ELISA analysis (hands on lab)

https://www.science.purdue.edu/science-express/labs/labs/ELISA_simulation_kit.pdf Student worksheets and patient profile sheets

Elaboration : Differentiate between serum samples, antibodies, antigens

Evaluation: Students will have computer access and go to the website (http://media.hhmi.org/biointeractive/vlabs/immunology/index.html?_ga=2.198629743.1190452864.1530895030-853823094.1530293813) to complete the assessment questions based on virtual ELISA on autoimmune disorder lupus

Lesson 5: (Part 1) Autoimmunity Flip Flop and the (Part 2) Imperfect Storm

Content Objective(s): SWBAT:

- understand T/B cell development and immune tolerance
- the function of MHC molecules
- autoimmune diseases result from interplay between genetic and environmental factors
- recognize conditions that mimic initiation of cytokine storm

Part 1: Autoimmunity Flip Flop

FLIP	FLOP
Section 1 **T/B Cell Development And Immune Tolerance** Healthy people /rodents have an effective **immune tolerance** towards body's own antigens (i.e. proteins in blood, and proteins in tissues, such as kidney, brain, and retina). Tolerance means the immune cells (T cells, B cells) do not mount a response against these antigens, so 'tolerate' them, unlike an immune response to bacteria, viruses and fungi, all of which comprise antigens foreign to the host (i.e. person, mouse, rat). **Immune tolerance** is the result of multiple processes occurring at the level of developing immune cells (e.g., T cells that develop and mature in the thymus, and B cells that develop and mature in the bone marrow) (central tolerance) as well as at the level of mature T cells in the blood and peripheral lymphoid tissues after existing the thymus and the bone marrow (peripheral tolerance)". **Section 2 Central Tolerance** The function of MHC molecules[1] is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T cells[1]. T cells that do not recognize self MHC and/or react with self-antigens presented by thymic APCs with high avidity are killed (central deletion); likewise, self-reactive B cells are killed in the bone marrow. However, these processes are not perfect, so some self-reactive T and B cells escape into the periphery. Such immune cells are controlled by multiple mechanisms operative in the periphery (**peripheral tolerance**).	***Big Idea Question*** 1. **What are antigens?** 2. **Give two examples of immune cells, and draw a quick sketch of each.** 3. **What is peripheral tolerance?** 4. **What are the functions of MHC molecules?** 5. **What can happen to T cells that do not recognize self-MHC and/or react with self-antigens?**

These mechanisms include but are not limited to T regulatory cells (Treg), immunoregulatory cytokines (like IL-4 and IL-10), and induction of anergy. T cell **anergy**[2] is a tolerance mechanism in which the lymphocyte is intrinsically functionally inactivated following an antigen encounter, but remains alive for an extended period of time.

6. Give two examples of immunoregulatory cytokines.

7. What do autoimmune responses target?

Section 3 Autoimmunity

Autoimmune responses target self-antigens, and thereby, they are the result of a break in self-tolerance. Potentially self-reactive T and B cells exist in all healthy individuals, but they are kept in check by mechanisms of tolerance. However, when these cells are unrestrained via a breakdown of tolerance, then they can attack self- tissues. Self-reactivity can be controlled by the immune system (this phase is called **sub-clinical autoimmunity**), but when a certain threshold is crossed, clinical signs of autoimmunity start appearing.

8. Describe sub-clinical autoimmunity.

Autoimmune diseases result from interplay between **genetic** and **environmental factors**. Most important genetic factors are the HLA genes. The human leukocyte antigen (HLA) system or complex is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface-proteins are responsible for the regulation of the system in humans[3]. There are many environmental factors including pathogens, cigarette smoke, toxins, dietary products, etc. Also, '**Hygiene hypothesis'**[4] has been proposed suggesting that activation of the immune system by certain microbial agents or worms can offer protection against autoimmunity. This might explain in part the lower incidence of autoimmunity in under-developed countries in terms of environmental hygiene compared too much higher incidence of autoimmunity in industrialized countries.

9. What is the importance of HLA genes?

10. Describe the hygiene hypothesis.

Breakdown of self-tolerance can occur because of defects in central and/or peripheral tolerance, as well as following infection by certain pathogens (bacteria, viruses). Infections

can break tolerance in different ways, including **molecular mimicry** in which the antigen of a pathogen molecularly resembles a self-antigen, and therefore the immune cells cannot discriminate between foreign and a self-antigen. The immune cells activated by foreign antigen can then attack self-antigen/self-tissue. Examples of molecular mimicry are rheumatic fever following streptococcal infection and Lyme arthritis after Borrelia infection (carried by a tick).	**11. What is molecular mimicry?**

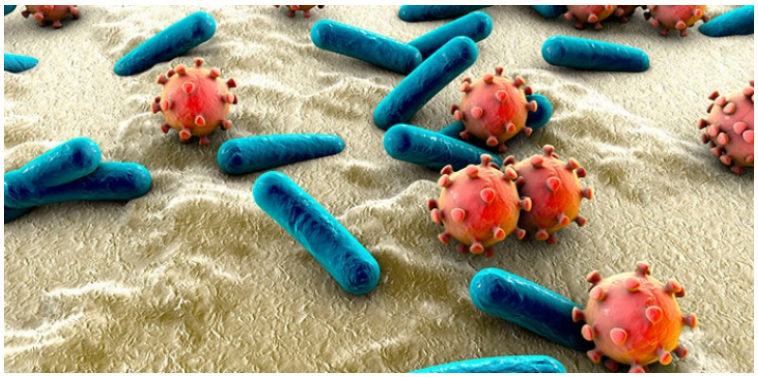
1. Immunobiology: The Immune System in Health and Disease. 5th edition Janeway CA Jr, Travers P, Walport M, et al.New York: Garland Science. 2001
2. T Cell anergy. Schwartz RH. Annu Rev. Immunol. 2003; 21:305-34. Epub 2001 Dec 19. Review
3. *Human Leukocyte Antigen.* Wikipedia *https://en.wikipedia.org/wiki/Human_leukocyte*
4. H Okada, C Kuhn, H Feillet, and J-F Bach. Clin Exp Immunol. 2010 Apr; 160(1): 1–9. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update

Immunobiology: The Immune System in Health and Disease. 5th edition Janeway CA Jr, Travers P, Walport M, et al.New York: Garland Science. 2001

The Imperfect Storm: Sepsis and Cytokines

Sepsis:

The Imperfect Storm



Read the article about sepsis

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916383/

Questions:

What is the major cause of death in most intensive care units in the U.S.?

