

Using Rheumatoid Arthritis to Explore Autoimmunity

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I. Science Background

The immune system is the body's defense system against various pathogens. The first line of defense is our physical barriers, including our skin and mucous membranes. When a pathogen penetrates one of our physical barriers, the immune system uses recognition tools to identify the invader. In the second line of defense, the innate immune response, there are many different leukocytes that take part in actively combatting and destroying the invader. These white blood cells include: neutrophils, macrophages, basophils, mast cells, eosinophils, dendritic cells, and natural killer cells. If these cells are unable to rid the infection, then the third line of defense is initiated: the adaptive immune response. This response is exclusive to vertebrates who have acquired a more specialized response to various pathogens. Major cells of the adaptive immune response include plasma cells, memory B cells, helper T cells, cytotoxic T cells, and memory T cells. Each of these lymphocytes has special roles in taking down the invader. As this is a customized response, it takes longer to produce initially, but the memory cells that are developed can trigger a rapid response upon repeat exposure to the pathogen.

Sometimes, the immune system “goes wrong” when it identifies a part of your own body (self-antigen) as something that is foreign, and an immune response is triggered. In this unit, we will explore the concept of autoimmunity and how it occurs. We will take a “deep dive” into rheumatoid arthritis, examining how this disease is researched in the lab, as well as how this disease can be diagnosed using biotechnology. Finally, we will raise awareness about other autoimmune diseases and how they impact many people living in the US and all over the world.

II. Student Outcomes

A. Science Concepts Covered

- What is Autoimmunity?
- Molecular Mimicry Hypothesis
- T cell differentiation (cell types and signaling)
- Types of autoimmune diseases
- Rheumatoid Arthritis
 - Causes
 - Immunological breakdown
 - Animal Models
- Purpose and types of ELISA

B. Next Generation Science Standards

HS-LS1 From Molecules to Organisms: Structures and Processes

- Disciplinary Core Idea LS1.A: Structure and Function
 - *Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)*

- *All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain instructions that code for the formation of proteins, which carry out most of the work of cells. (HS-LS1-1)*
- *Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level. (HS-LS1-2)*
- **Cross-cutting concept: Systems and System Models**
 - *Models (e.g. physical, mathematical, computer models) can be used to simulate systems and interactions – including energy, matter, and information flows – within and between systems at different scales. (HS-LS1-2) (HS-LS1-4)*

HS-LS3 Heredity: Inheritance and Variation of Traits

- **Science and Engineering Practices**
 - Analyzing and Interpreting Data
 - Engaging in Argument from Evidence
- **Disciplinary Core Idea LS3.A: Inheritance of Traits**
 - *Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function (HS-LS3-1)*
- **Disciplinary Core Idea LS3.B: Variation of Traits**
 - *Environmental factors also affect expression of traits, and hence affect probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depends on both genetic and environmental factors. (HS-LS3-2) (HS-LS3-3)*
- **Cross-cutting concept: Science is a Human Endeavor**
 - *Technological advances have influenced the progress of science and science has influenced advances in technology (HS-LS3-3)*

C. Course Placement

This unit was planned for a senior elective course in Immunology. The focus on autoimmunity would be appropriate for upper-level students who have basic knowledge of the immune system. This would allow students to be exposed to what happens when the immune system “goes wrong” or becomes dysfunctional.

D. Relevance

The NIH estimates that up to 23.5 million Americans are afflicted by autoimmune disease. Rheumatoid arthritis specifically affects approximately 1% of the world population. It is highly likely that many students know someone else with an autoimmune

disease, and are curious about how autoimmunity occurs. In this unit, students will take a closer look into autoimmunity through learning about rheumatoid arthritis in depth and practicing how to diagnose a patient using ELISA. Finally, students will get to study the autoimmune disease of their choice for their final project.

III. Learning Objectives

- Students will connect what they have learned in adaptive immunity to what they are learning about autoimmunity, related to immune cell types and communication.
- Students will perform an active simulation of how rheumatoid arthritis occurs.
- Students will be able to distinguish different types of animal models.
- Students will use real data from an animal model to determine the effectiveness of a treatment for rheumatoid arthritis.
- Students will perform an anti-CCP ELISA to diagnose patients with rheumatoid arthritis.
- Students will research and present on an autoimmune disease of their choice.

IV. Time Requirements

This unit is based on a schedule with 70 minute class periods. Using this time frame, the unit should have approximately five days of material, with 2-3 additional days for the final assessment. My recommendation is to use 1-2 classes as working periods for the autoimmune disease project, so students can research their autoimmune disease thoroughly and ask thoughtful questions about the immunological breakdown. The final class will be used for presentations.

V. Advance Preparation

The Bio-Rad Biotechnology Explorer™ ELISA Immuno Explorer™ Kit will require the most advance preparation. This should be ordered before the autoimmunity unit and shipped at the appropriate time to ensure the materials are ready to go by Day 5.

Download the Autoimmune Disease Unit PowerPoint to use the teaching slides.

Advance preparation for the *Active Simulation of Rheumatoid Arthritis* will require labeling and gathering equipment (see next section). This should be set up prior to Day 2's class.

