BIOLOGY 404 SYLLABUS SUMMER 2022

CONTACT INFORMATION

David Allman: 215-746-5547 (office)

Email: dallman@pennmedicine.upenn.edu

OFFICE HOURS (for Dave)

Mondays 10:00AM-11:00AM
230 John Morgan Building

Teaching assistant:

To be determined

BIOLOGY 404
BASIC IMMUNOLOGY

Tuesdays - 5:00 TO 8:50 PM

LECTURES HELD VIA ZOOM:
https://sasupenn.zoom.us/j/6837392595?pwd=cU9meCtrb1I0MGI2UitHaHpxTlRSdz09

COURSE GOALS: The goals of this course are several: First, I hope to introduce you to basic principles and current concepts in the field of immunology. Second, I would like to stimulate your thinking, especially from an experimental standpoint, about how these principles and concepts are formed. Finally, I hope you will leave with a foundation, for both the field of immunology and general experimental approaches, which will enable you to learn more on your own, through critical appraisal of the literature.

COURSE DESCRIPTION: The course will begin with a general overview of immunity, followed by in-depth considerations of the underlying cellular, molecular, and genetic events. Finally, later discussions will focus upon more specialized issues in immunology, such as disease states involving the immune system, as well as particularly interesting problems in modern immunology.

EXAMS: There will be two exams, EACH WITH A TAKE-HOME AND AN IN-CLASS PORTION. The exact format of these will be announced prior to each exam, but will be primarily essay or brief answer questions. Take-home exams will be distributed two weeks before the in-class exam dates and must be turned in along with the in-class exam. *Take-home exams will not be accepted late.*

QUIZZES: **There will be an open book quiz that is due at the beginning of every session at 5pm.** These are mainly for self-evaluation and to introduce new ideas to you, and can only improve your final grade. Each will be corrected and returned by the next class. Each quiz will become available 34 hours before it is due.

ONLINE THREADED DISCUSSION GROUPS: We will be utilizing the Canvas web site (https://canvas.upenn.edu/) in which we will be able to discuss, as a group, basic principles and problems that guide our current understanding of the immune system. Each week the class will be given a question or problem to work on as a group. For some weeks, I may also pose a question or describe an experiment in class, and then each student will be expected to attempt to address the issue online. These are threaded discussions in the sense that all members of the class will be able to view, and respond to, comments made by others in the class or within their specified group. In addition, the course director and teaching assistant may guide the discussion when appropriate. We may also have certain "guests" with expertise in a relevant area add comments. I think that these exercises will be fun and will add to your understanding of scientific principles and your ability to learn from your colleagues. Participation is mandatory: Roughly 33% of your final grade will be derived from your participation, or lack thereof, in this activity.

SCHEDULE: Each session will be divided as follows:

* Up to the first 20 minutes will be devoted to an open discussion of the previous lecture/homework problems/quiz questions.

*A 10-minute break.

READINGS: Readings in the primary text are given in the table on page 6. I strongly suggest you use these, as you see fit, to strengthen your grasp and broaden your perspective of the concepts conveyed in class.

TEXT: The recommended text is **Immunobiology 8th or 9*th edition*, by Janeway et al.**, published by Garland Publishing, Inc. Copies are available in the University bookstore, Amazon, you name it . . .

ATTENDANCE: Attendance is not required, although participation in the quizzes will allow us to determine if excessive absences might be the basis for poor performance - and could mitigate against any special consideration if this is the case.

GRADING

The following "straight scale" grading system will be used:

>97 = A+	87-89 = B+	77-79 = C+		
93-96 = A	83-86 = B	73-76 = C	<70 = D	<60 = F
90-92 = A-	80-85 = B-	70-72 = C-		

Throughout the course you may collect chips. At the end, you cash in your chips, and your grade will be computed by taking your total "chips" and dividing by 3.

CHIP SOURCES AND MAXIMA

Exams: Each exam has two parts: an in-class portion consisting of short answer questions similar to the quiz questions you have had, and a take-home portion consisting of three sets of essay questions. On each take-home exam, the question sets will be progressively more challenging. You may answer as many as one question from each set. *The in-class portion of each exam is worth a maximum of 55* chips. The take-home questions are worth 15 chips/question. Thus, on each exam, *the maximum from each take-home exam is 45 chips.*
Threaded Discussion Groups: There will be 8 threaded discussions/debates, each worth 10 chips for a total of 80 possible chips. At the end of the class your grade will be normalized to 100. Each week you will be graded on your participation in these online discussions and debates of current topics in immunology. Each discussion/debate will be posted by 10pm on the night of a lecture. Responses from each individual registered for the class are due by 10pm the following Monday.

