

AUTOIMMUNITY

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WHAT IS AUTOIMMUNITY?

Understanding Autoimmune Diseases (NIH)

Autoimmunity occurs when the immune system loses the ability to distinguish “self” from “non-self” and mistakenly attacks one’s own cells or tissues.

There are over 80 autoimmune diseases currently identified. Some are organ-specific whereas others are systemic (occurring throughout the body).

http://autoimmune.pathology.jhmi.edu/whatis_spectrum.cfm

MOLECULAR MIMICRY HYPOTHESIS

Molecular mimicry occurs when the structure of a foreign antigen is very similar to antigen present on our own cells or tissues:

- Upon presentation of the foreign antigen, lymphocytes are activated to target this antigen
- These lymphocytes could potentially also react to self-antigen
- This leads to the destruction of one's own cells or tissues

Molecular Mimicry as a Mechanism for Autoimmune Disease

(Cusick, M., Libbey, J., and Fujinami, R. Clin Rev Allergy Immunol. 2012 Feb; 42(1): 102–111.)

T CELL IMBALANCE & AUTOIMMUNITY

Another potential issue that contributes to the breakdown of tolerance seen in autoimmunity is an imbalance between Th17 and Treg cells

https://openi.nlm.nih.gov/detailedresult.php?img=PMC2670432_nrs07003.f6&req=4

The following autoimmune diseases are known to be related to Th17/IL-17 production:

- Rheumatoid Arthritis
- Inflammatory Bowel Disease
- Multiple Sclerosis
- Psoriasis
- Ankylosing Spondylitis
- Sjögren's Syndrome

A CLOSER LOOK INTO AUTOIMMUNITY: RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory condition that impacts the lining of the joints. Many of our joints are surrounded by a synovial membrane – and when this area becomes inflamed, it leads to cartilage thinning and bone loss.

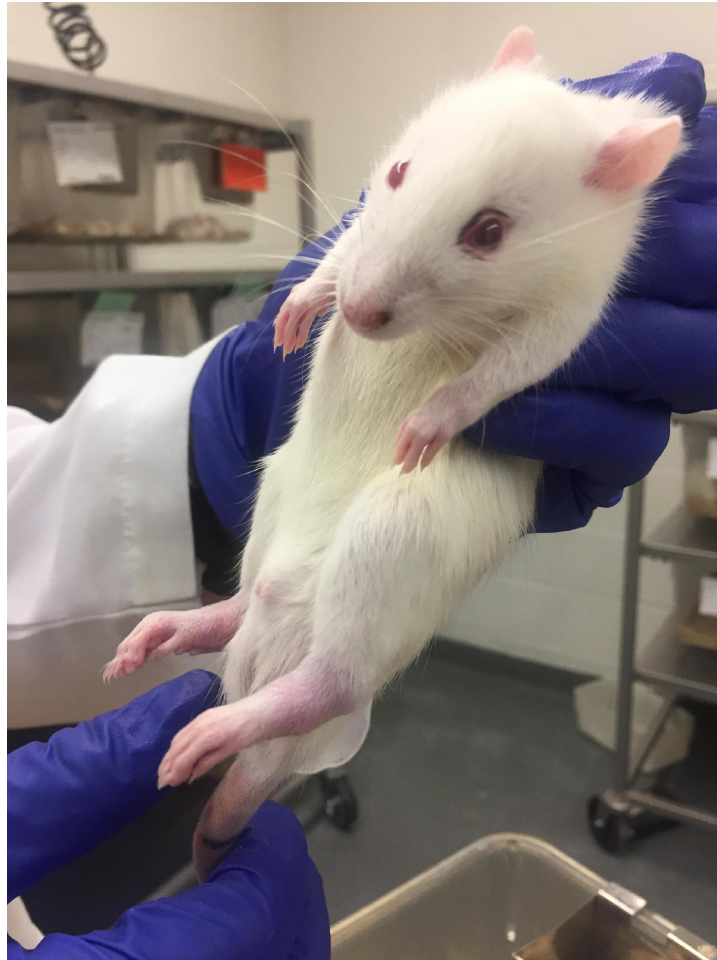
https://www.niams.nih.gov/health_info/Rheumatic_Disease/default.asp