Answer: Sepsis is the major cause of death in most intensive care units in the U.S.

What mediates the initial immune recognition response?

Answer: Pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs)

Differentiate between hyper-inflammatory and hypo-inflammatory responses in septic patients?

Answer: In the initial pro-inflammatory response of sepsis, both the adaptive and innate immune systems are rapidly activated in some septic patients; these normal homeostatic counter inflammatory mechanisms remain

References

Virulence. 2014 Jan 1; 5(1): 45–56. Published online 2013 Sep 25. doi: 10.4161/viru.26516

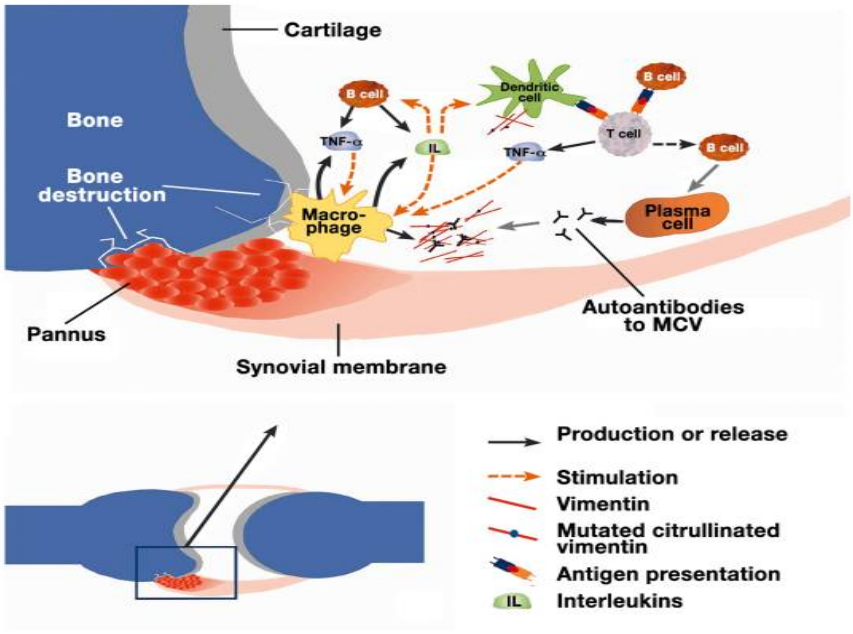
Lesson 6: Modeling of the Joints: An Activity of cells involved in rheumatoid arthritis

Content Objective(s): SWBAT: -understand that your body's immune system can mistakenly attack normal cells.

Engagement

Autoimmune diseases cause your body's immune system to mistakenly attack normal cells. In autoimmune arthritis, such as rheumatoid arthritis (RA), your immune system attacks the lining of your joints. This leads to inflammation that can affect your entire body. **With an elbow partner, turn and talk and describe what is happening in the image below.**

What cells are involved and describe the cascade of cellular events?



clip art image Microsoft office

Explanation: In depth analysis of autoimmunity

Exploration: Rheumatoid arthritis activity (see student section)

Elaboration: Differentiate between normal joint, osteoarthritis, and rheumatoid arthritis.

Extension: Students will have computer access to the websites below to compare and contrast normal joint, osteoarthritis, and rheumatoid arthritis.