Quizzes: There will be 9 quizzes, each worth 10 chips. At the end of the course, 20% of your quiz chips are added to your other chips **PRIOR TO** division by 3. The maximum chips from quizzes is thus (90 X .20), or 18. **Therefore the maximum the quizzes can add to your final grade is 18/3, or 6.0.**

SUMMARY OF CHIP SOURCES AND MAXIMA

Quizzes	18
Threaded Discussions (normalized)	100
Exam I 2 week portion	45
Exam I 24-hour portion	55
Exam II 2 week portion	45
Exam II 24-hour portion	55
MAX POSSIBLE TOTAL CHIPS	318
MAX POSSIBLE FINAL SCORE	106 (318/3)

LECTURE SCHEDULE

May 24 INTRODUCTION TO BASIC IMMUNOLOGY
KINETICS AND PROPERTIES OF IMMUNE RESPONSES -
AB STRUCTURE AND MOLECULAR BASIS FOR AB SPECIFICITY

May 31 INTRODUCTION TO CELLULAR IMMUNOLOGY
CELLS AND TISSUES OF THE IMMUNE RESPONSE
CONTRAST: INNATE AND ADAPTIVE IMMUNE MECHANISMS
CLONAL SELECTION HYPOTHESIS & RECEPTOR DIVERSITY

June 7 THE GENETIC BASIS FOR AG-RECEPTOR DIVERSITY
MECHANISMS OF AG RECEPTOR DIVERSITY
B CELL DEVELOPMENT - LINKING DEVELOPMENT WITH RECEPTOR DIVERSITY

June 14 THE B CELL RESPONSE, EFFECTOR FUNCTIONS MEDIATED BY ANTIBODIES
THE GERMINAL CENTER & AFFINITY MATURATION
ANTIBODY ISOTYPES, THEIR GENERATION AND FUNCTION
ANTIBODY MEDIATED EFFECTOR FUNCTIONS AND COMPLEMENT
COMMUNICATION BETWEEN B CELLS AND INNATE IMMUNE CELLS
HAND OUT TAKE-HOME EXAM #1

June 21 TOOLS, TESTS AND EXPERIMENTAL SYSTEMS USED IN IMMUNOLOGY

June 28 **MID-TERM IN-CLASS EXAM - TAKE-HOME EXAM #1 DUE**

July 5 THE MHC AND T CELL BIOLOGY
ALLOREACTIVITY AND IMMUNE RESPONSE GENES
"MHC RESTRICTION" AND ITS IMPLICATIONS
THE MHC, A GENETIC LOCUS CONTROLLING T-DEPENDENT IMMUNE RESPONSES

July 12 CONTROL OF T CELL-MEDIATED IMMUNITY
POSITIVE SELECTION OF IMMATURE T CELLS
ANTIGEN RECOGNITION BY T-CELLS - STRUCTURE OF THE T CELL RECEPTOR
ANTIGEN PROCESSING AND PRESENTATION
COSTIMULATION
CYTOKINES AND T CELL SUBSETS

July 19 THE PROBLEM OF SELF VERSUS NON-SELF DISCRIMINATION
B AND T CELL DEVELOPMENT VIS A VIS TOLERANCE
MECHANISMS OF PERIPHERAL TOLERANCE
HAND OUT TAKE-HOME EXAM #2

July 26 INNATE IMMUNITY AND ITS RELATIONSHIP TO ADAPTIVE IMMUNITY
INFLAMMATORY CYTOKINES
PATTERN RECOGNITION MECHANISMS
IMMUNE MEMORY

