CAMOSUN COLLEGE - CHEMISTRY & GEOSCIENCE DEPARTMENT

CHEMISTRY 251 - *IMMUNOLOGY*

Course Outline, Fall Semester 2004

Course Description

This course describes the basic concepts of immunology and the application of immunochemistry to molecular, medical, and veterinary biotechnology. Topics include antigens and antibodies, immune responses, vaccines, antibody diagnostics, immunosuppression, hypersensitivity, transplants, cancer, auto-immune diseases, immunodeficiencies including AIDS, and current immunological technologies.

Semester offered	Fall 2004
Credits	4 credits
In-class workload	Approx. 6 hours per week • There are three 50-minute lectures per week. • Laboratory periods are 2 hours and 50 minutes.
Out-of-class workload	6 hours per week
Number of weeks	14 weeks
Pre-requisite	Chem 120 - College Chemistry 1

Intended Learning Outcomes

- Students successful in this course will be able to evaluate fundamental aspects of the human immune system, and relate these to a wide variety of immunologically-based clinical conditions including allergies, transplant rejections, autoimmune diseases, and immunodeficiencies including AIDS.
- Students will be able to compare and contrast various types of antibody-based diagnostic tests, and various vaccine formulations.
- Students will have the hands-on experimental skills required to conduct the most commonly used immunological techniques including enzyme-linked immunosorbent assays (ELISA), latex bead agglutination assays, and Western-blotting detection of antigens.
- Students will have the ability to evaluate experimental design, design control experiments, and interpret data arising from basic immunological technologies.
- Students will be capable of working in a level-1 biosafety laboratory.
- Students will be experienced in the preparation, handling and storage of many types of solutions, buffers, reagents, and with equipment used immunological experimentation.

Instructor

Jamie Doran, Ph.D.

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Office hours: Regular office hours are posted on the office door.
These are: Monday, 12:30 to 1:20
Tuesday, 12:30 to 2:20
Friday, 11:30 to 1:20
Students are welcome whenever the door is open.
Appointments may be made to meet at other times.
Office hours will be extended before exam times.

Course times and locations

Lecture times Tuesday
9:30 AM to 10:20 PM
Fisher Building, Room F360

Wednesday
9:30 AM to 10:20 PM
Fisher Building, Room F200

Thursday
9:30 AM to 10:20 PM
Wilna Thomas Building, Room WT103

Laboratory Periods* Monday
2:30 AM to 5:20 AM
Fisher Building, Rooms F360 and F358

*This time period will be used primarily for laboratory experiments, and will also host midterm exams.

Please see the laboratory and midterm exam schedules below.

Textbook (Required)

Understanding Immunology. 2001 Edition. Au. Peter Wood, Prentice Hall, Harlow, Essex, UK.

Laboratory Manual & Selected Course Notes (Required)

A booklet of experimental procedures and selected course notes is available for purchase through the College Bookstore.

General Materials and Supplies

Safety glasses Safety glasses are required when handling hazardous chemicals or biochemicals. The students are required to purchase their own pairs of glasses. Students lacking safety glasses when they are required will not be permitted to work in the laboratory.

Lab coats Lab coats are required for any experiments involving potentially chemically or biologically hazardous materials. Students are required to provide their own lab coats. Students lacking lab coats when required will not be permitted to work in the laboratory.

Latex gloves Latex or similar gloves will be available in the lab and are to be used when appropriate to protect the skin from hazardous chemicals or biochemicals. They are also required at times to protect valuable immuno-chemicals from becoming contaminated with biomolecules or bacteria on the skin, or from becoming degraded by enzymes from the skin.

Scientific calculator Calculators may be required in the lab, in class or during exams. Students are required to provide their own calculators.

Course evaluation

Attendance at the final exam is mandatory. Appropriate documentation must accompany any explanation for absence.

If either of the midterm exams is missed due to illness or for any other justifiable reason (accompanied by appropriate documentation), a student may either take a substitute test to be written at a mutually agreeable time, or choose to add the percentage value of that midterm exam to the percentage value of the final exam.

Course evaluation:

Midterm Exam #1	(90 min)	25 %	Monday, October 18th .
Midterm Exam #2	(110 min)	30 %	Monday, November 15th .
Final Exam	(3 h)	45 %	To be scheduled by the College.

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[The percentage values indicate the proportion of the final grade determined by each exam.]

Curriculum tested on the first midterm exam will not be part of the curriculum tested on the second midterm exam.

The final exam is a comprehensive exam.