- Day 1: Print [Understanding Autoimmune Diseases](#) and *Rheumatoid Arthritis Fact Sheet*; Teaching Slides 2-5
- Day 2: Print *Active Simulation of Rheumatoid Arthritis*
- Day 3: Print *Animal Models of Rheumatoid Arthritis*; Teaching Slides 6-9
- Day 4: Print Bio-Rad Protocol III (ELISA Antibody Test): Student Introduction and Lab Guide, *ELISA Pre-Lab Questions*; Teaching Slides 10-14
- Day 5: Print *ELISA Post-Lab Questions, Autoimmune Disease Projects*

VI. Materials and Equipment

- For the *Active Simulation of Rheumatoid Arthritis*
 - Mock “antigen” (such as a spikey ball)

- 1 sports pinny
- Three cans of tennis balls (9 balls total)
- 6 small boxes (holding containers for tennis balls)
- Round stickers or labeling tape
- Pair of goggles
- Frisbee
- Stackable plastic cups
- Sharpie to label tennis balls
- Cones to mark off areas (lymph node vs. joint)
- Clipboards to fill out worksheet
- For the ELISA
 - Follow the materials listed under Protocol III in the Bio-Rad Biotechnology Explorer™ ELISA Immuno Explorer™ Kit. Distribute appropriate amount of materials based on class size.

VII. Student Prior Knowledge and Skills

Students should have prior knowledge in general biology as well as basic immunology. Students will need to have a working understanding of innate cells (ex. macrophages, neutrophils) as well as lymphocytes (especially T cells). Students should also know how antibodies function in preparation for the ELISA. Basic lab skills are also important; students must have an understanding of lab safety, know how to use lab equipment (such as a micropipette), and practice sterile technique.

VIII. Daily Unit Plans

Day 1: Introduction to Autoimmunity (Teaching Slides 2-5)

- A. Read “Understanding Autoimmune Diseases”
 - Discussion: What is autoimmunity? How many of you have heard of one of these autoimmune disorders? How many of you know someone with an autoimmune disorder?
- B. Molecular Mimicry Hypothesis
 - How could the immune system mistake its own tissues for a foreign invader?
 - One hypothesis is *molecular mimicry*. This occurs when a pathogen is molecularly very similar to one of our own tissues, and therefore, the lymphocytes that are activated to target this pathogen could potentially become self-reactive and destroy one’s own tissues.
 - Discuss a couple examples from “[Molecular Mimicry as a Mechanism of Autoimmune Disease](#)” – Table 1.
- C. T Cell Imbalance & Autoimmunity
 - Another potential issue that contributes to the breakdown of tolerance seen in autoimmunity is the imbalance of Th17 and Treg cells.
 - There are many known IL-17/Th17-associated autoimmune diseases.

D. A Closer Look into Autoimmunity: Rheumatoid Arthritis

- Complete Rheumatoid Arthritis Fact Sheet

Day 2: Active Simulation – How Rheumatoid Arthritis Occurs

- Have students perform the active simulation (following directions)
- Complete the simulation worksheet at each station
- Perform a full “run through” if time
- Review worksheet back in the classroom

Day 3: Animal Models in Rheumatoid Arthritis

- Introduction: Read excerpt from Animal Models chapter and answer questions
 - Source: Rajaiah R. and K.D. Moudgil. Animal Models, In: Rheumatoid Arthritis (eds. M.C. Hochberg, A.J. Silman, J.S. Smolen, M.H. Weisman, and M.E. Weinblatt), Mosby, Inc., an affiliate of Elsevier, Inc., 2008, Ch. 8N.
- Observations: view pictures of rheumatoid arthritis animal models
 - Teaching Slides 6-9
 - Compare control and treatment trials
- Data and Analysis: using data from the lab, make a graph comparing control and treatment trials. Calculate significant difference using a t-test.
- Conclusions: determine if this treatment is effectively reducing symptoms for rats affected by rheumatoid arthritis; should this treatment be pursued?

Day 4: Diagnosing Rheumatoid Arthritis in Patients (Teaching Slides 10-14)

- Discuss potential markers for rheumatoid arthritis
- Introduce ELISA
 - Purpose
 - Types of ELISA
 - ELISA analysis
- Introduce anti-CCP ELISA
- ELISA Pre-Lab questions

Day 5: Perform anti-CCP ELISA using Bio-Rad Biotechnology Explorer™ ELISA Immuno Explorer™ Kit.