August 2 **FINAL IN-CLASS EXAMINATION - TAKE HOME EXAM 2 DUE**

SUGGESTED READINGS

LECTURE	TOPIC	PAGES IN JANEWAY 8TH EDITION	PAGES IN JANEWAY 9TH EDITION
1	Intro / Kinetics / Ab-Ag interactions	Pages 1-10, 18-22; Skim Chapter 1	Pages 1-24 Pages 141-151
2	Cells and tissues / development Cell interactions / clonal selection Innate immunity	Pages 127-138; Pages 1-30 Chapter 11	Chapter 2 (all) Pages 11-24 (repeat)
3	Genetic basis for receptor diversity	Chapter 1 (all); Pages 157-168	Pages 174-186 Pages 296-313
4	Ab isotypes and isotype switching / germinal center reaction Antibody-mediated effector mechanisms	Pages 48-71; Chapter 10; Pages 179-189	Pages 191-198 Chapter 10
5	Immunologist's Toolbox	Appendix 1	Skim pages 740-779
6	MHC and T cell receptor function	Pages 652-654; Pages 169-173 Chapter 6 Pages 138-151	Pages 214-242 Pages 152-170 Pages 328-340
7	T cell mediated immunity	Chapter 9	Pages 282-290 Chapter 9
8	T Lymphocyte development and B and T Cell tolerance	Chapter 8	Chapter 15
9	Integrating innate and adaptive immunity	Chapter 11	Chapter 11

LECTURE 1

SESSION I ADVANCE ORGANIZER:

Most animals have protective systems to survive attack by foreign materials (pathogens, etc.). Some are "constitutive" mechanisms, serving to protect against any type of invasion, such as the keratinized layer of the epidermis and associated secretions. Others, including the immune system of vertebrates, are INDUCIBLE - becoming active only after the offending agent is present.

These protective mechanisms show different degrees of specificity. Some are virtually nonspecific; i.e.; they act against any and all offending agents in the same fashion. Others, again including the immune system, are SPECIFIC - possessing the ability to recognize subtle differences between different offending agents, tailoring the response to these differences.

Finally, the immune system displays two additional important properties: MEMORY, implying that upon a second or further exposure to a given foreign substance, the immune system responds more rapidly and more avidly; and NON-RESPONSIVENESS TO SELF, implying that structures which are a normal part of the host organism are not attacked and destroyed by its own immune system, even though they *could* be attacked by the immune system of an individual in which they were not "self".

I. INTRODUCTION TO BASIC MOLECULAR AND CELLULAR IMMUNOLOGY

A. PROTECTIVE MECHANISMS - GENERAL CONSIDERATIONS

1. PROTECTIVE MECHANISMS EXIST IN MOST ANIMAL FORMS.

a. SPECIFIC *vs* NON SPECIFIC
nonspecific mechanisms - not cognitive
keratinized skin layer inflammation processes
specific -requires recognition

b. INDUCIBLE *vs* CONSTITUTIVE PROTECTIVE SYSTEMS
constitutive - always active, require no stimulus
inducible - require an activating stimulus

c. IMMUNITY AMONG VERTEBRATES

B. IMMUNITY IS A PROTECTIVE MECHANISM

1. The notion of immunity is not new. That disease exposure could lead to subsequent disease resistance was recorded by the ancient Greeks. Accounts of deliberate exposure to disease-producing materials as a means of inducing resistance occur throughout history in many cultures. Perhaps the best known (but by no means the first) is the account of Edward Jenner, who defended the practice of smallpox vaccination. It is now evident that immunity is mediated by specialized organs, cells, and molecules, which together produce the phenomena described by these early observers.

2. The basis for immunity is the recognition, at a sub-molecular level, of subtle structural differences between "self" and "non-self". It is this ability to distinguish small molecular differences that makes the molecules and cells of the immune system good candidates for use as specific molecular probes in research, therapeutics, diagnostics, and industry.

C. AN EXPERIMENT CHARACTERIZING IMMUNE RESPONSES

1. The immune system is characterized by four major properties: *inducibility, specificity, memory, and non-responsiveness to self.* These properties reflect the cellular and molecular events that comprise the immune response. Your own tetanus vaccination serves as an example to demonstrate these properties:

a. INDUCIBILITY

Inducibility is inferred from the observation that immunity is not a constitutive phenomenon: i.e.; exposure to an immunogenic agent, or antigen, must occur before immunity is expressed.

b. SPECIFICITY

Since immunization with one substance does not provide immunity to another, specificity is inherent in the immune response. This property is one reason that the immune response is so well suited to the production of specific tests and tools for the detection of small molecular differences.

c. "*MEMORY*"

The property of memory may be deduced from the observation that subsequent to initial antigen exposure, evidence of immune activity is more rapidly detectable upon re-exposure.

d. NON-RESPONSIVENESS TO SELF

Non-responsiveness to self, one of the most important yet puzzling properties of the immune system, is clearly requisite for a system designed to act against substances by recognizing their molecular structures. Obviously, if such a system were reactive to molecular structures of the host, serious injury or death might result. Indeed, the serious clinical symptoms of diseases characterized by immune reactivity to one's own molecular constituents (autoimmune diseases) are evidence of this. How the immune system manages to distinguish "self" from "non-self" remains one of the major problems in immunology.