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967437/

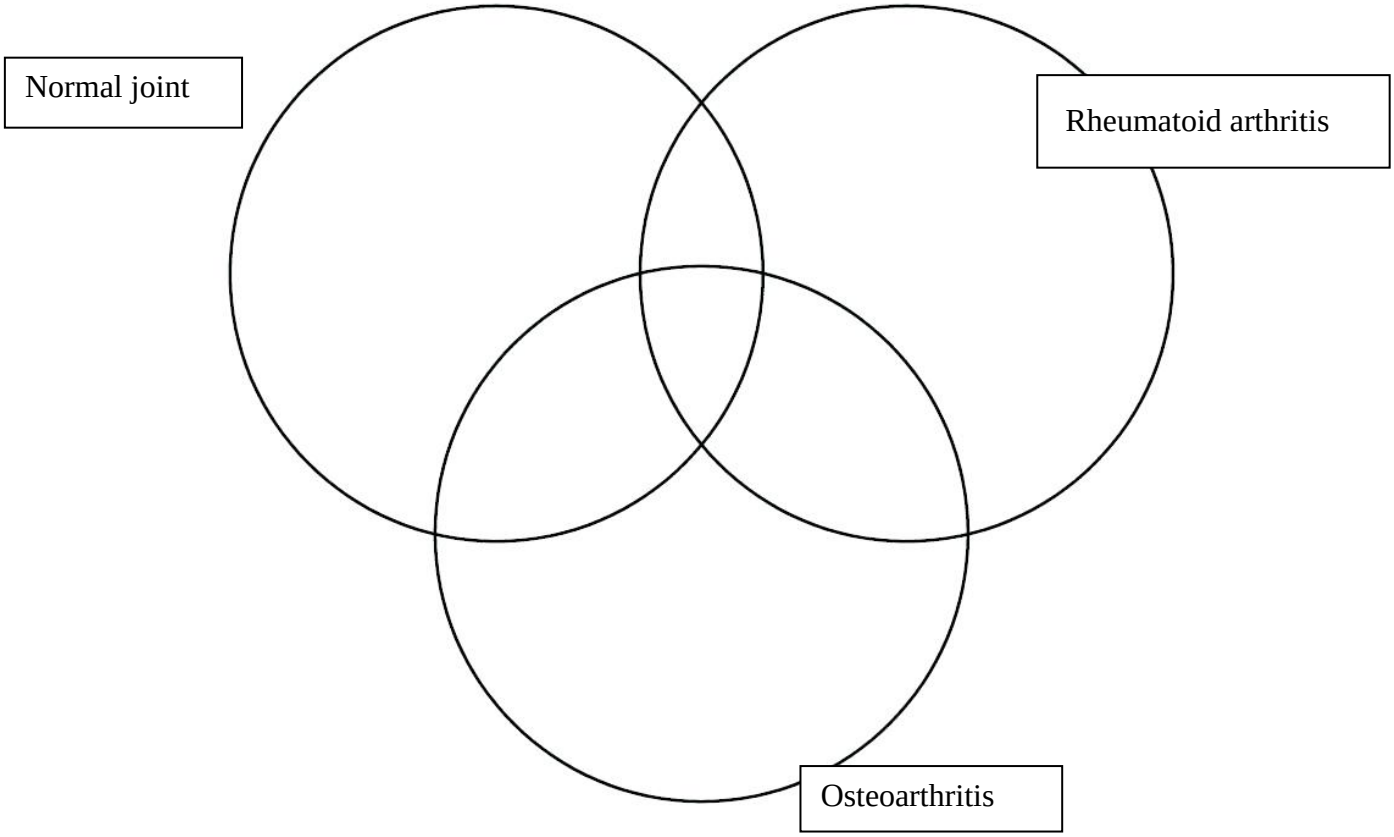
Interdiscip Toxicol. 2013 Sep; 6(3): 111–125

2. https://www.mayoclinic.org/diseases-conditions/osteoarthritis/symptoms-causes/syc-20351925

1998-2018 Foundation for Medical Education and Research (MFMER)

3 https://www.ncbi.nlm.nih.gov/books/NBK459454/

Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al., editors.
Bogota (Colombia): El Rosario University Press; 2013 Jul 18. Autoimmunity: From Bench to Bedside



Worksheet for Lesson 6: Name: _____ Date: _____ Pd: ___

Factors Influencing the Joint Susceptibility in Autoimmune Arthritis

Objectives:

- To model rheumatoid arthritis and to see which cells have been found to massively infiltrate synovial membranes and be central to the pathophysiology of inflammation
- To compare rheumatoid arthritis and a normal joint