RHEUMATOID ARTHRITIS: ANIMAL MODEL

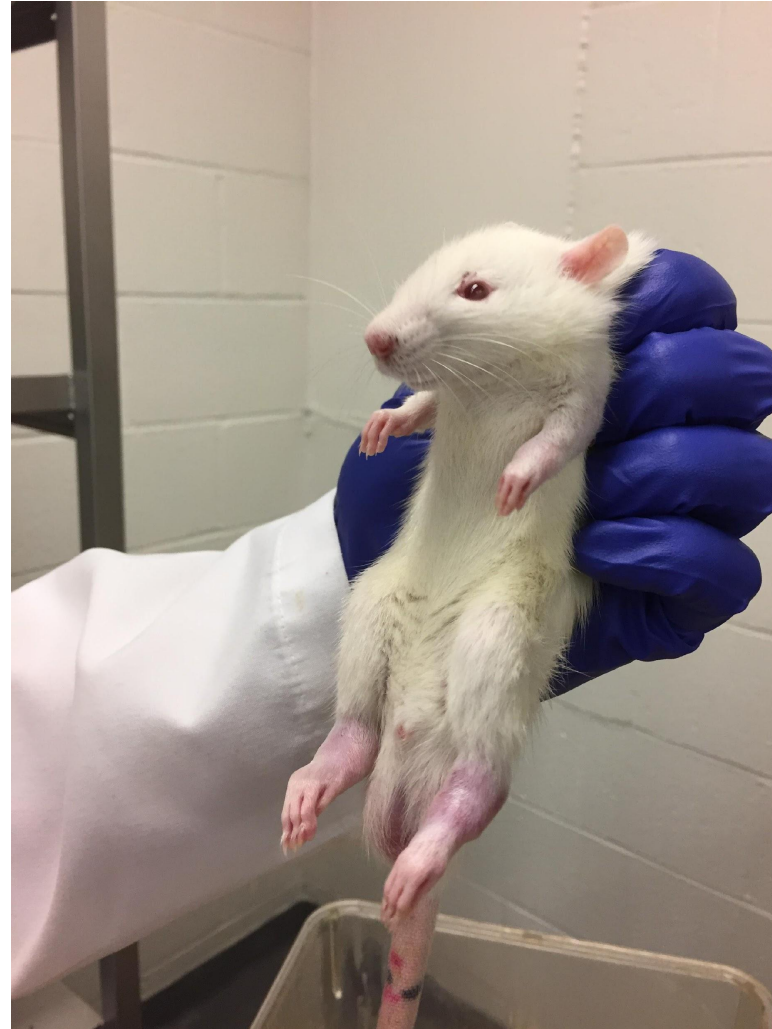
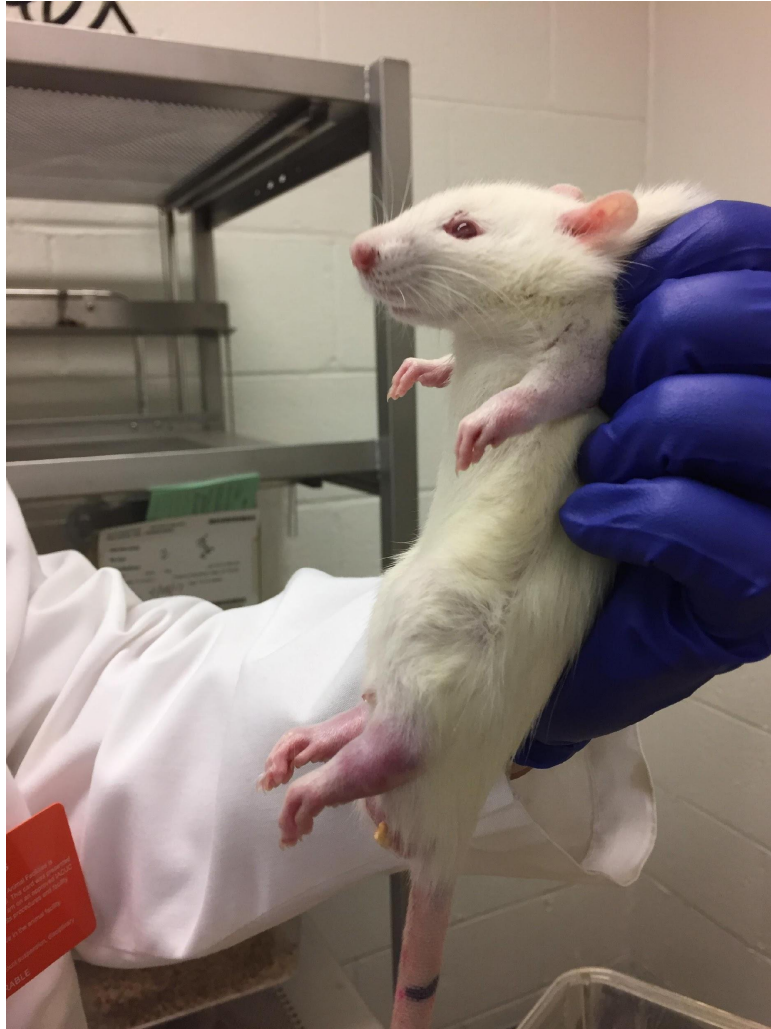
- Adjuvant-induced Arthritis in Lewis Rats