SESSION II ADVANCE ORGANIZER:

The immune system operates *via* two general mechanisms.

One involves the production of molecules which appear in the tissue fluids (the 'humors'). These molecules specifically interact with the offending agent to destroy or inactivate it. This is called the *HUMORAL* IMMUNE RESPONSE.

The second involves the activation of cells (as opposed to soluble molecules) which attack other cells bearing foreign materials, and is thus termed CELLULAR IMMUNITY.

Immune responses follow characteristic kinetics, if immune activity is plotted as a function of time following antigen administration.

An understanding of the antibody molecule and antibody-antigen interactions shows how specificity can be explained at a molecular level. The precipitin reaction may be used to demonstrate the properties of antibody-antigen interactions and make inferences about the general structure of antibodies.

The major take-home message from the precipitin reaction experiment is that humoral immune responses are inducible, specific. The major point of hapten inhibition of precipitation is that antibody-antigen interactions are reversible and that antibodies are multivalent.

I. INDUCTION AND EFFECTOR PHASES OF IMMUNE RESPONSES

A. **An immune response may be operationally divided into two phases**:

1. **The *induction phase*.** This involves specific interactions between structures on the antigen molecule and receptors on the cells which mediate immunity. These interactions stimulate the cells to differentiate and divide. This antigen-induced activation of cells is the first step in an immune response.

2. **The *effector phase*.** This entails direct interaction of the immune system's molecules and cells with an antigen, as well as the activation of other cellular and molecular systems, which collectively serve to destroy and remove the antigen.

II. 'HUMORAL' *vs* 'CELL MEDIATED' IMMUNE RESPONSES

A. **The immune system acts *via* two major effector mechanisms.**

1. **The humoral immune response** is characterized by the appearance of soluble molecules, called antibodies, in the serum (the fluid constituent of blood) of the immunized individual. Antibodies are proteins which interact specifically with structural features on the surface of the antigen.

2. **Cell mediated immunity** results in the activation of cells which do **not** secrete soluble antigen-specific mediators of immunity, but instead directly lyse other cells expressing foreign materials upon their surface.

B. In addition, **the immune system contains a large regulatory component** consisting of cells, molecules and receptors, which serve to positively or negatively regulate the activities of these two major effector arms of the response.

III. KINETICS OF IMMUNE RESPONSES

A. IMMUNE RESPONSES FOLLOW CHARACTERISTIC KINETICS

FIG. 1

Immune responses follow characteristic kinetics. After the first, or primary, exposure to antigen, several days pass before effector activity (either antibody or cell mediated immunity) can be detected. Following this, there is a steady rise, plateau, and subsequent fall in specific immune activity. Upon secondary exposure, however, several differences are noted. First, the lag period is considerably shorter. Second, the activity rises at a more rapid rate and reaches higher levels than in the primary response.

B. COMPARE AND CONTRAST PRIMARY *vs* SECONDARY RESPONSES

The differences between the primary and secondary response have several implications for immunity, as well as for any attempt to manipulate or exploit the immune system's capabilities or components.

***1. The primary response*, because of its slower rise time, is rarely able to prevent disease** if the offending antigen is pathogenic. The primary response may, however, help in the recovery from disease and the restriction of disease-producing materials to a particular site.

***2. The secondary response* will frequently prevent any overt symptoms of disease**, because the offending agent is rendered innocuous by the immune system's effector functions before these symptoms are apparent. This is the basis for vaccination - the deliberate exposure of individuals to nonpathogenic forms of materials that usually cause disease. This intentional exposure 'primes' the subject, so that subsequent exposure will induce a secondary, disease-preventing response.

C. WHAT CELLULAR AND MOLECULAR EVENTS UNDERLIE THIS?

These kinetic differences between primary and secondary responses reflect a preferential expansion and differentiation of the cells within the immune system that are specifically reactive with the immunizing anti- gen. Most of these changes occur during the primary response. Thus, upon secondary exposure this already expanded, specific armamentarium reaches detectable levels of activity more rapidly.