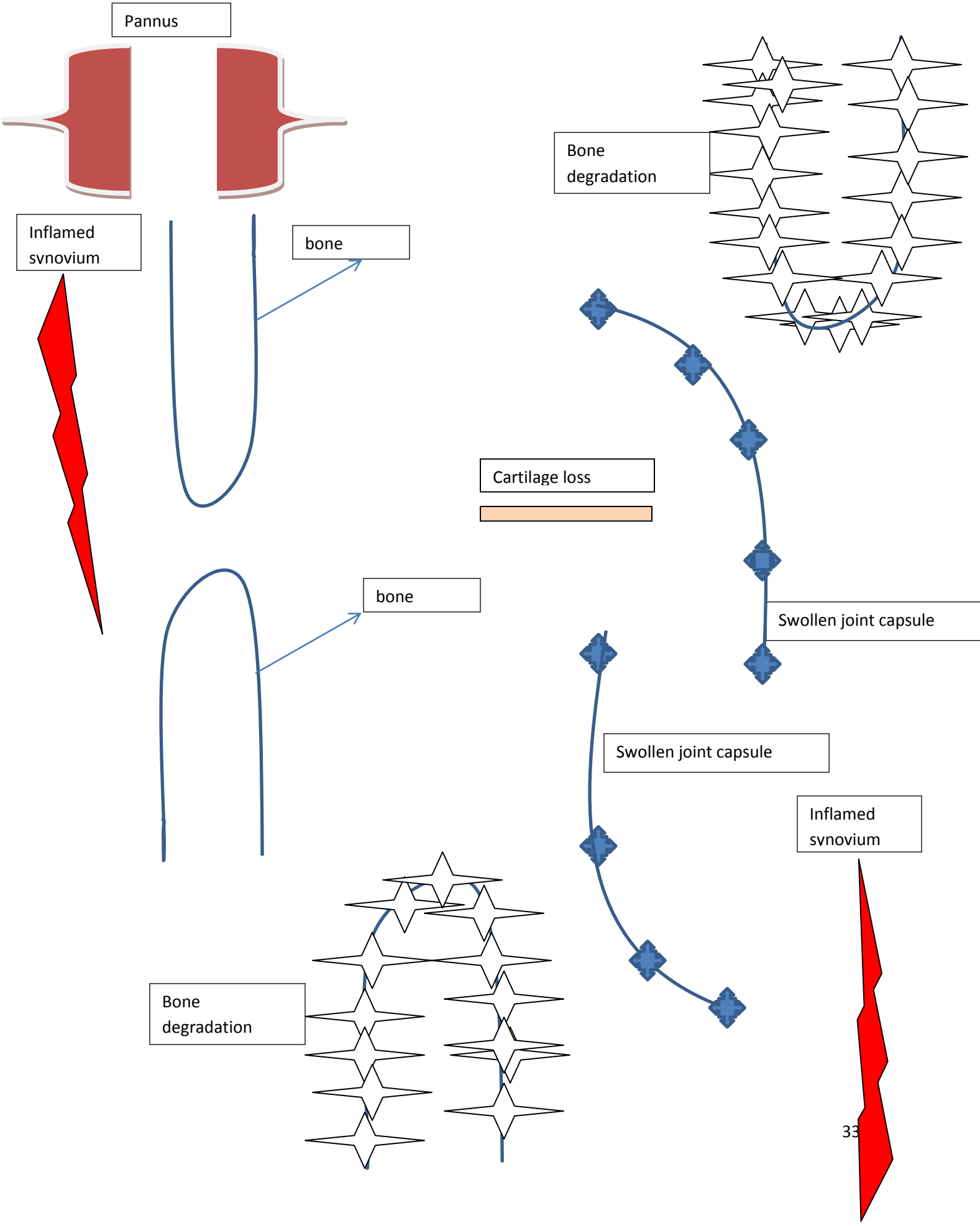
Introduction

Autoimmune diseases develop when your body's immune system mistakenly attacks normal cells. In autoimmune arthritis, such as rheumatoid arthritis (RA), your immune system attacks the lining of your joints. This leads to inflammation that can affect your entire body.

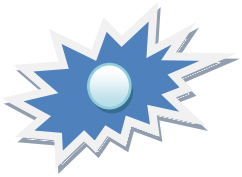
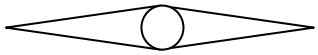
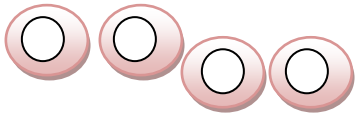
Part 1: Define the following terms and then explain their role in rheumatoid arthritis. You can also compare the terms for normal joints compared to rheumatoid arthritis using the following terms.

- **Extracelluar matrix (ECM)**
- **Pannus**
- **Angiogenesis**
- **Endothelial cells**
- **Synovial fluid**
- **IL-1**
- **Macrophages**
- **Chondrocytes**
- **Dendritic cells**

Part 2: After defining the terms above model rheumatoid arthritis with cut outs below. You are provided an image of a normal joint for your reference. You will be modeling rheumatoid arthritis in your model. (See last page.)


Pannus
Inflamed synovium
bone
Bone degradation
Cartilage loss
bone
Swollen joint capsule
Swollen joint capsule
Inflamed synovium
Bone degradation

Part 3: Once you have made your model go back and add the following cells to the area of inflammation including:

- macrophages 
- dendritic cells 
- synovial fibroblasts 
- chondrocytes 
- T Cells 
- B cells 
- neutrophils 

Part 4:

Based on what you have learned in the model you built, your understanding of key vocabulary, and analyzing image A, compare and contrast image B and image C. Make sure to provide evidence form the image key features to support which image is from a normal rat, and which image is from a rheumatoid arthritis model.

Example of rheumatoid arthritis model **Image A**

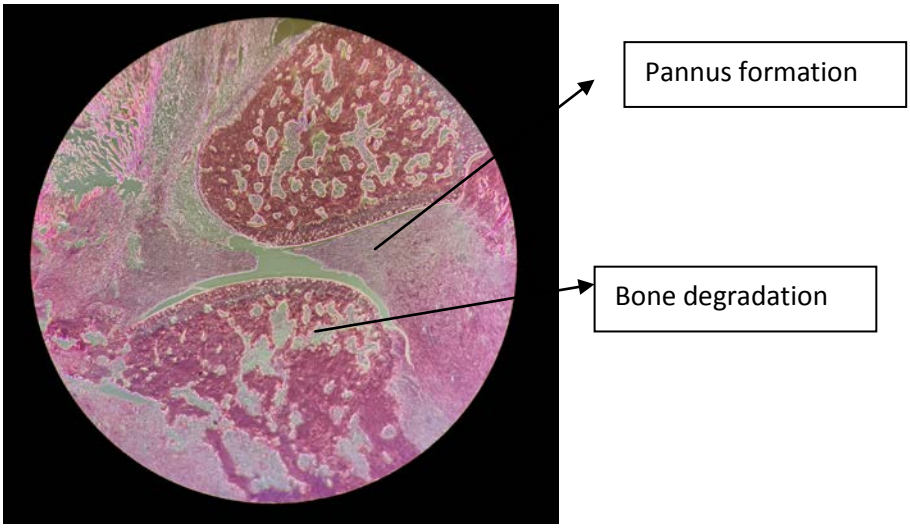


Image B

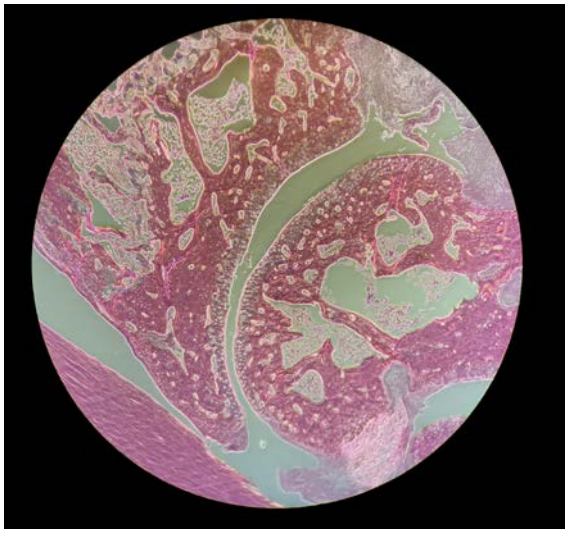
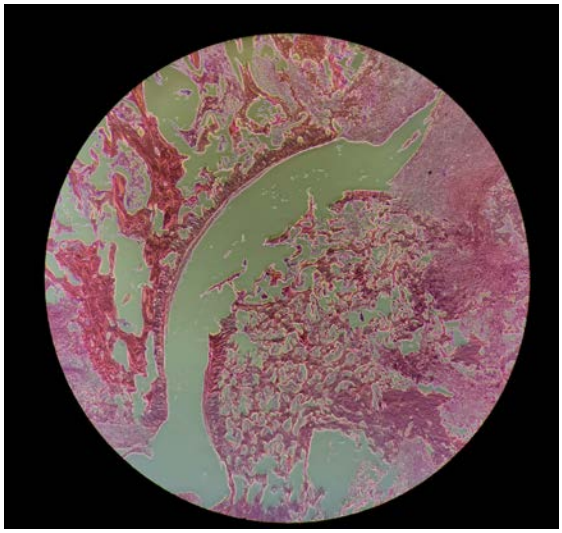