Participation in laboratory experiments is **mandatory**. No laboratory experiment can be missed without an acceptable reason submitted in writing (e.g. a note from medical doctor). There are no laboratory reports to be handed in. However, students are responsible for understanding the principles, practical aspects, design, and expected results of each experiment. These aspects of the laboratory work will be subject to examination on the midterm and final exams.

Grade scale

The percentage marks for the course will be converted to grades according to the School of Arts & Science scale:

A+	= 95% to 100%	B-	= 70% to 74%
A	= 90% to 94%	C+	= 65% to 69%
A-	= 85% to 89%	C	= 60% to 64%
B+	= 80% to 84%	D	= 50% to 59%
B	= 75% to 79%	F	= 0% to 49%

Lecture Outline

HISTORICAL PERSPECTIVE

- Early historical evidence of immunity in humans
  - Survivors of the bubonic plague in Athens 430 BC do not reacquire the disease
  - Certain African tribes practice immunization against snake venoms
  - Mithradates IV (ancient Greece) uses immunization against poisons

- Variolation
  - Origins in China and India≤ 1500 AD to prevent smallpox infections
  - Approach spreads to Persian region
  - In the 1720's variolation moves from Turkey to England, but never adopted

- Other approaches to small pox vaccination
    - Jenner's work with a cowpox vaccine
    - WHO determines to rid the world of small pox in 1970's
    - Issue of what should be done with existing stocks of small pox virus

- The development of the field of immunology
    - Meaning of the term 'immunity'
    - Recognition of the four basic tenants of adaptive immunity
    - Louis Pasteur creates the field of immunology with seminal experiments demonstrating vaccination and acquired immunity in animals and humans. He develops attenuated bacteria or viruses are vaccines. [His germ theory of disease laid the groundwork for these studies.]
    - Pasteur and Koch compete to create vaccines, as vaccination becomes widely accepted in Europe and abroad.
    - Elie Metchnikoff initiates the field of cellular immunology with studies of phagocytes; his efforts also initiate the study of comparative immunology
    - Nuttal, Von Behring contribute to the discovery of humoral immunity
    - Wright proves that <u>both</u> cellular and humoral immunity make up the human immune system
    - Ramon develops toxoids (attenuated chemicals) as vaccines
    - A history of vaccine use proves to prevent major human infectious diseases

GENERAL ROLE FOR THE IMMUNE SYSTEM IN MAINTAINING BODY INTEGRITY

- Challenges to health: infectious organisms, cancer, toxins
- Implications for transplantation, allergy, autoimmunity, pregnancy
- Blood, serum, plasma, antiserum
- Innate immunity versus adaptive immunity

THE INNATE IMMUNE RESPONSE

- General characteristics of nonspecific physical and chemical defenses
    - Physical barriers
        - Skin
        - Mucous membranes
    - Defensive chemicals
        - pH
        - Lysozyme
        - Iron-binding compounds
        - Oxygen
    - Natural bacterial flora and microbial antagonism

- White blood cells (leukocytes) involved in innate immunity
    - Phagocytic cell types – monocytes & macrophage, neutrophils (PMN's), dendritic cells (brief mention)
    - Nonphagocytic leukocytes – eosinophils, natural killer cells
    - Inflammatory leukocytes – Mast cells, basophils
    - Lymphocytes – B-cells and T-cells
    - Origins of myeloid and lymphoid cell lines

- Humoral factors involved in innate immunity (not responsible for at this point)
    - Complement
    - Interferons
    - Acute phase proteins

- The innate, acute, inflammatory response
    - Constriction and local dilation of vessels
    - Roles for cells and soluble factors from the blood
    - Margination
    - Extravasation (diapedesis)
    - Chemotaxis
    - Mast cell activity

- The process of phagocytosis by macrophage
    - Oxygen-dependent and oxygen-independent killing mechanisms
    - Antigen presentation links innate immunity with adaptive immunity
    - Ways that microbes can prevent phagocytic killing

ANTIBODIES AND ANTIGENS

- The lymphatic system
    - Primary and secondary lymphatic tissues
    - Structure and function of the lymphatic system

- Antibodies
    - Roles as adaptor molecules
    - Structure and function of a prototypic, divalent Ab molecule
        - ◊ Fab and Fc fragments
        - ◊ Globular constant domains
        - ◊ Variable and hypervariable Regions
    - Isotypes (classes) of antibodies
        - ◊ Types of heavy and light chains
        - ◊ Immunological characteristics and functions
    - Genetic basis of antibody diversity

- Antigens, immunogens, and haptens
    - Definitions of terms
    - Epitopes (antigenic determinants)
    - Characteristics and properties of immunogens
    - Experimental conditions that affect the immunogenicity of immunogens
    - Vaccination conditions that affect the immunogenicity of immunogens