IV. THE MOLECULAR BASIS FOR SPECIFICITY IN HUMORAL RESPONSES

A. THE ANTIBODY MOLECULE

Understanding the chemical nature of antibodies and their interactions with antigen is important for several reasons. First, their structure demonstrates how exquisite specificity may be expressed at a molecular level. Second, they provide a conceptual framework for more general considerations of molecular recognition in solution and at cell surfaces.

B. EXPERIMENTAL APPROACH TO UNDERSTANDING Ab STRUCTURE:

1. PRECIPITIN REACTION

Antigen-antibody interactions may be demonstrated and studied by a variety of tests. Detailed descriptions of all such tests are beyond the scope of this course. The following discussion presents the fundamental properties of antibody-antigen interactions using an elegantly simple test, *the precipitin reaction*. The results of this test make many inferences about the nature of the antibody molecule's structure and chemical behavior.

C. IMMUNIZATION - PRACTICAL ISSUES

DEFINITIONS

ANTIGEN - *Any substance which will induce a specific immune response*. when administered to an immunocompetent individual.

HAPTEN - A substance, frequently a small organic group, which *cannot itself act as an antigen* , but which will serve as an antigenic determinant when coupled to a larger, "carrier", molecule. [e.g.; in the antigen, DNP-BSA (dinitrophenyl - bovine serum albumin), the DNP group is the *hapten* and the BSA is the *carrier* molecule. The entire complex is the *antigen*.]

CARRIER - A substance, usually a large molecular weight protein, to which small groups (haptens) may be coupled in order to make them antigenic.

ANTIGENIC DETERMINANT - Any small region of a molecule which forms a *3-dimensional array of structure and charge which will be recognized and bound by an antibody combining site*. Note that unlike the above entities, this term has no meaning except when spoken of in the context of a particular antibody's combining site.

D. A SIMPLE PRECIPITIN REACTION SHOWS:

INDUCIBILITY

There is no precipitation formed in the pre-immune serum sample, only in the samples after immunization.

SPECIFICITY

As the molecular vehicle of humoral immunity, it stands to reason that the interaction of an antibody with antigen displays specificity. (This specificity is a function of the amino acid composition within particular areas of the antibody molecule, as seen below. The combining site thus formed presents a particular spatial array of structure and charge which is available to interact with small structures on the antigen surface.) In the precipitin test we are doing, the specificity is shown by a lack of reactivity against materials which were not used to immunize.

HETEROGENEITY

This is actually more a property of the humoral immune response in general than of antibody-antigen interactions per se. In general, most antigens elicit a large variety of antibodies when used to immunize an individual. For example, even very small organic molecules such as 2-,4-dinitrophenyl (DNP), if coupled to an appropriate carrier molecule, will elicit literally *thousands* of different antibodies - each of which binds DNP with measurable affinity but each of which is slightly different than the other. This extensive HETEROGENEITY allows a great deal of selectivity within immune responses, since it is thought that those antibodies of higher affinity will gradually predominate as antigen levels decrease within the individual. Indeed, a gradual increase in the average affinity of an antisera is observed as the primary response proceeds and presumably reflects this process. Heterogeneity is shown in our example by the reaction of the antisera with the hapten - even on a carrier which we know there are no antibodies against, as well as with the carrier molecule we used in the immunization - even when no hapten molecules are coupled to it.

E. HAPTEN INHIBITION SHOWS:

REVERSIBILITY

Perhaps the most important property (at least in terms of your understanding what the term "specificity " means in an immunological sense) of antibody-antigen interactions is their reversibility. This property is often ignored by veteran as well as novice immunobiologists - and is frequently rediscovered by experience.

Reversibility of antibody-antigen interactions may be demonstrated by various types of competitive inhibition experiments. The reversibility of antibody-antigen interactions indicates that the binding of an antibody to its ligand is a non-covalent process mediated primarily by charge interactions and other weak chemical forces. This in turn indicates that antigen-antibody interactions are an equilibrium process, and may be described (as any other equilibrium process) by the law of mass action. Thus, for any particular antibody-antigen combination, it should be possible to derive an affinity constant based upon the strength of the interaction between the combining site and the ligand.