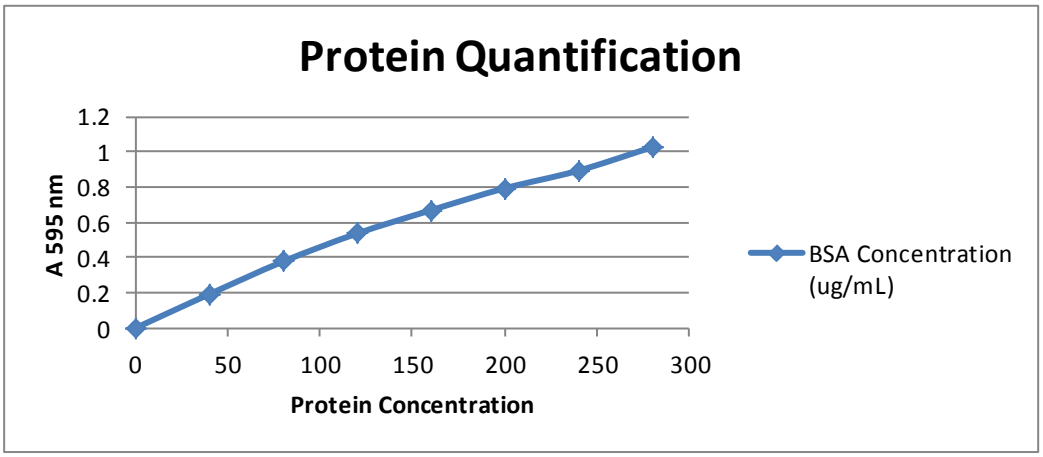
Image C



Lesson 7

College and Career Readiness: Science Aptitude Questions

Human endothelial cells are used as a cell line in some immunological assays.



What are trends in the data?

Graph the following from the data table below

	15 min	30 min	1 Hour	2 Hour	3 Hour
1 uL	0.024	0.039	0.044	0.044	0.044
5 uL	0.2115	0.19	0.1995	0.1975	0.1945
1 uL + Cel	0.045	0.041	0.048	0.0605	0.054
5 uL + Cel	0.201	0.204	0.21	0.204	0.227

Lesson 8: The Great Debaters

Engagement: watch video -http://www.pbs.org/wgbh/pages/frontline/vaccines/view/

Introduction

Science Connection:

Some people have suggested that vaccines can stimulate autoimmune reactions if some of the antigen fragments in vaccines resemble a person's self-antigens. It is unclear why an immune system that is tolerant of its own self-antigens would respond to a self-antigen mimic in a vaccine. [1]

An autoimmune response might be instigated by a vaccine or by a natural infection if the microbial agents bind to self-antigens in infected cells and change the antigens' shape such that they are no longer tolerated and can elicit an immune response. Although the immune systems of two people may respond to the same protein in a vaccine, their T cells may respond to different portions of that protein.

Rationale: What's going on?

There has been a very steep decrease in the rate of vaccinations recently, particularly within communities of affluent, well-educated parents:

"It's that whole natural, BPA-free, hybrid car community that says 'we're not going to put chemicals in our children 'I'm going to be pure and I want to keep my child pure."[2]

The school board has received a proposal to not require parents to vaccinate children if it's against the parents' wishes. The Prince George County community is truly divided. Vaccinations can be beneficial to many people within the state due to population demographics of a high elderly population and people from emerging countries and diverse areas; however, it does propose a risk factor due to the fact that some people get vaccinated at later ages.

There will be a meeting within the local state school board to decide upon whether or not to limit requirements of vaccinations. The state is planning to take this to a public debate in which representatives of specific groups of people will take a stand on whether or not vaccination is necessary for school aged children.

Objective

1. The class will gain experience on vaccination risks and decision making in the health policy and education community in order to test decision-making skills when it comes to debatable medical issues.

2. Recognize that some autoimmune diseases may be stimulated by viruses

Procedure

1. After reading over the activity's introduction and objectives as a class, begin the meeting by dividing the class into 6 different interest groups.
2. Once divided, the groups should prepare their research discussion/argument based off the description given in their interest groups.
3. When all groups are prepared, the debate will begin with a five minute statement by each group debating their side of the issue at hand. After, all other groups are permitted to ask two questions each to get further information upon the proposal.
4. After hearing all arguments, the school board will reach a decision on whether or not to pass this proposal and provide a brief explanation as to why or why not.