- Review lab procedure; reminders on handling equipment
- Perform ELISA
- Answer Post-Lab questions

IX. Final Assessment

Autoimmune Disease Project

The goal of this project is to make an informative poster or brochure that will promote awareness for a particular autoimmune condition. The following information should be addressed:

- Basic definition of the condition

- Signs & symptoms
- Causes
 - Provide specific detail on immunological breakdown
 - Potential genetic factors
- Diagnostics
 - How doctors diagnose the condition; any particular tests
- Treatments
 - How they target the causes and/or symptoms
- Foundations, organizations, or support groups
 - How they support dealing with this condition; any events (such as walks)
- Misc. information
 - Other ways to cope (e.g. dietary changes, lifestyle changes)
 - Any population group “at risk” for this condition
 - Any notable people with this condition

Your project will be evaluated on the following factors:

1. Depth and accuracy of information **(70%)**
2. Visual content: use of pictures and/or diagrams **(10%)**
3. Overall appearance: clear organization of content, readability (e.g. font size), attention to detail (spelling, grammar, etc.) **(10%)**
4. Citations: provide sources for all information in APA format **(10%)**

Autoimmune Disease Project Teacher Rubric:

Each major category is worth 5 points. This scale is generalized so that it can be applied to all autoimmune disease projects, however, teachers should familiarize themselves with the autoimmune conditions chosen by the students, so each presentation can be evaluated appropriately. Many of these categories have several factors that need to be addressed, and teachers must evaluate not only if the factors are addressed, but also how well they are addressed through different levels of detail and supporting visuals.

	5	4	3	2	1	0
Basic definition	Mentions all major details	Missing an important distinguishing characteristic	“An autoimmune disease that affects -----”	“An autoimmune disease”	“A condition”	None
Signs & symptoms	All major symptoms provided	Missing 1 major symptom	Missing more than 1 major symptom	A few symptoms provided	1 or 2 symptoms provided	None
Causes	Names mutation(s), names affected chromosome(s), says which cell(s) are impacted, and describes how the immune	Names mutation(s), names affected chromosome(s), and which cell(s) are impacted	Names a mutation and which cell is impacted	“a mutation causes a breakdown in the immune response”	“caused by a mutation”	None

	response is impacted					
Diagnostics	Names several tests, their purpose, and relates back to signs/symptoms	Names several tests and their general purpose	Names 2 tests and their purpose	Names more than 1 test	Names 1 test	None
Treatments	Names several treatments and their specific purposes	Names at least 2 treatments with specific purposes	Names 2 treatments, general purpose	Names more than 1 treatment	Names 1 treatment	None
Foundations/ Organizations/ Support groups	Names several foundations/ organizations in addition to support groups; includes specific events	Names several foundations/ organizations in addition to support groups	Names 1 foundation/ organization; Mentions support groups	Names 1 foundation/ organization	“There are support groups”	None
Misc. info	Details other ways to cope (lifestyle changes); provides, population group at risk, provides notable person and their profession	Provides all 3 pieces of misc. info with good detail. Either missing 1 key point or provides incorrect info.	Provides all 3 pieces of misc. info, but missing details (ex. need to expand on ways to cope)	Provides 2 pieces of misc. info	Only provides 1 piece of misc. info	None
Visual content	Several pictures and/or diagrams that illustrate key points	Includes diagram with pictures / good quality	More than 1 picture / good quality	1 picture / large but poor quality	1 picture / small and poor quality	None
Overall appearance	Draws viewers in, clear organization, large font size, attention to detail	One key factor could be improved (ex. organization)	Somewhat organized, readable font size, minimal grammar/ spelling mistakes	Somewhat organized, small font, some grammar/ spelling mistakes	Disorganized with labels, small font, many grammar/ spelling mistakes	No labels, small font, many grammar/ spelling mistakes
Citations	Correct APA format; at least 3 full citations	Small formatting error; more than 1 full citation	Incorrect compilation of author name, title, links OR Only 1 full citation	Titles and links	Only links	None

Total points: ____/50

X. Student Section

A. Rheumatoid Arthritis Facts Sheet

Rheumatoid Arthritis Fact Sheet

In groups, use the recommended resources to answer the following questions:

1. There are approximately _____ million people in the US who are affected by rheumatoid arthritis.
2. This disease is characterized by chronic inflammation that impacts the _____ of the body, particularly the hands, wrists, knees, ankles, and feet.
3. Rheumatoid arthritis typically appears in people between _____ - _____ years old.
4. There are about three times as many _____ compared to _____ who have rheumatoid arthritis.
5. There are certain genetic factors that make someone more susceptible to rheumatoid arthritis, including:
 - a.
 - b.
 - c.
6. There are certain environmental factors that make someone more susceptible to rheumatoid arthritis, including:
 - a.
 - b.
 - c.
7. Rheumatoid arthritis is typically diagnosed with the help of laboratory tests, such as the _____ factor test, the anti-_____ antibody test, and other blood tests such as _____ blood cell count.