MULTIVALENCY REQUISITE FOR PRECIPITATION

Since monovalent ligand will competitively inhibit the precipitation of complexes that occurs when multivalent ligands are used, it may be inferred that precipitation requires multivalent interactions, and more importantly, that antibody molecules are likely to have more than one combining site per molecule.

SESSION III ADVANCE ORGANIZER

Immunoglobulins are large multi-chain glycoproteins. Each molecule has two identical heavy and two identical light chains. The amino terminal portions of both types of chains are highly variable in amino acid sequence, and are where the combining site for antigen is formed. The carboxy-terminal portions of each chain are fairly constant from molecule to molecule and dictate effector functions. Several general classes of heavy and light chain constant regions exist, and define the heavy and light chain "isotypes".

IMMUNOGLOBULIN STRUCTURE

I. GENERAL FEATURES

All immunoglobulins consist of at least one basic four-chain monomer. Each monomer contains two identical large, or heavy, chains; and two identical small, or light, chains. The heavy chains have a molecular weight of 50K daltons, and the light chains have a molecular weight of 25K daltons. Thus, each such four-chain molecule has a weight of about 150Kd. The chains are linked by interchain disulfide bonds as shown in Figure 2. In addition, each chain has several intrachain disulfide bonds which occur at regular intervals and cause the molecule to fold in distinct globular domains of about 110 amino acids each.

FIG. 2
(DRAW AND LABEL AN IG MOLECULE IN THIS SPACE)

II. PRIMARY STRUCTURE

The antigenic specificity of a given immunoglobulin molecule is determined by the amino-terminal 110 amino acids of both chains. In this region of both heavy and light chains, the primary amino acid sequence differs markedly from molecule to molecule. This 110 amino acid long region of each chain is called the variable region. In addition, three areas of particularly great variability exist within this 110 amino acid long variable region, which are termed hypervariable regions. These hypervariable areas contain the amino acid residues actually involved in forming the antigen combining site once the immunoglobulin molecule has folded.

FIG. 3
(DRAW AND LABEL A "VARIABILITY PLOT" IN THIS SPACE)

The carboxy terminal 110 amino acids of the light chains, and 330 amino acids of the heavy chains, are relatively constant from molecule to molecule. However, there are several different possible constant regions which may be used for each. These different constant regions are termed light- and heavy-chain isotypes.

Among light chains, two major types of constant regions are found. Among heavy chains, at least eight types of constant regions may exist. The constant regions of the heavy chains dictate the effector function of the immunoglobulin molecule.

I. HEAVY AND LIGHT CHAIN ISOTYPES

The two types of **light chain** constant regions are termed kappa (κ) and lambda (λ). These are the *light chain isotypes*. The average proportion of antibodies bearing kappa vs. lambda light chains varies greatly between species. For example, in mice over 95% of all antibodies bear kappa light chains, whereas in humans the proportions of kappa and lambda are roughly equal. No functional distinctions have been ascribed to the different light chain isotypes.

It is the constant region of the heavy chain which is used to characterize a particular antibody molecule as IgM, IgG1, IgA, etc. These are termed heavy chain isotypes. When used in reference to a complete molecule, the Roman alphabet symbols shown above are used. When reference is made to the heavy chain alone, the Greek alphabet counterpart is generally used (e.g.; gamma, mu, alpha, etc.).

Particular quaternary structures are associated with particular heavy chain isotypes. Most isotypes consist of only the single four-chain monomer already described. Notable exceptions to this are IgM and, under certain conditions, IgA.

The IgM molecule is a pentamer of the four-chain subunit, held together by short peptides termed the J-chain. Thus, each IgM molecule contains ten antigen combining sites.

IgA molecules are initially produced as a monomer. However, this isotype is capable of being secreted across mucosal membranes. During this process, two monomers are joined to form a dimeric, tetravalent molecule.

The potential effector functions mediated by an immunoglobulin molecule are dictated by the heavy chain isotype. This is because many effector functions involve the interaction of antigen-antibody complexes with other cells or molecules, and the sites of these interactions are created by the tertiary and quaternary structure of the constant region domains. Thus, constant regions of different primary sequence will differ with respect to these sites.