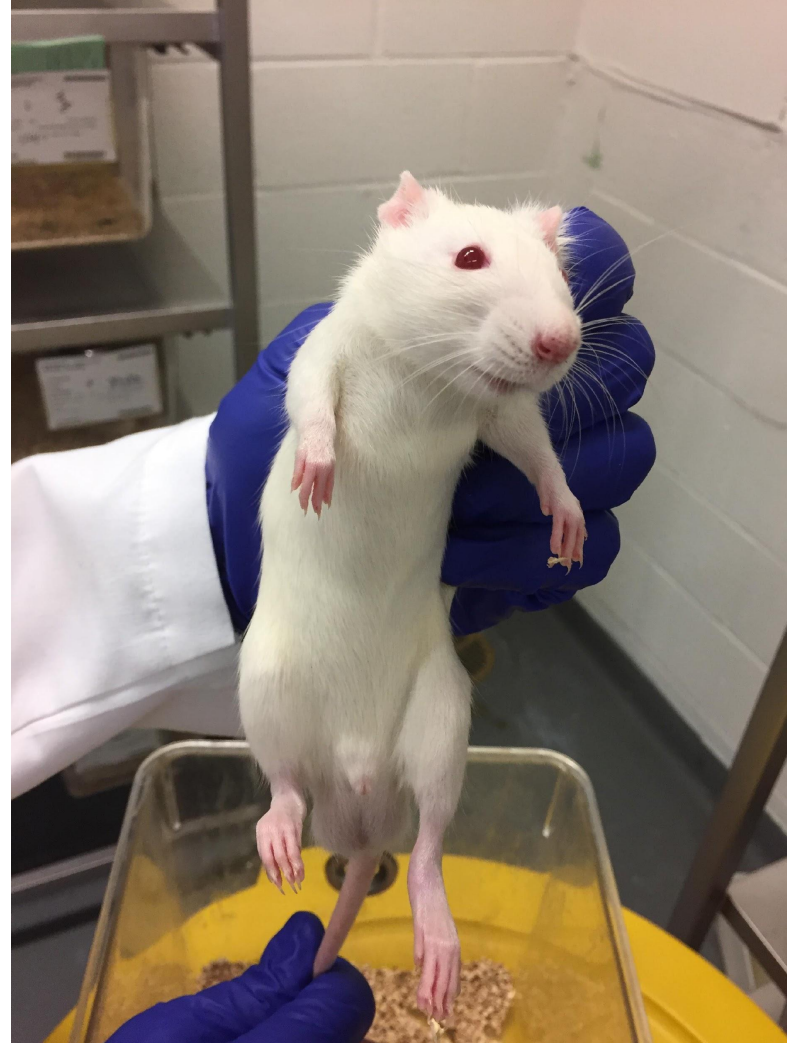
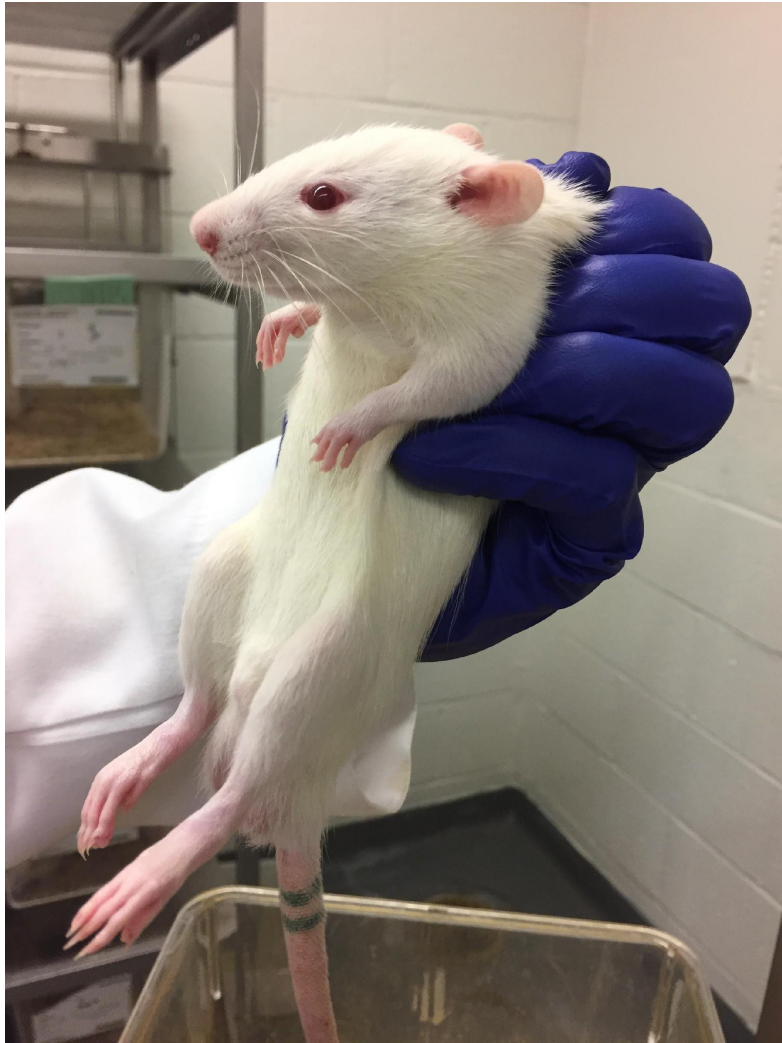
ONSET OF DISEASE & ADMINISTERING TREATMENT