Resources:

Arthritis Foundation. *Rheumatoid Arthritis*. <http://www.arthritis.org/about-arthritis/types/rheumatoid-arthritis/>

National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Handout On Health: Rheumatoid

Arthritis. https://www.niams.nih.gov/health_info/Rheumatic_Disease/default.asp#ra_9

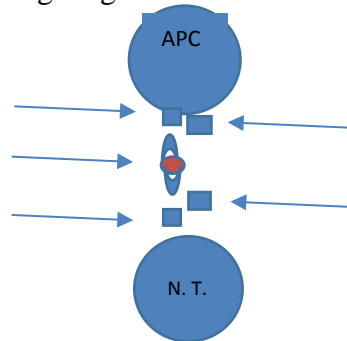
Rheumatoid Arthritis Support Network. *Rheumatoid Arthritis Facts and Statistics*. <https://www.rheumatoidarthritis.org/ra/facts-and-statistics/>

B. Active Simulation of Rheumatoid Arthritis

Simulation of Rheumatoid Arthritis

Station 1

1. What two signals must the Naïve T cell receive from the APC in order to become activated?
2. Label the following diagram:



3. Where does T cell activation occur?

Station 2

4. What three cytokines does the APC send the Activated T cell?
5. Provide two transcription factors that have been upregulated and explain why this is important.
6. What kind of effector cell has been developed?

Station 3

7. Why does the Th17 cell “run a lap?”

8. Where does the Th17 cell end up?

Station 4

9. What are two important cytokines released by Th17?

10. Which innate immune cells cause inflammation in the joint using IL-1 and TNF?

11. When receiving pro-inflammatory cytokines, the osteoclast cells will contribute to bone erosion through releasing enzymes such as MMPs (matrix metalloproteinases). In this simulation, what is representing the enzyme? What is representing the bone matrix?

C. Animal Models

Animal Models in Rheumatoid Arthritis

Introduction

Source: Rajaiah R. and K.D. Moudgil. *Animal Models*, In: *Rheumatoid Arthritis* (eds. M.C. Hochberg, A.J. Silman, J.S. Smolen, M.H. Weisman, and M.E. Weinblatt), Mosby, Inc., an affiliate of Elsevier, Inc., 2008, Ch. 8N.

1. What are the two major types of arthritis models and what is the main difference between these models?
2. What are three common features seen in these arthritis models across the board?
3. Observe Table 8N-1.
 - a. Which rat strain is most widely used in the experimentally induced arthritis models?
 - b. What type of cell is typically drives the development of disease?
 - c. The spontaneously induced arthritis models will use either *transgenic* mice (such as K/BxN) or *knockout* mice (such as BALB/cA IL-1Ra^{-/-}). In each case, what do you think is happening to the mouse genes?
4. We will focus our studies on adjuvant-induced arthritis (p.218).
 - a. What kind of microbial product is used?
 - b. How long does it take to develop the disease?
 - c. What is the scale used to rate the severity of AA?
 - d. How could the paw be quantitatively measured?
5. See Figure 8N-1 to distinguish each phase of adjuvant-induced arthritis.

Data and Analysis

Day	Arthritic control				Anti-arthritic drug			
	Rat 1	Rat 2	Rat 3	Average	Rat 1	Rat 2	Rat 3	Average
0	0	0	0		0	0	0	
2	0	0	0		0	0	0	
4	0	0	0		0	0	0	
6	0	0	0		0	0	0	
8	0	0	0		0	0	0	
10	0	1	1		1	1	0	
12	1	2	2		2	2	1	
14	2	3	3		2	2	2	
16	4	5	4		3	3	2	
18	5	6	6		2	2	1	
20	4	5	5		2	3	2	
22	3	4	4		1	2	1	

6. Using the data above, calculate averages and create a graph that best displays the progression of adjuvant-induced arthritis in the control and treatment groups.

7. Compare the control group and treatment group using a t-test and calculate the statistical significance in arthritis scores.

Conclusions

8. Referencing the data, does this particular treatment appear to be effective in reducing the symptoms of arthritis? Why or why not? Should this treatment be pursued in further research?

D. ELISA Pre-Lab Questions

Pre-Lab Questions:

1. ELISA is an acronym that stands for _____ - _____
_____.
2. In immunology, what basic knowledge of antibodies is key to understanding how an ELISA works?
3. Describe the respective roles of the primary antibody and a secondary antibody.
4. What is the name of the enzyme and the substrate used to produce a color change?
5. Which samples are being used as the positive control and the negative control in this experiment? What is the purpose of these controls?
6. Anti-CCP antibodies are a specific kind of autoantibody that target abnormal citrullinated peptides prevalent in RA patients. What are autoantibodies, in general?

E. ELISA Post-Lab Questions

Post-Lab Questions:

1. Why did you test each patient's serum in 3 wells instead of 1 well?
2. What is the importance of the wash step? Discuss three major reasons, based on when the wash step is used in the procedure.
3. Looking at the class results, which patients' serum contained anti-CCP antibodies?

4. Were there any unexpected results (ex. false negative, false positive)? What are three potential sources of error in this experiment?
5. Anti-CCP antibodies are present in some people even before developing symptoms of RA.¹ Who could benefit from testing for anti-CCP antibodies? How might this change the course of the disease?