List of Diseases [3]

Anthrax	Cervical Cancer	Diphtheria
Hepatitis A	Hepatitis B	Haemophilus influenzae type b (Hib)
Human Papillomavirus (HPV)	Influenza (Flu)	Japanese Encephalitis (JE)
Lyme Disease	Measles	Meningococcal
Monkeypox	Mumps	Pertussis (Whooping Cough)
Pneumococcal	Poliomyelitis (Polio)	Rabies
Rotavirus	Rubella (German Measles)	Shingles (Herpes Zoster)
Smallpox	Tetanus (Lockjaw)	Tuberculosis
Typhoid Fever	Varicella (Chickenpox)	Yellow Fever

Special Interest Groups

1. The School Board – The public meeting holders that must listen to all arguments upon making the final decision of whether or not to allow school aged children to start school without proper vaccination and immunization records or only certain immunizations. Along with the decision-making aspect, this group must also provide background research on the topic such as the history of vaccinations, the pioneers in the field of microbiology at the beginning of the meeting to show comprehension of subject matter. Must be able to ensure flow of debate and educate class on what vaccines and immunizations are.

2. Research Scientists and Physicians at the American Medical Association – Define the organization's overall purpose and goals. Also, provide the pros for vaccinations. You must have an understanding of the flowing diseases that have been prevented through vaccinations

3. Parents Against all required Immunizations – Must provide the cons of the immunizations meaning they must debate the risk of vaccinating children. You are the group that feels vaccinations cause autism and give us background why you feel this. Why are you opposing this, back up your findings and give examples of current practices of communities not vaccinating children in the country. You must have a working knowledge of the diseases listed above. How will this affect other children that may be immunocompromised?

4. Parents for Selective vaccinations – Undefined with siding. The group as a whole must decide what specific immunizations they will give their children. This group much research common vaccinations for school aged children and defend which vaccinations are necessary and which aren't and explain why? How will this affect other children that may be immunocompromised? You must have a working knowledge of the diseases listed above

5. Parents for all required vaccinations – – Must provide the pros of the immunizations meaning they must debate the benefits of vaccinating children. You are the group that feels vaccinations not only protect your children but is a moral and civic issue. You take the stand that we are a privileged society to have access to vaccines. In many places around the world, people don't have easy access to them, and there are even some places where aid workers are killed for trying to administer vaccinations, you must have a working knowledge of the diseases listed above.

6. Tax Payers: Undefined with siding. The group as a whole must decide whether to support immunization of children financially and provide education al programs to nurses and health care workers that will administer the shots. Due to budget constraints where will the current funds come from despite the increase number of families moving into Maryland?

References:
1. Immune Response to Vaccine Antigens: Vaccine Safety Forum: Summary of Two Workshops. The National Academies of Science, Engineering, and Medicine. Washington Dc, 1997.

2. The Truth about vaccinations, your physician knows more than the university of google. www.violentmetaphors.com . Aug 14, 2013

3. Vaccine Preventable Diseases. http://www.cdc.gov/vaccines/vpd-vac/default.htm

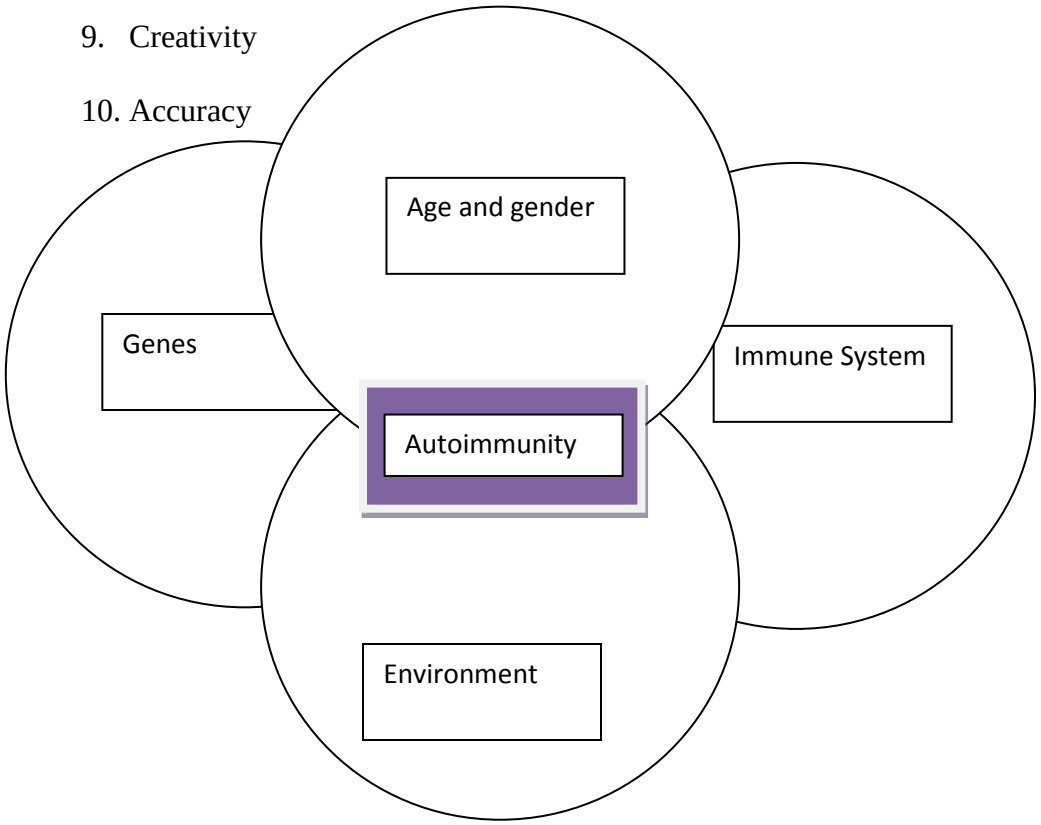
Lesson 9: PowerPoint Autoimmmunity

Content Objective(s): SWBAT: To understand that your body's immune system can mistakenly attack normal cells.

