Immunology Laboratory

Introduction of the immune system

Lab 1. Organ of immune system
Lab 2. Peripheral blood smear
Lab 4. Blood cell separation

Functions of the immune system

Lab 5 Microbial Killing by macrophages
Lab 14. Lymphocyte proliferation
Lab 15. Antibody-mediated cytolysis

The properties and applications of antibodies

Lab 6. Precipitation
Lab 7. Agglutination
Lab 9. Immunofluorescence microscopy
Lab 10. ELISA
Lab 11. Immunoprecipitation
Lab 12. Western blot
Lab 13. Flow cytometry

Case Study

Purpose:
How are the knowledge and technologies that we have learnt from this course applied in the real word.

How to do it?
Each student choose an immune system-related disease out of a list of 20.

Search and study the information about this disease.

Sources of information
Books
Research papers
Web

Present the information as a poster on May 13.

The following information should be included in your poster

1. The name of the disease
2. The typical symptoms of the disease
3. The immunological mechanism of the disease
4. The diagnostic test for the disease
   Principle
   Method
   Major Reagents
5. Treatment
   Fundamental mechanism
   Targets
   Expected results

List of immune system-related diseases

1. Congenital Asplenia
2. X-linked agammaglobulinemia
3. Hyper IgM immunodeficiency
4. MHC class I deficiency
5. MHC class II deficiency
6. X-linked severe combined immunodeficiency
7. Toxic shock syndrome
8. Contact sensitivity to poison ivy
9. AIDS
10. Wiskott-Aldrich syndrome
11. Allergic asthma
12. Systemic lupus erythematosus
13. Multiple myeloma
14. T cell lymphoma
15. Chronic Granulomatous disease
16. Insulin-dependent diabetes mellitus
17. Interferon-γ receptor deficiency
18. Acute systemic anaphylaxis
19. Graft-Versus-Host Disease
20. Selective IgA deficiency
21. Rheumatoid arthritis