XI. Teacher Answer Keys

A. Rheumatoid Arthritis Facts Sheet

Rheumatoid Arthritis Fact Sheet

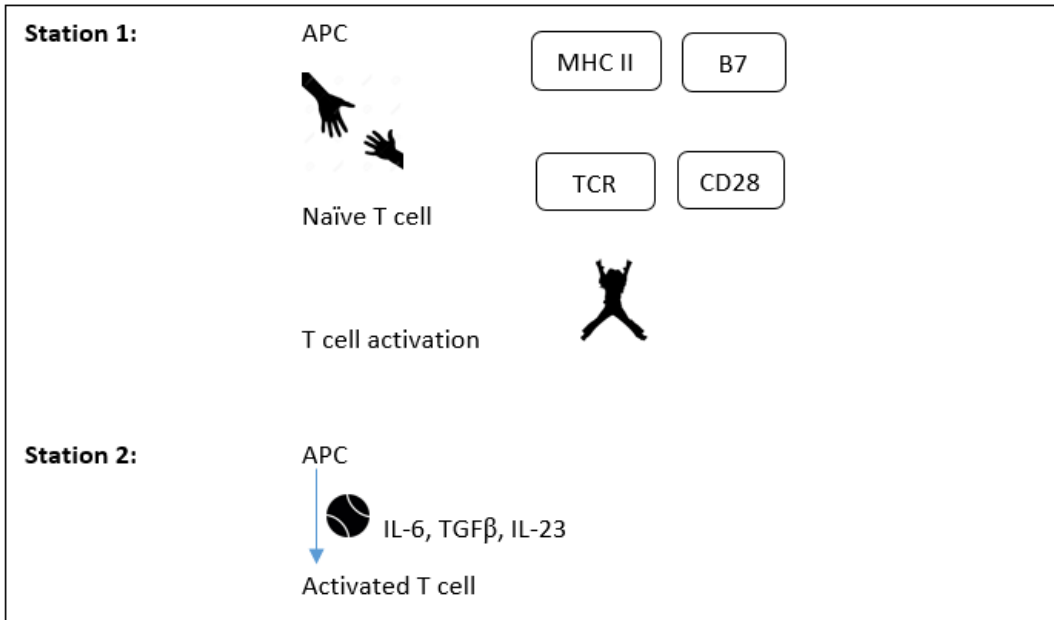
1. There are approximately 1.3 million people in the US who are affected by rheumatoid arthritis.
2. This disease is characterized by chronic inflammation that impacts the joints of the body, particularly the hands, wrists, knees, ankles, and feet.
3. Rheumatoid arthritis typically appears in people between 30-60 years old.
4. There are about three times as many women compared to men who have rheumatoid arthritis.
5. There are certain genetic factors that make someone more susceptible to rheumatoid arthritis, including:
 - a. HLA (MHC gene)
 - b. STAT4
 - c. TRAF1
 - d. PTPN22
6. There are certain environmental factors that make someone more susceptible to rheumatoid arthritis, including:
 - a. Cigarette smoke
 - b. Insecticides
 - c. Silica
7. Rheumatoid arthritis is typically diagnosed with the help of laboratory tests, such as the rheumatoid factor test, the anti-CCP antibody test, and other blood tests such as white blood cell count.

¹ Mandl LA, Chibnik L, Schur P, Karlson EW. Anti-cyclic citrullinated peptide (Anti-CCP) antibodies are strongly associated with risk of rheumatoid arthritis after adjusting for hormonal and behavioral factors, *Arthritis Rheum*, 2005, vol. 52 suppl.pg. S732

B. Active Simulation of Rheumatoid Arthritis

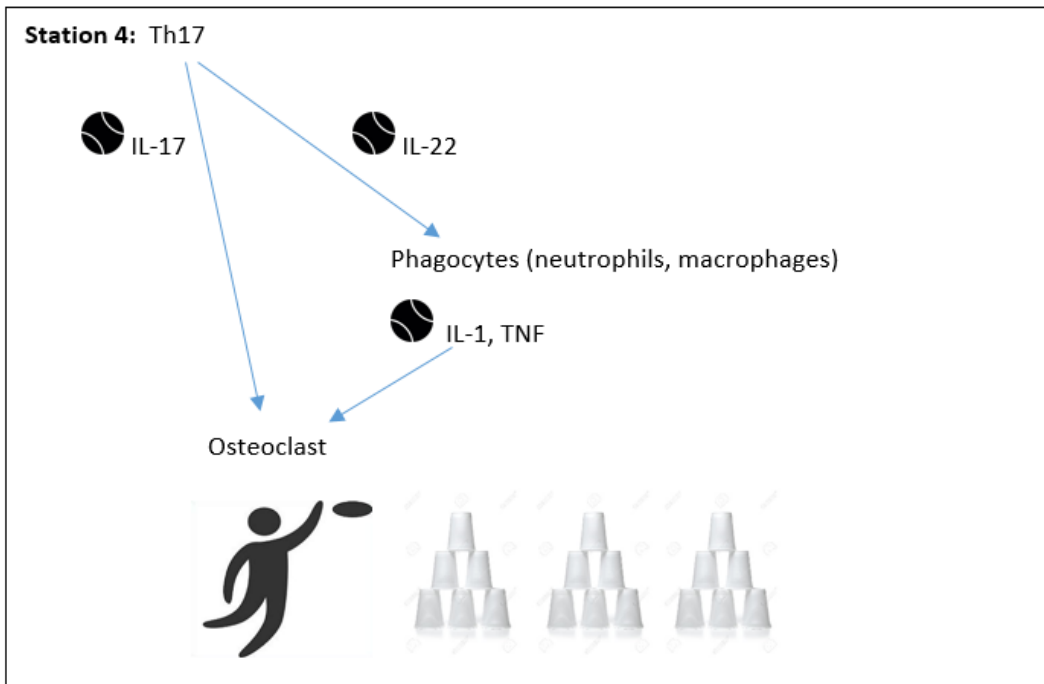
Active Simulation of Rheumatoid Arthritis: Game Plan