DEVELOPMENT OF DISEASE: CONTROL



DEVELOPMENT OF DISEASE: TREATMENT



DIAGNOSING RHEUMATOID ARTHRITIS

As seen in the active simulation, one of the most important cytokines released by Th17 cells is IL-17, which promotes bone erosion through osteoclasts. However, there are many IL-17/Th17-mediated autoimmune diseases.

Other potential markers for rheumatoid arthritis include:

- Rheumatoid Factor (RF)
 - Occurs in ~80% of people with RA, but also present in other inflammatory diseases
- Anti-CCP antibodies
 - Occurs primarily in people with RA (stronger indication)

These markers can be used alongside other tests to diagnose someone with rheumatoid arthritis.

- How are these markers found?

ENZYME-LINKED IMMUNOSORBENT ASSAY

The enzyme-linked immunosorbent assay (ELISA) is used to detect the presence of an antibody or antigen in a particular blood sample.

- Presence of antibody can be used to identify allergies or serious infections
- Presence of antigen can be used for drug testing, pregnancy tests (hCG hormone)

http://www.elisa-antibody.com/uploads/Clean_Lilaic/ELISA-Home%20Pregnancy%20Test.jpg

http://www.biology.arizona.edu/immunology/activities/elisa/graphics/elisa_plate.gif

In the case of rheumatoid arthritis, the ELISA technique can be used to look at many markers, including IL-17, rheumatoid factor, and anti-CCP antibodies.

TYPES OF ELISA

- Direct ELISA
- Indirect ELISA
- Sandwich ELISA

<https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html>

To compare Direct and Indirect ELISA: watch this [animation](#) and take the quiz.

Sandwich ELISAs are considered to be very effective because they have high sensitivity and specificity.