COMPLEMENT

- Classical Complement Pathway
- Alternative Complement Pathway
- Lectin-Mediated Complement Activation Pathway
- Roles of Various Products of Complement Activation and other Acute Phase Proteins in the Inflammatory Response and other Aspects of Innate Immune Cell Function

ROLES OF B-CELLS AND T-CELLS

- Processing of B-lymphocytes and T-lymphocytes

- Antibody production by B-cells
    - The Clonal Selection Theory of Antibody Synthesis
    - B-cell receptors and antigen binding
    - B-cell activation and maturation
        - Plasma cells
        - Memory B-cells
        - Role of T-helper cells and macrophage
    - The Primary Immune Response
    - The Secondary Immune Response
    - Affinity Maturation

- Antibody production by B-cells
    - Affinity Maturation
    - Relationship of Affinity Maturation to Class switching
    - The role of T-helper cell - B-cell interactions in Preventing Harmful Effects of Affinity Maturation
    - T-independent B-cell Antigens
    - The Secondary B-cell -Mediated Immune Response
    - Genetics of human antibody formation
        - ◊ Multi-gene organization of immunoglobulin genes
        - ◊ Variable region gene rearrangements
        - ◊ Generation of antibody diversity
        - ◊ Antibody gene expression

- Cell-mediated immune response
    - General Role in the Human Immune Response
    - Cytotoxic T-cells: General Nature and Roles
    - NK-cells: Roles, killing mechanism
    - ADCC (antibody-dependent cell-mediated cytotoxicity)
    - 'Killer-cells', a form of NK cell
    - Eosinophils and Phagocytes Exhibit ADCC

- T-cell subclasses
    - Cytotoxic T-cells, Helper T-cells, and T-cells exhibiting Immuno-suppressive Activities (Suppressor Activities)
    - Clonal selection Applies to Cytotoxic T-cells
    - Role of MHC I presentation in Tc-cell Activation
    - Role of Helper T-cells in Tc-cell Activation

- MHC Presentation and T-cell Surface Proteins CD4 and CD8
    - T-helper cells possess CD4 proteins
    - Cytotoxic T-cells possess CD8 proteins
    - Role of CD4 in recognition of MHC II
    - Role of CD8 in recognition of MHC I
    - Co-stimulatory roles of CD4 or CD8 in T-cell Activation
    - 'T-cell restriction' and Its Importance
    - Cytotoxic T-cell Receptors Recognize T-cell Antigens Held in the Cleft of MHC I Molecules Through Six Hypervariable Regions in the Variable Domains of the T-Cell Receptor (TCR)
    - Endogenous Antigen Processing
    - Exogenous Antigen Processing
    - Role of Macrophage in Cytotoxic T-cell Activation

- Cytokines
    - General Nature and Characteristics
    - Autocrine and Paracrine Functions
    - General Effects on an Immune Response
    - Classic Characteristics: Pleotrophy, Redundancy, Synergy, Antagonism
    - Interferons: ◎, ◎ and ◎ - Nature and Roles in Innate or Adaptive Immunity

- TH1 vs. TH2 Responses
    - Humoral vs. Cellular Immune Responses
    - Cytokine Profiles
    - Polarization (Humoral vs. Cellular) of Immune Responses
    - Functions of Cytokines in Mediating Polarization

- Immunosuppression
    - Immunological Silence
        - Central Tolerance
            - ◊ Thymic processing
            - ◊ Neonatal tolerance
        - Peripheral Tolerance

    - Acquired Immunotolerance
        - Low-Zone Tolerance
        - High-Zone Tolerance
        - Immunotolerance Created by Certain Immunization Regimes
        - Natural Acquisition of 'Immunotolerance' in Adults

- Blood Group Antigens
    - Rh Antigens and Fetal Hemolytic Disease
    - The ABO System and Compatible Blood Donors

HYPERSENSITIVITY (Allergy)

- The nature of hypersensitivity and allergens

- Types of hypersensitivity
    - Immediate Type Hypersensitivity
        - Type 1 - Anaphylactic hypersensitivity
            - ◊ Systemic Anaphylaxis
            - ◊ Localized Anaphylaxis
        - Type 2 - Antibody-Dependent Cytotoxicity Hypersensitivity
        - Type 3 - Complex-Mediated Hypersensitivity
            - ◊ Systemic
            - ◊ Localized
    - Delayed Type Hypersensitivity
        - Type 4 - Cell-Mediated Hypersensitivity