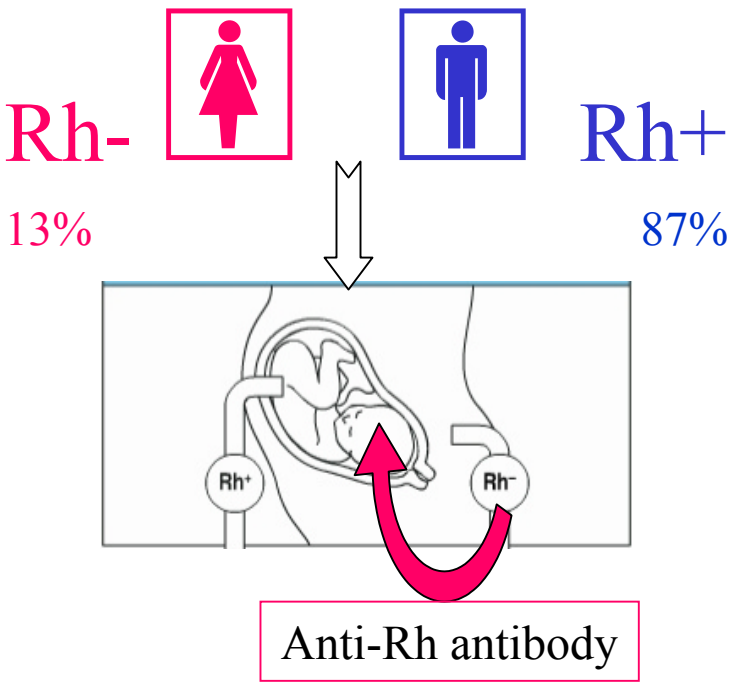
Hemolytic disease of new born

1. The typical symptoms of the disease

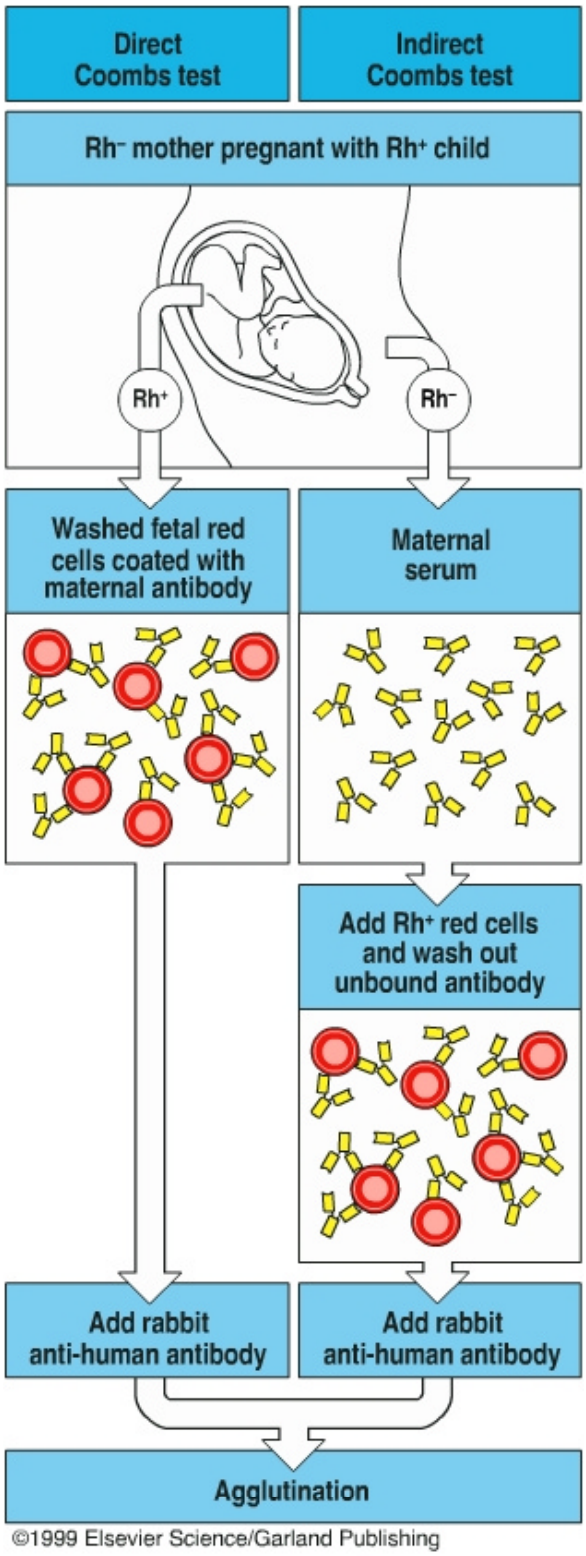
During the second and third of pregnancy, fetus becomes profoundly anemic, and Amniotic fluid has increasing amount of bilirubin, a pigment derived from the breakdown of heme, indicating that the fetus' red blood cells were being hemolyzed.

2. The immunological mechanism of the disease

Hemolytic disease of new born is caused by alloantibodieds induced by a fetus in the pregnant mother.



3. The diagnostic test for the disease



Method:
Agglutination assay

Major Reagents:

Direct Coombs test: Fetus red cells and human IgG-specific antibody

Indirect Coombs test: Maternal serum, Rh+ red cells amd human IgG-specific antibody

4. Treatment

Transfusion with Rh-negative packed red blood cells until the fetus is sufficiently mature to sustain extrauterine life without difficulty