LYMPH NODE



Station 3: Effector T cell (Th17)

THE JOINT



Station Descriptions

STATION 1: Get two student volunteers. Label one person as the **APC** (Antigen Presenting Cell) and the other person as the **Naïve T cell**. Using stickers or labeling tape, mark the hands of the APC as “**MHC II**” and “**B7**.” Mark the hands of the Naïve T cell as “**TCR**” and “**CD28**.” Provide a spikey ball or other unique object to the MHC II hand to be presented as the “**antigen**.” Then, have students recall which ‘hand’ must connect with which in order for T cell activation to occur. Once the Naïve T cell becomes activated, he/she will do **10 jumping jacks** and puts on a **sports pinny**.

STATION 2: Using the same student volunteers, instruct the **APC** to pull each tennis ball from the bin and shout its name as it is being tossed to the **Activated T cell**. The Activated T cell will catch the tennis ball, repeat the name, and put the tennis ball into its respective bin. This will allow students to write down the names of the tennis balls (**IL-6, TGFβ, IL-23**) being transmitted from cell to cell. Once the Activated T cell has received all three cytokines, he/she will put on a pair of **goggles** which have been labeled with transcription factors **RORγT** and **STAT3** (labeling tape can be used to mark the sides of the goggles).

STATION 3: The **Effector T cell** (now sporting a pinny and goggles) will run a lap around the floor to mimic circulation before moving to the joint area.

STATION 4: Upon entering the joint, the **Effector T cell/Th17 cell** will find a bin with two tennis balls: **IL-17** and **IL-22**. Two students may play the role of phagocytes (neutrophils and macrophages, respectively). The phagocytes will have their own bin of tennis balls (**IL-1** and **TNF**) sitting at about the midpoint of the station. Another student will play the role of the **osteoclast**, located towards the bottom of the station. At the very end will be a large stack of **plastic cups** (or several stacks next to each other). The Th17 cell will send IL-22 to the phagocytes, which in turn will send IL-1 and TNF to the osteoclast. In addition, the Th17 cell will send IL-17 to the osteoclast. Upon receiving these three cytokines, the osteoclast has 3 **frisbee** throws to knock down the plastic cups. The frisbee represents MMPs (matrix metalloproteinases) which help to break down proteins in the bone matrix, represented by the cups.

Teacher’s Note: You may decide to have a “walk through” with one group of students and then do a “run through” with a different group of students to get everyone to participate. You could also have “station masters” for those who might prefer directing others at each station. To make this competitive, you could see which group of students is able to move through the whole sequence the fastest. Make sure students have time to answer questions at each station.

Extension Activity: If time, have another student act as a **Treg** cell. This would be most important at the joint – Treg can send **IL-10 & TGFβ** to **Th17** and the **osteoclast** which would then subdue their activity. For instance, if the osteoclast receives these cytokines, it will only be permitting one frisbee throw instead of three. Ask students how increasing the population of Tregs might change the overall damage occurring to the bone in rheumatoid arthritis.

Simulation of Rheumatoid Arthritis

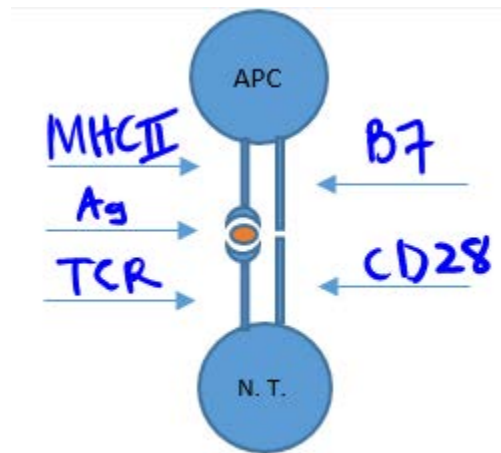
Station 1

1. What two signals must the Naïve T cell receive from the APC in order to become activated?

Signal 1: MHC II presenting antigen

Signal 2: B7

2. Label the following diagram:



3. Where does T cell activation occur?

In the lymph node

Station 2

4. What three cytokines does the APC send the Activated T cell?

IL-6, TGF β , IL-23

5. Provide two transcription factors that have been upregulated and explain why this is important.

ROR γ T and STAT3. These transcription factors determine the type of effector T cell by promoting the expression of certain cytokines.

6. What kind of effector cell has been developed?

Th17 cell.

Station 3

7. Why does the Th17 cell “run a lap?”

The Th17 cell must go through circulation first before going to the target site.