AUTOIMMUNITY

- Major Sources of Autoimmunity (eight)

- Autoimmune Diseases
    - Tissue-Specific Diseases
        - Aspermatogenesis
        - Sympathetic Opthamalia
        - Hashimoto's Thyroditis

        - Insulin-Dependent Diabetes
        - Autoimmune Anemias
            - Pernicious Anemia
            - Hemolytic Anemias
        - Goodpasture's Syndrome
        - Graves Disease
    - Systemic Autoimmune Diseases
        - SLE (Lupus)
        - MS
        - Rheumatoid Arthritis

TRANSPLANTATION IMMUNOLOGY

- Autograft, Isograft, Allograft, Xenograft

- Privileged Sites & Privileged Tissues

- Graft Rejection
    - Hyperactive Rejection
    - Acute Rejection
        - First-Set Rejection
        - Second-Set Rejection
    - Chronic Rejection

- Prevention of Rejection
    - Tissue Typing
    - Immunosuppressive Agents

- Clinical Transplantation
    - Examples and Success Rates
    - Graft vs. Host Reaction

CANCER IMMUNOLOGY

- Tumour-Specific Transplantation Antigens
    - Viral Antigens
    - Chemically-induced Tumour Antigens

- Tumour-Associated Transplantation Antigens
    - Carcinofetal Antigens
    - Embryonic Antigens
    - Alpha-feto Protein Antigen

- Immune Response to Tumours

- Cancer Immunotherapy
    - Cytokine Therapy
    - Interferon Therapy
    - Tumour Necrosis Factor Therapy
    - Monoclonal Antibody-Based Therapies
    - Anti-cancer Vaccines

MONOCLONAL ANTIBODIES

- Basis and procedure For Monoclonal Antibody Production

VACCINES

- Needs, Benefits, and Potential Risks

- Type of Vaccines
    - Killed or Inactivated Vaccines
    - Live Attenuated Vaccines
    - Subunit Vaccines
        - Purified Biomolecules
        - Recombinant Vaccines
        - Heterologous Vaccines
        - Peptide Vaccines
        - DNA Vaccines

IMMUNODEFICIENCY

- Primary Immunodeficiencies
- Secondary Immunodeficiencies
    - HIV & AIDS
        - Approaches and Challenges Associated With Developing An HIV Vaccine

IMMUNOTECHNOLOGY

- *In vitro* antibody-antigen reactions
  - Gel precipitin reactions
    - Principles
    - The Precipitin Curve
    - The Lattice Theory
  - Applications of the Precipitin Reaction
    - Ouchterlony double-diffusion method
      - Principle
      - Experimental design
      - Qualitative interpretation of results
        - ◊ Reaction of identity
        - ◊ Reaction of non-identity
        - ◊ Reaction of partial identity
    - Radial immunodiffusion (RID)
      - Principle (Mancini single-diffusion method)
      - Experimental design
      - Quantitative interpretation of results
  - Agglutination reaction
    - Principle
    - Experimental design
    - Interpretation of positive or negative results

- ELISA
  - Principle of ELISA
  - Standard ELISA for Analysis of Antigen: Indirect & Direct Assays
  - Capture ELISA for Analysis of Antigen: Indirect & Direct Assays
  - General Procedure for Standard, Indirect ELISA for Analysis of Antigen

- Western blotting detection of antigens
  - Principle of Western Blotting Analysis of Antigen(s)
  - General Procedure for Western Blotting
  - Interpretation of results

Other Immuno-Diagnostic Techniques:

Radioimmunoassay (RIA); Immunofiltration assays; Immunochromatographic assays; Affinity chromatography; Immuno-electron microscopy; Immuno-fluorescence microscopy; Fluorescence-activated cell sorter; Rocket electrophoresis; Two-dimensional immuno-electrophoresis.

Some Suggested Reading:

NB. No reading is assigned per se. It is up to you to possess a good set of lecture notes and use the course text and laboratory manual and selected course notes package to read relevant material.

The following is a guide to help identify much of the relevant information. However, it is not intended to be comprehensive as it is anticipated that students have the ability to undertake their own studies.