Wiskott-Aldrich syndrome

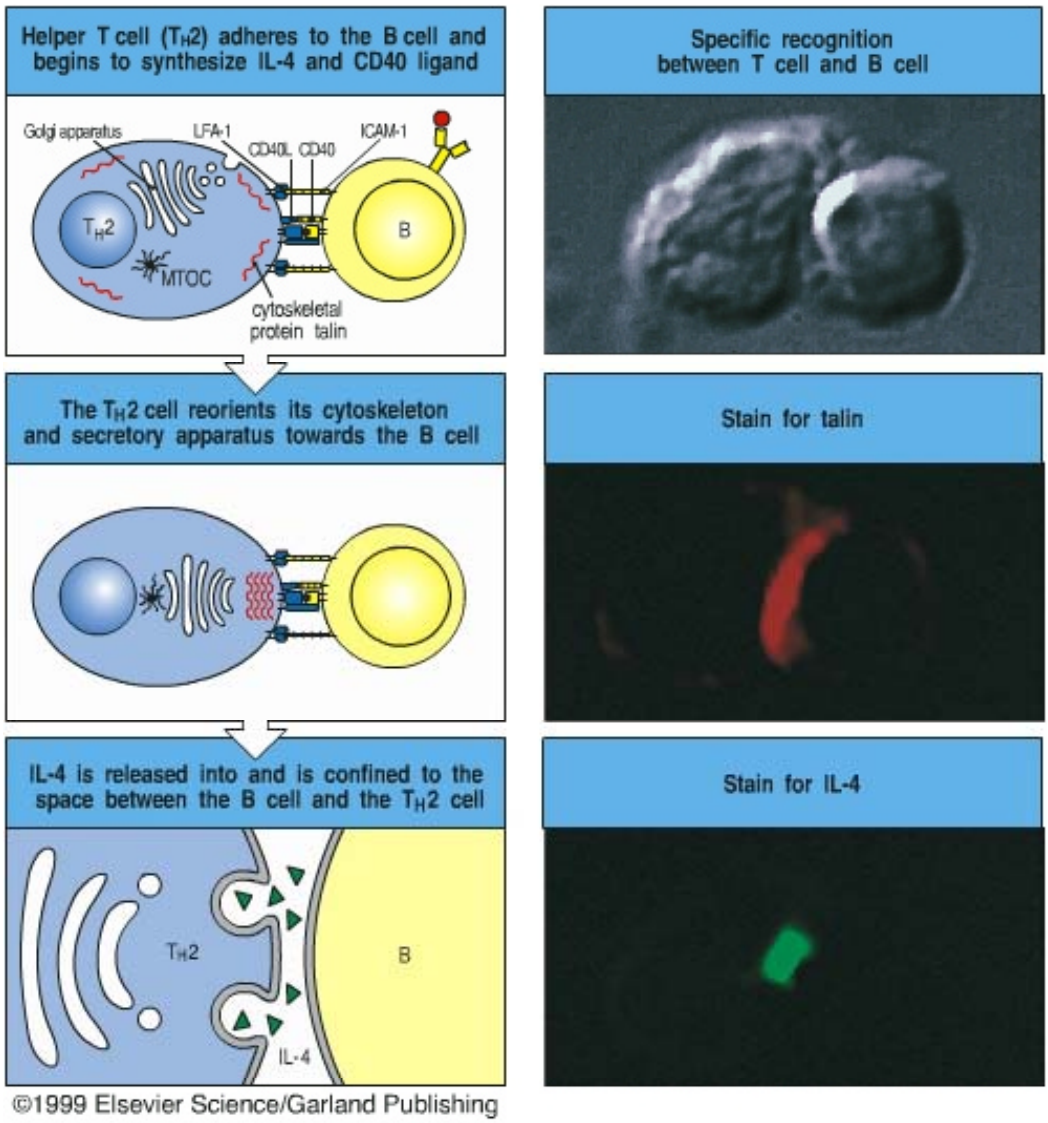
1. The typical symptoms of the disease

Normally developed male infants have recurrent bacterial and viral infections, eczema and asthma. Late they develop frequent severe nosebleeds. By the middle age, they may develop B cell lymphoma.

2. The immunological mechanism of the disease

Patients with Wiskott-Aldrich syndrome have a defective gene in the short arm of X-chromosome. The defective gene encodes a protein named the Wiskott-Aldrich syndrome protein or WASP. WASP is expressed only in white blood cells and megokaryocytes and involved in the reorganization of the actin cytoskeleton. WASP is essential for the function of T cells and platelets.