8. Where does the Th17 cell end up?

The joint.

Station 4

9. What are two important cytokines released by Th17?

IL-17 and IL-22.

10. Which innate immune cells cause inflammation in the joint using IL-1 and TNF?

Phagocytes: macrophages and neutrophils.

11. When receiving pro-inflammatory cytokines, the osteoclast cells will contribute to bone erosion through releasing enzymes such as MMPs (matrix metalloproteinases). In this simulation, what is representing the enzyme? What is representing the bone matrix?

The frisbee represents the enzyme, whereas the stacks of cups represent the bone matrix. The osteoclast releases this enzyme to weaken (and eventually destroy) the bone matrix.

C. Animal Models

Introduction

Source: Rajaiah R. and K.D. Moudgil. *Animal Models, In: Rheumatoid Arthritis* (eds. M.C. Hochberg, A.J. Silman, J.S. Smolen, M.H. Weisman, and M.E. Weinblatt), Mosby, Inc., an affiliate of Elsevier, Inc., 2008, Ch. 8N.

1. What are the two major types of arthritis models and what is the main difference between these models?

Two major types of arthritis animal models are:

- (i) Experimentally induced arthritis model
- (ii) Spontaneously induced arthritis model

The main difference between these models is how the disease ‘comes about’ or how it is triggered in the animal model. Experimentally induced arthritis models use microbial products or other antigens known to trigger the immune system to attack the joint. Spontaneously induced arthritis models depend on a genetic mutation or a deficiency that would cause issues such as inflammation or autoreactive T cells.

2. What are three common features seen in these arthritis models across the board?

- (i) Synovial hyperplasia (enlargement of the synovium due to an increasing # of cells)
- (ii) Mononuclear cell infiltration (mononuclear cells include lymphocytes as well as monocytes)
- (iii) Cartilage and bone erosion

3. Observe Table 8N-1.
 - a. Which rat strain is most widely used in the experimentally induced arthritis models?
Lewis rat
 - b. What type of cell is typically drives the development of disease?
T cells
 - c. The spontaneously induced arthritis models will use either *transgenic* mice (such as K/BxN) or *knockout* mice (such as BALB/cA IL-1Ra^{-/-}). In each case, what do you think is happening to the mouse genes?
Transgenic mice: gene is being manipulated (ex. gene that affects the TCR)
Knockout mice: gene is nonfunctional (ex. IL-1Ra)

4. We will focus our studies on adjuvant-induced arthritis (p.218).
 - a. What kind of microbial product is used?
Heat-killed *Mycobacterium tuberculosis*

 - b. How long does it take to develop the disease?
10-12 days

 - c. What is the scale used to rate the severity of AA?
0 to 4 with the maximum arthritic score of 16 per rat (x4 paws)

 - d. How could the paw be quantitatively measured?
Paw thickness and paw volume can be physically measured

5. See Figure 8N-1 to distinguish each phase of adjuvant-induced arthritis.
Naïve (Day 0) 🔴 Onset (Days 10-12) 🔴 Peak (Days 16-18) 🔴 Recovery (Days 19-29)

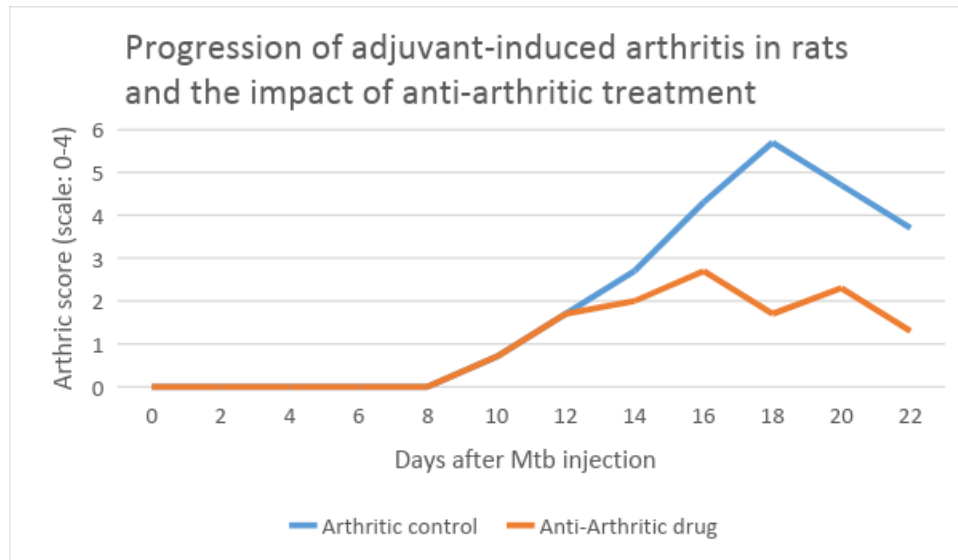
Data and Analysis

Day	Arthritic control				Anti-arthritic drug			
	Rat 1	Rat 2	Rat 3	Average	Rat 1	Rat 2	Rat 3	Average
0	0	0	0	0.0	0	0	0	0.0
2	0	0	0	0.0	0	0	0	0.0
4	0	0	0	0.0	0	0	0	0.0
6	0	0	0	0.0	0	0	0	0.0
8	0	0	0	0.0	0	0	0	0.0
10	0	1	1	0.7	1	1	0	0.7
12	1	2	2	1.7	2	2	1	1.7
14	2	3	3	2.7	2	2	2	2.0
16	4	5	4	4.3	3	3	2	2.7
18	5	6	6	5.7	2	2	1	1.7
20	4	5	5	4.7	2	3	2	2.3

22	3	4	4	3.7	1	2	1	1.3
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Figure 1. Impact of anti-arthritic drug on arthritic score in Lewis rats.