Project Guidelines

Your project should not contain more than twelve slides and must discuss one of the following autoimmune disorders: rheumatoid arthritis, Graves' disease, Lupus, myasthenia gravis, type 1 diabetes, multiple sclerosis, rheumatic fever, colitis psoriasis, scleroderma

	Point Value
1. Give the name of your autoimmune disorder.	5 points
2. Symptoms of your disorder/ parts of the body that are affected	15 points
3. Are there periods of relapse or remission? Explain.	10 points
4. Describe the infected tissues.	15 points
5. Illustrate how cryptic epitopes can lead to autoimmunity.	15 points
6. What cytokines are involved? Describe any other cells involved in the response.	15 points
7. Identification of the MHC allele involved	10 points
8. Pictures	10 points
9. Creativity	10 points
10. Accuracy	10 points



Assessments

1. IgM

A) has five antigen-binding sites.
B) has the ability to cross the placenta.
C) attaches to mast cells and basophils.
D) has five constant regions.
E) is secreted into saliva.

2. An antigen is

A) a molecule that reacts specifically with an antibody or immune lymphocyte.
B) a hypervariable region of an antibody.
C) the constant region of an antibody.
D) a cytokine.
E) none of the above.

3. Immunoglobins

A) are glycoproteins.
B) recognize and bind to foreign substances.
C) increase phagocytosis.
D) neutralize toxins.
E) all of the above.

4. The epitope is

A) the part of the antibody that binds to the antigen.
B) the part of the antibody that binds to the T helper cell.
C) C) part of the antigen that is bound by the antibody.
D) part of the hypervariable region of the antibody.
E) none of the above.

5. Opsonisation

A) is the killing of target cells by cytotoxic T cells.
B) is the secretory component of IgA.
C) helps increase phagocytosis.
D) is the interaction that allows IgG to cross the placenta.
E) none of the above.

Answers: 1. D 2. A 3. E 4. C 5. C

Lab Evaluation Sheet: Assays, Micropipettes, and Dilution rations

Immunological and Histological Lab Practices

Self-Assessment

1. **What role did you have in your lab group today? _____**
2. **What actions did you carry out in your lab group today? _____**

_____

_____

3. **On a scale of 1 -10 (1 being the lowest) what score do you feel you earned for your contribution to the lab group? _____**
4. **Explain why you feel you earned the above score.**

_____

Group Assessment

1. **Did your group meet all of the posted objectives for the lab? (yes or no)**
2. **Explain why you feel the group met the entire objective for today._____**

_____

_____

3. **On a scale of 1 -10 (1 being the lowest) what score do you feel the group earned for you're the lab assignment? _____**
4. **Explain why you feel the group earned the above score.**

_____

_____

_____

Teacher Assessment

I agree/disagree with the above score for the self-assessment. Why?

I agree/disagree with the above score for the group assessment. Why?

Oral defense rubric for PowerPoint of autoimmune disorder

Category	Scoring Criteria	Total Points	Score
Organization (10 points)	The type of presentation is appropriate for the topic and audience.	5	
	Information is presented in a logical sequence.	5	
Content (40 points)	Introduction is attention-getting, lays out the problem well, and establishes a framework for the rest of the presentation.	5	
	Technical terms are well-defined in language appropriate for the target audience.	5	
	Presentation contains accurate information.	10	
	Material included is relevant to the overall message/purpose.	10	
	points made reflect well their relative importance.	10	
Presentation (40 points)	Speaker maintains good eye contact with the audience and is appropriately animated (e.g., gestures, moving around, etc.).	5	
	Speaker uses a clear, audible voice.	5	
	Delivery is poised, controlled, and smooth.	5	
	Good language skills and pronunciation are used.	5	
	Visual aids are well prepared, informative, effective, and not distracting.	5	
	Able to answer questions about presentations from peers	5	
	Able to answer questions about presentations from teacher	10	
Score	**Total Points**	**90**	

STUDENT SECTION

Rationale: Introduction and overview of appropriate science background on autoimmunity

I. T/B cell development and immune tolerance

Healthy people/rodents have an effective **immune tolerance** toward the body's own antigens [i.e. proteins in blood, and proteins in tissues (kidney, brain, and retina)]. Tolerance means the immune cells (T cells, B cells) do not mount a response against these antigens, so the body 'tolerates' them. This is unlike the immune response to bacteria, viruses and fungi, which comprises foreign antigens.

II. Immune tolerance is the result of multiple processes occurring at the level of developing immune cells (i.e. T cells that develop and mature in the thymus and B cells that develop and mature in the bone marrow; central tolerance). Immune tolerance also occurs at the level of mature T cells in the blood and peripheral lymphoid tissues after existing the thymus and the bone marrow (peripheral tolerance).

III. Central tolerance occurs when T cells do not recognize self MHC and/or react with self-antigens presented by thymic APCs with high affinity are killed in the bone marrow (central deletion); likewise, self-reactive B cells are killed in the bone marrow. These processes are not perfect, and some self-reactive T and B cells escape into the periphery. Such immune cells are controlled by multiple mechanisms operating in the periphery (**peripheral tolerance**). These mechanisms include, but are not limited to, T regulatory cells (Treg), immunoregulatory cytokines (like IL-4 and IL-10), and induction of anergy.

IV. Autoimmunity

A. Autoimmune responses target self-antigens, and are the result of a break in self-tolerance. Potentially self-reactive T and B cells exist in all healthy individuals, but they are kept in check by mechanisms of tolerance. However, when these cells are unrestrained because of a breakdown of tolerance, then they can attack host tissues. To some extent this self-reactivity can be controlled by the immune system (subclinical autoimmunity), but when a certain threshold is crossed, clinical signs of autoimmunity start appearing.