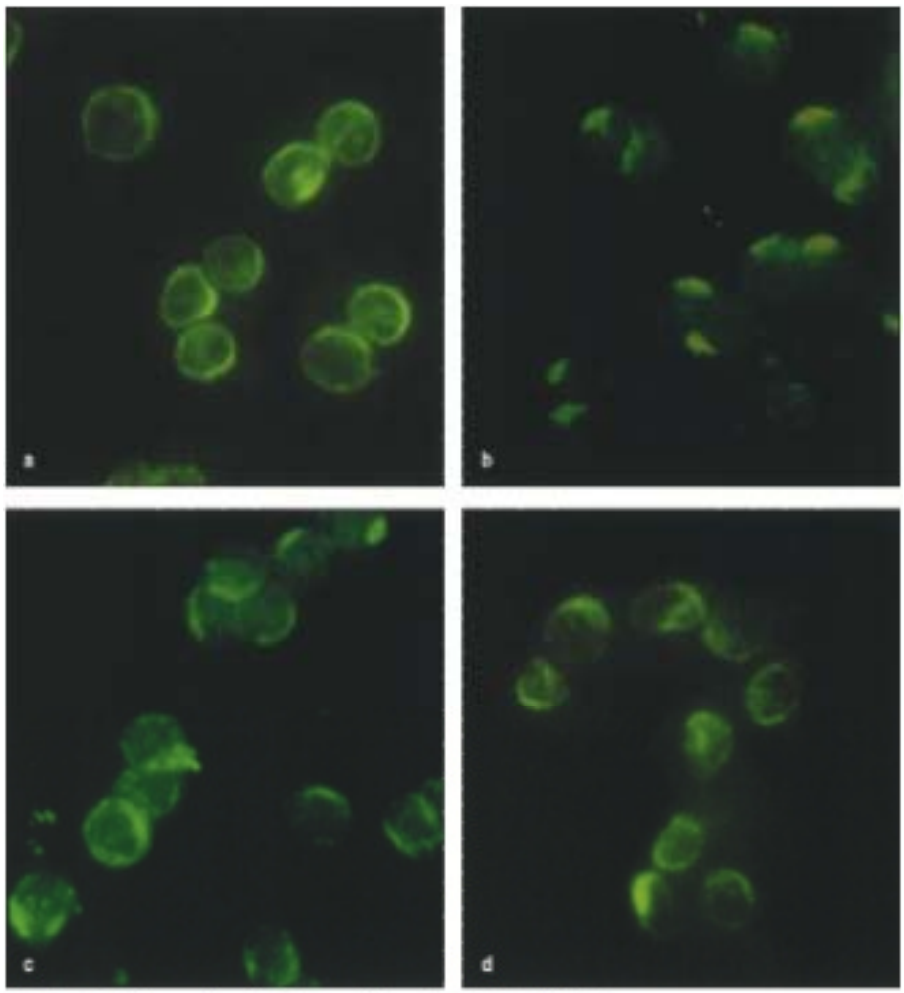
Activation of T cells



-stimulation +stimulation

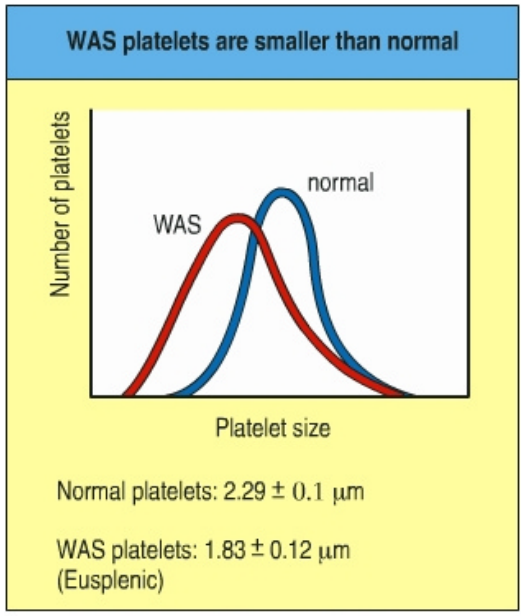
Normal

WASP-/-



3. The diagnostic test for the disease

a. Sizes of platelets
Method:
Microscopy

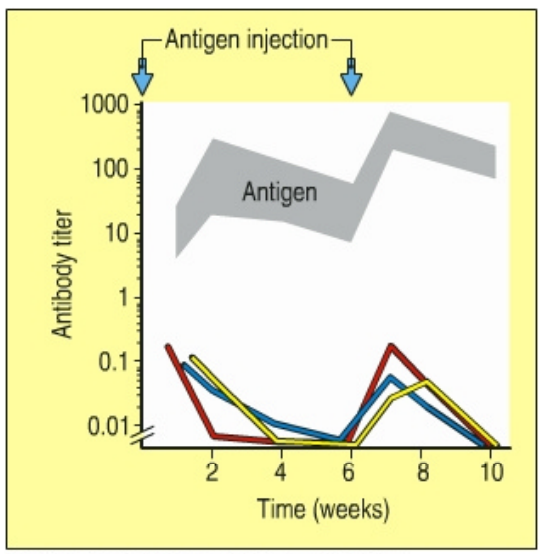


b. Antibody responses after immunization

ELISA will be used to determine antigen-specific antibodies in patients' sera.

Patients will be immunized twice with the antigen bacteriophage ox174, and their titer of anti-bacteriophage antibodies is measured at 2, 4, 6 weeks after the infection.

Antigen: bacteriophage
Primary antibody: patients' sera
Secondary antibody: HRP-conjugated anti-human IgG



3. The diagnostic test for the disease

c. T cell proliferation

MTT assay will be used to determine the ability of T cells from patients in response to stimulation, such as a mitogen concanaviallin (ConA).

4. Treatment

Removal of the spleen

Intravenous gamma globulin

Antibiotics

Treatment of symptoms

Gene therapy?

Study Questions for the Final Exam

1. Everything on the web site www.wam.umd.edu/~wenxsong/ImmunLab.html

2. Commons and Differences among different techniques
   - Principle
   - Applications
   - Reagents

3. How to use what you learnt to design assays
   - Purpose
   - Method
   - Major reagents
   - Expected results
   - Interpretation of results

4. Understanding and interpreting experimental data