- Using the data above, calculate averages and create a graph that best displays the progression of adjuvant-induced arthritis in the control and treatment groups.



- Compare the control group and treatment group using a t-test and calculate the statistical significance in arthritis scores.

T-test values show statistical significance ($p < .05$) on days 16-22.

Day 16: $p = 0.012$

Day 18: $p = 0.0005$

Day 20: $p = 0.004$

Day 22: $p = 0.004$

Conclusions

- Referencing the data, does this particular treatment appear to be effective in reducing the symptoms of arthritis? Why or why not? Should this treatment be pursued in further research?

Students must explain effectiveness of treatment based on the data provided. Statistical significance between control and treatment trials is shown around peak phase. Treatment should be pursued in further research.

D. ELISA Pre-Lab Questions

1. ELISA is an acronym that stands for _____ - _____
_____.

Enzyme-linked immunosorbent assay.

2. In immunology, what basic knowledge of antibodies is key to understanding how an ELISA works?

Antibodies target a specific antigen. If the antibody and antigen have complementary structures, they will bind to form an antibody-antigen complex. In an ELISA, one can determine whether or not a patient's serum contains the antibody of interest by providing the specific antigen.

3. Describe the respective roles of the primary antibody and a secondary antibody.

A primary antibody is specific for the antigen being placed in the well. A secondary antibody attaches to the constant region of the primary antibody. The secondary antibody is conjugated with an enzyme that will react with a substrate to produce a color change.

4. What is the name of the enzyme and the substrate used to produce a color change?

HRP (horseradish peroxidase) enzyme and TMB (tetramethylbenzidine) substrate.

5. Which samples are being used as the positive control and the negative control in this experiment? What is the purpose of these controls?

The serum containing anti-CCP antibodies is the positive control. The serum from the unaffected person is the negative control. We must compare our test results with the positive and negative control. If the positive control is colorless, or the negative control contains color, this indicates an experimental error. In this case, the experiment would need to be performed again to get more reliable results.

6. Anti-CCP antibodies are a specific kind of autoantibody that target abnormal citrullinated peptides prevalent in RA patients. What are autoantibodies, in general?

Autoantibodies are created in an autoimmune response (auto- meaning "self"). This occurs when a person's immune cells mistakenly view their self-antigen as foreign. After an immune response has been triggered, activated plasma cells will produce autoantibodies to target this antigen.

E. ELISA Post-Lab Questions

1. Why did you test each patient's serum in 3 wells instead of 1 well?

It is important to have three trials of each sample for consistency. If there is a color difference between the three wells of the same person, this indicates variation within the

samples or potentially experimental error.

2. What is the importance of the wash step? Discuss three major reasons, based on when the wash step is used in the procedure.

The wash step is used so that only the bound antigen and antibody remain in the well. The first major wash step gets rid of any unbound antigen. The second major wash step gets rid of any primary antibody that has not bound to the antigen (i.e. irrelevant antibodies). The third major wash step gets rid of free-floating secondary antibody (when there is no primary antibody to bind to). This ensures that the color change is only seen in wells that have the antigen, primary antibody, and secondary antibody bound together.

3. Looking at the class results, which patients' serum contained anti-CCP antibodies?
Students discuss their results.

4. Were there any unexpected results (ex. false negative, false positive)? What are three potential sources of error in this experiment?
Students discuss their results.

Three potential sources of error include: using the same tip for different samples, samples splashing between wells, not waiting 5 minutes for antigen or antibodies to bind.

5. Anti-CCP antibodies are present in some people even before developing symptoms of RA.² Who could benefit from testing for anti-CCP antibodies? How might this change the course of the disease?

Close family members could benefit from testing for anti-CCP antibodies (for example, if your mother or sibling has RA). In addition, those who are exposed to certain environmental factors (mineral oil, silica, cigarette smoke, air pollution) over long periods of time could be tested. This might change the course of disease by allowing the person to take preventative measures and even get early treatment in order to delay the onset and reduce the severity of rheumatoid arthritis.

² Mandl LA, Chibnik L, Schur P, Karlson EW. Anti-cyclic citrullinated peptide (Anti-CCP) antibodies are strongly associated with risk of rheumatoid arthritis after adjusting for hormonal and behavioral factors, *Arthritis Rheum*, 2005, vol. 52 suppl.pg. S732