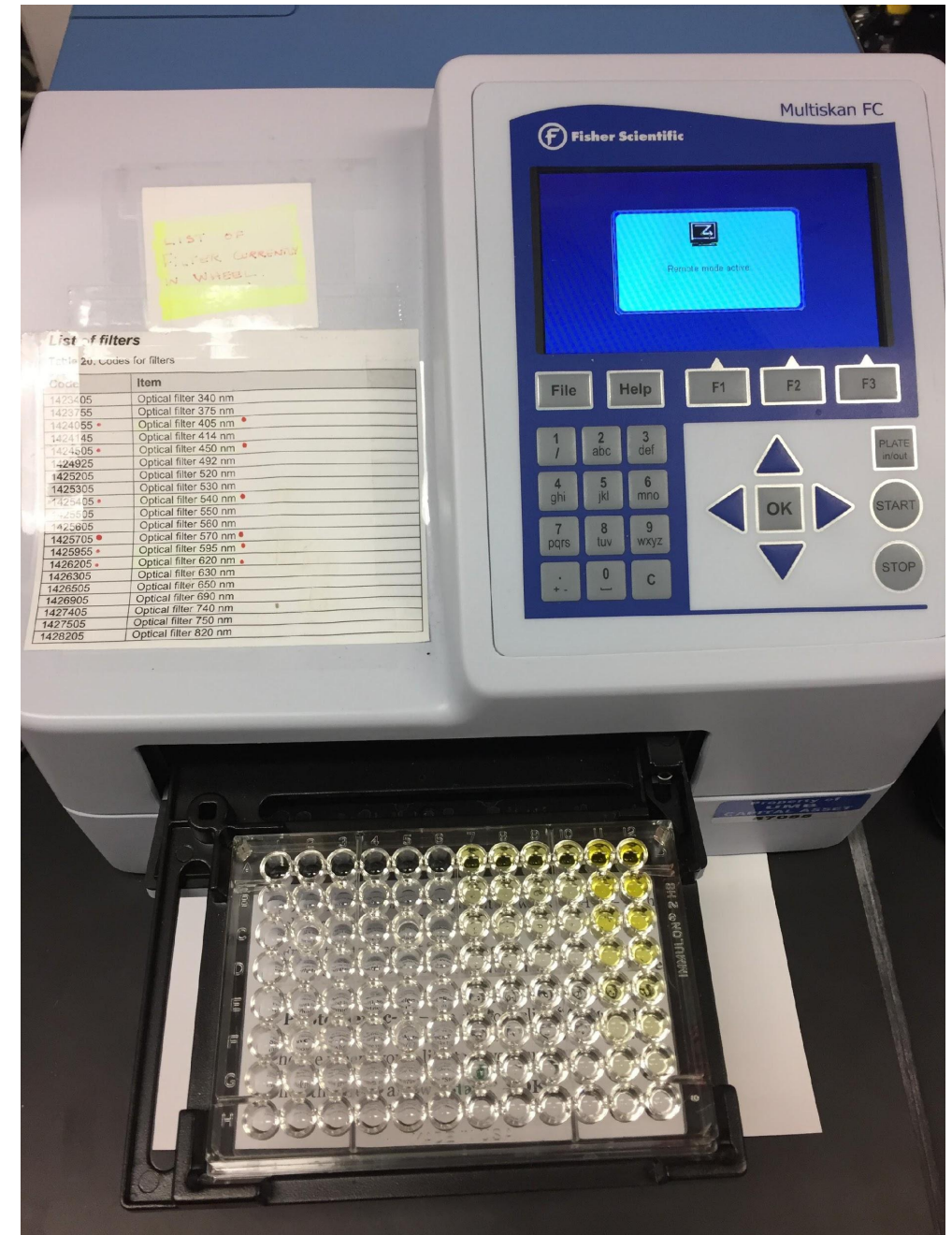
- Two antibodies (capture antibody and detection antibody) must interact with different epitopes of the antigen
- Possible experiment using rats as an animal model:
 - Coat plate with antibody to rat IL-17
 - Put healthy and diseased rat serum in wells
 - Put secondary antibody for IL-17, conjugated with enzyme
 - Put in substrate to produce color change
 - Determine if concentration of IL-17 is higher in diseased rats compared to healthy rats

ELISA ANALYSIS

The reaction of the enzyme binding to the substrate produces a color change which can be analyzed **qualitatively or quantitatively**.

Qualitatively, it is possible to see large differences in color production to determine the presence or absence of the target antigen/antibody.

Quantitatively, a special machine can be used to determine the optical density of each sample. Researchers use a standard curve to relate the optical density to antibody titer.



DIAGNOSING RA: ANTI-CCP ELISA

We will perform an indirect ELISA to test patient's serum samples for anti-CCP.

Our simulated reagents for this lab activity include:

- Purified CCP (cyclic citrullinated peptide)
- Serum samples from patients (potentially containing anti-CCP antibodies)
- Anti-human immunoglobulin antibodies w/conjugated HRP enzyme
- TMB substrate
- Serum containing anti-CCP antibodies
- Serum from an unaffected person