B. Autoimmune diseases result from interplay between genetic and environmental factors. The most important genetic factors are the HLA genes. There are many environmental factors (i.e. pathogens, cigarette smoke, toxins, and dietary products). Also, the **'hygiene hypothesis'** has been proposed, suggesting that activation of the immune system by certain

microbial agents or worms can offer protection against autoimmunity. This might explain, in part, the lower incidence of autoimmunity in under-developed countries in terms of environmental hygiene compared too much higher incidence of autoimmunity in industrialized countries.

C. **Breakdown of self-tolerance** can occur because of defects in central and/or peripheral tolerance, as well as following infection by certain pathogens (bacteria, viruses). Infections can break tolerance in different ways, including **molecular mimicry** in which an antigen of a pathogen molecularly resembles a self-antigen, and therefore the immune cells cannot discriminate between a foreign and a self-antigen. The immune cells activated by foreign antigen can then attack self-antigen. Couple examples of molecular mimicry are rheumatic fever following streptococcal infection and Lyme arthritis after Borrelia infection, which is carried by a tick.

Materials: See Section V. Advance Preparation and Materials and Equipment.

Procedures:

Lesson 1:	Students will work in lab groups to read the scenario of an infection and determine the appropriate antibody based on the type of infection introduced. Ask students what they think antibodies are and how they might work.
Lesson 2	Video describes the immune system https://www.youtube.com/watch?v=G4jobV6-bFA Lecture notes on immune system and antibodies. Students work in lab groups to complete the class switching lab activity (formative assessment immunity quiz).
Lesson 3	https://www.youtube.com/watch?v=bex0itUMxmI how to micropipette Lab stations can share the food coloring or the colors can be diluted to make several sets for each station - one pipette per lab station. **Self and group analysis from Summative Assessment lab sheet.**
Lesson 4	Students wear lab gloves and then wok in lab tables to complete ELISA (simulation) to test each patient sample for antibodies against the H5N1 avian influenza virus.
Lesson 5	Give each student a copy of the flip flop student worksheet. Sepsis and the imperfect storm article reading and analysis.
Lesson 6	Students go to various websites to compare and contrast normal joint, osteoarthritis, and rheumatoid arthritis.
Lesson 7	Analyze the questions based on the article and data table and graphs.
Lesson 8	http://www.pbs.org/wgbh/pages/frontline/vaccines/view/ view video Students divided into groups and do background research on their assigned topic. Teacher ensures the discussion is respectful of different perspectives. The class is divided into groups of six, and the school board will lead the debate.
Lesson 9	Students work in maximum group of three, will pick topic based off sheet and use chrome books in class to research. PowerPoint Activity: Autoimmune disorder and oral defense (will review rubric for PowerPoint and oral defense).

Data Collection:

Lesson 2 Key

Fluorescence Label	Amount of solute 10X dye (µl) dye	Amount of solvent (distilled H_2O) µl	Total volume µl
Red	23	207	230
Blue	56	504	560
Yellow	400	3600	4000
Green	125	1125	1250

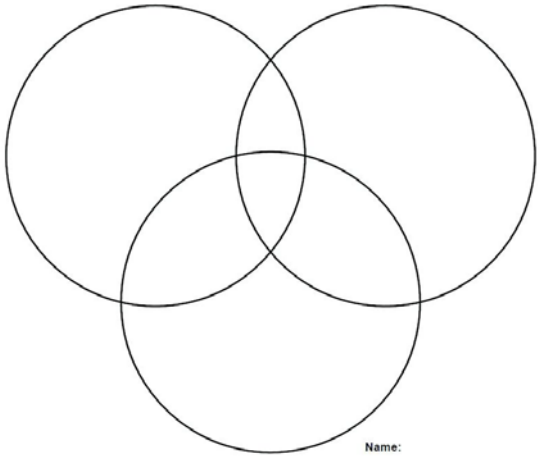
Lesson 3 Key

Normal rat	**Mild stage progression**	**Longer onset progression**
Normal synovium	Synovitis	Synovitis
No pannus or bone destruction	Mild pannus or bone destruction	Severe pannus or bone destruction
Noninflamatory	Inflammation and cartilage damage	Inflammation and severe cartilage damage

Lesson 4

Sample	Color	Test Result
Positive control	Dark purple	positive
Negative control	Light green	Negative
Patient A		
Patient B		
Patient C		
Patient D		
Patient E		
Patient F		

Lesson 6



Responses may vary based on similarities and differences

Discussion/Analysis

Lesson 1

Think of a way to differentiate between humoral and cell-mediated immunity

Lesson 2

What is the importance of diluting concentrated solutions?

Lesson 3

Why do you think the two week window was used for the study?

What if the study was only three days? Do you think the data would be similar? Explain.

Lesson 4

What basic principles of antibody-mediated immunity are utilized in an ELISA assay?

How does an ELISA indirectly detect infection by a disease-causing agent?

What is the function of the secondary antibody and chromogen in an ELISA?

Lesson 5

In-depth analysis of autoimmunity - probe for responses with audience.

Lesson 6

Remind students that the body's immune system can mistakenly attack normal cells.
With an elbow partner, turn and talk and describe what is happening in the image below.

What cells are involved, and describe the cascade of cellular events?

Lesson 7

What is being tested? What are the outcomes?

Lesson 8

Ask students to think about where they go for health information and who they trust to give them medical advice. Have students explain their answers. Then, have students compare and analyze different sources of information and evidence presented in the video.
http://www.pbs.org/wgbh/pages/frontline/vaccines/view/

Lesson 9

Able to answer questions about presentations from peers?
Able to answer questions about presentations from teacher?