Lecture Topic	***Understanding Immunology* by P. Wood**	***Selected 251 Course Notes***
History	This subject is not covered. Section 1.1 provides some perspective.	p. 154 - 160
Overview of Immunology	Chapter 1. Be selective and appreciate this discussion as setting the scene for much that is to follow in the course.	
Innate immunity	Sections 1.3, 2.1, 2.2, 2.4, 2.7, 2.8, 3.1	p. 161 - 168
Antibodies & Antigens	Chapter 3 lymphatic system intro. - sections 8.1, 8.2	
B-cell function	Chapter 4 & references to Ab genes from Chapter 3	
Affinity Maturation	Sections 4.6, 4.8	
Secondary Ab Response	Sections 4.9, 7.2	
T-helper Cell - B-Cell Interactions	Section 7.3, 8.3.2	
Cytotoxic Cells	Sections 2.7.2, 10.2	
ADCC	Section 9.8	
CD4 and CD8 & MHC Presentation	Chapter 5, & Sections 7.2, 7.4	

Cytokines	Section 2.3 (also see T-cell functions)	
Interferons	Section 2.7.1	
Th1 and Th2 Responses	Sections 7.2, 7.6	
Ab-Mediated Effector Responses	Sections 9.2 to 9.5	
Complement	Section 9.6	p. 169 - 176
Acute-Phase Proteins	Sections 2.5, 9.7	
Immunosuppression/Immunotolerance	Sections 11.1, 11.3.1, 11.4.1 to 11.4.4, 11.5	
Jerne's Network Hypothesis		p. 206 - 207
Blood Group Antigens	Sections 13.8.1, 13.8.2	
Hypersensitivity	Chapter 13	P. 177 - 178
Autoimmunity	Chapter 12	p. 193 - 200
Transplantation	Chapter 15, Section 3	
Cancer Immunology	Chapter 15, Section 4	
Monoclonal Antibodies	Chapter 15, Box 15.2	p. 127 - 131
Vaccines	Chapter 15, Section 2	p. 179 - 192
Immunodeficiency	Chapter 14	p. 201 - 205
ELISA	p. 212, 214, 237	Expts. 4 & 6
Other Immunodiagnostic technologies		Expts. 1, 2, 3, 5 & 7. p. 142 - 153

Laboratory Schedule

'Week 1.' *Labour Day Holiday*

Week 2 **Monday, September 13th.**
Experiments 1 & 2 Gel Immunodiffusion and the Identification of Antigens by Precipitin Reactions
Experiment 1. The Ouchterlony Reaction
Experiment 2. The Radial Immunodiffusion Assay

Week 3 **Monday, September 20th.**
Experiment 1 (continued). Interpretation of the Ouchterlony Reaction
Experiment 2 (continued). Interpretation of the Radial Immunodiffusion Assay
Experiment 3. Identification of *Aeromonas salmonicida*, a Bacterial Pathogen of Salmon, by Developing a Latex Bead Agglutination Assay:
Part I. Manufacture of Antibody-Coated Beads

Week 4 **Monday, September 27th.**
Experiment 3. Part II. Latex Bead Agglutination Assay:

Experiment 4. Detection of *A. salmonicida* Antigens and Determination of Anti-*A. salmonicida* Polyclonal Antibody Titre Using an Indirect Enzyme-Linked Immunosorbent Assay (ELISA).
Part I. Coating of microtiter wells with antigens.

Week 5 **Monday, October 4th.**
Experiment 4. Part II. ELISA Assay.

Week 6 **Monday, October 11th.** *Thanksgiving Day Holiday*

Week 7 **Monday, October 18th** **<u>Midterm Exam #1</u>**

Week 8 **Monday, October 25th**
Experiment 4. Discussion of ELISA results.

Experiment 5. Western Blotting Analysis of *Aeromonas salmonicida* Proteins.
Part I. SDS-polyacrylamide gel electrophoresis separation of proteins.

Week 9 **Monday, November 1st.**
Experiment 5. Part II. Western blotting of proteins onto nitrocellulose.

Week 10 **Monday, November 8th.**
Experiment 5. Part III. Immuno-detection of antigens on Western blots

Experiment 6. Differentiation and Titre Determination of Atlantic Salmon and Rainbow Trout Sera Using Monoclonal Antibodies in an ELISA Assay
Part I. Dilution of antigens, and coating of microtiter plates.

Week 11 **Monday, November 15th** **Midterm Exam #2**

Week 12 **Monday, November 22nd**
Experiment 6. Part II. ELISA Assay.

Experiment 7. Part I. Propagation of Monoclonal Antibody Producing Hybridoma Cell Tissue Cultures.

Week 13 **Monday, November 29th**
Experiment 6. Discussion of ELISA results.

Experiment 7. Part II. Immunofiltration Affinity Chromatography Characterization of the Subtypes of the Monoclonal Antibodies in Tissue Culture Supernatants

Week 14 **Monday, December 6th**

Wrap up of experiments or discussion of experimental results (if needed).

Lab lecture - Other immuno-technologies.