Using Rheumatoid Arthritis to Explore Autoimmunity

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Table of Contents

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 path2( )sood c-4( )-10(r)-1(es)-

D. A Closer Look into Autoimmunity: Rheumatoid Arthritis
  " Complete Rheumatoid Arthritis Fact Sheet

Day 2: Active Simulation – How Rheumatoid Arthritis Occurs

A. Have students perform the active simulation (following directions)
B. Complete the simulation worksheet at each station
C. Perform a full "run through" if time
D. Review worksheet back in the classroom

Day 3: Animal Models in Rheumatoid Arthritis

A. 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.
B. Observations: view pictures of rheumatoid arthritis animal models
  " Teaching Slides 6-9
  " Compare control and treatment trials
C. Data and Analysis: using data from the lab, make a graph comparing control and treatment trials. Calculate significant difference using a t-test.
D. 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)

A. Discuss potential markers for rheumatoid arthritis
B. Introduce ELISA
  " Purpose j

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

	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/

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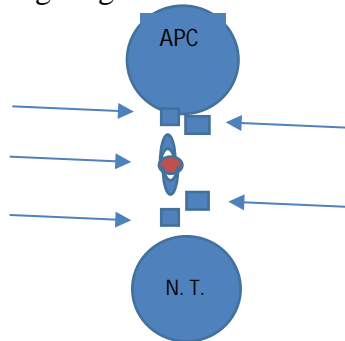
Rheumatoid Arthritis Support Network. *Rheumatoid Arthritis Facts and Statistics.* https://www.rheumatoidarthritis.org/ra/facts-and-statistics/

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?

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.

B. Active Simulation of Rheumatoid Arthritis

THE JOINT
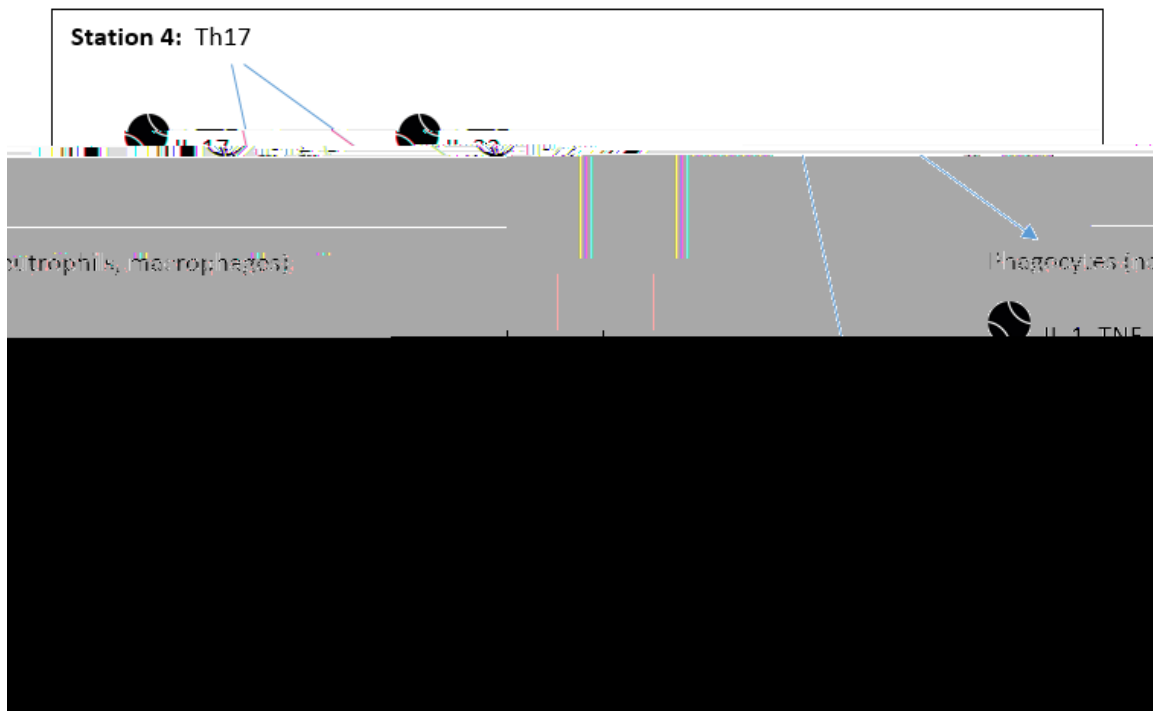
STATION 1: Get two student volunteers. Label one person as the **APC** (Antigen Presenting Cell) and the other person as the **Naïve T cell**. Using stickers or labeling tape, mark the hands of the APC as "**MHC II**" and "**B7**." Mark the hands of the Naïve T cell as "**TCR**" and "**CD28**." Provide 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-   7 *)   -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 5 2 5   7and **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 r2(N)2(4(y)20(t)-2Al)-2(4(r T(20(t)(t)-2(e)4ha)4(ve)4( )-1(, -15ha)4(o)-

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

22	3	4	4	3.7	1	2	1	1.3

Figure 1. Impact of anti-arthritic drug on arthritic score in Lewis rats.

6.

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?

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