Heavy chain constant region isotype	When in a whole molecule, the Ig is termed:	Major functional properties :
μ	IgM(κ or λ)	First isotype made in primary responses (pentameric form), good complement fixation; Surface receptor for antigen on B- lymphocytes (monomeric form).
δ	IgD(κ or λ)	Surface receptor for antigen on mature primary B-lymphocytes. Not secreted.
γ1 γ2a γ2b γ3	IgG_1(κ or λ) IgG_{2a}(κ or λ) IgG_{2b}(κ or λ) IgG_3(κ or λ)	These IgG subclasses are the major isotype found in the serum during secondary responses and late primary responses. These are the isotypes generally associated with protection to pathogens, except for certain bacteria, or for pathogens that enter *via* mucosal routes
α	IgA(κ or λ)	Associated with mucosal sites (e.g.; peyer's patches); Transported across basement membranes
ε	IgE(κ or λ)	Binds to receptors on mast cells and mediates immediate hypersensitivity and anaphylaxis (types of allergy).

II. ENZYME DIGESTION: Fab AND F(ab)'$_2$ FRAGMENTS

Several techniques which fragment immunoglobulin molecules are useful for both the study and the use of antibodies. These techniques produce predictable breaks in immunoglobulin molecules and may be used to alter the valency, in terms of antigen combining sites, as well as to separate the combining sites (amino terminal portions) from the areas of the molecule which mediate effector functions (carboxy terminal portions).

The most commonly used technique is enzymatic cleavage. By far the most widely used enzymes for fragmenting immunoglobulins are *papain* and *pepsin*. Each of these results in the cleavage of the molecule such that the amino terminal, antigen-binding end of the molecule is separated from the carboxy terminal regions of the heavy chains. Their cleavage points differ: pepsin retains the two combining sites in their usual dimeric configuration; whereas papain separates the two amino terminal arms - resulting in two *monovalent* antigen binding fragments. The antigen binding fragments produced are termed either F(ab) or $F(ab)_2$; for the monovalent and bivalent fragments respectively. The heavy chain constant fragment which results from such cleavage is called the Fc fragment.

These techniques are useful in situations where the effector functions of a molecule hinder the intended use. For example, some constant regions tend to stick to cell surfaces non-specifically, and will thus cause problems if one is trying to identify particular cell types using antibodies specific for particular cell surface structures. In these cases, the use of F(ab) or F(ab)2 fragments will frequently prevent false positives due to constant region binding to cells.

III. AFFINITY CONSIDERATIONS AND MEASUREMENT

By measuring the proportion of sites filled at given molar concentrations of antibody and antigen, the calculation of affinity constants is possible. The affinity constants of antibodies may vary over a wide range: from $10^4 M^{-1}$ to as great as $10^8 M^{-1}$. It is not essential that you know the appropriate equations and manipulations needed to derive an affinity constant. HOWEVER, THE CONCEPT OF HOW AFFINITY RELATES TO THE NOTION OF IMMUNOLOGIC "SPECIFICITY" IS VERY IMPORTANT.

LECTURE 2

SESSION I

INTRODUCTION TO CELLULAR IMMUNOLOGY

ADVANCE ORGANIZER

Immune responses primarily involve three types of cells: B-lymphocytes, T-lymphocytes, and “antigen-presenting” cells. All arise from pluripotential stem cells in the bone marrow. B-lymphocytes develop and mature in the bone marrow, and are the cells which produce antibody. T-cells mature in the thymus, and may differentiate to one of three subtypes of cells: Cytotoxic T-cells, which can lyse other cells of the body expressing foreign surface molecules; Helper T-lymphocytes, which are involved in the induction of both humoral and cell mediated immunity; or Suppressor T-lymphocytes, which down - regulate immune responses. APC’s may be of several types, although most are macrophage-like, and also mature in the bone marrow. These are important in the induction of immune responses through the “presentation” of antigen to helper T-cells. Some of them (e.g.; macrophages) serve as phagocytic cells in the effector phase of immune responses. All of these cell types are produced *continuously* during an individual's lifetime.

Tools that detect unique cell surface molecules may be used to distinguish the different cell types. These distinguishing surface molecules go by a variety of names, but are standardized by the cluster designation (CD) nomenclature. For example, all T-lymphocytes display a molecule called CD-3 on their surface.

I. CELLS AND TISSUES OF THE IMMUNE SYSTEM

A. CELLS AND THEIR GENERAL FUNCTIONS

All manifestations of immunity arise from cells within the individual. The major cell types involved in the immune response are lymphocytes and macrophages. The lymphocytes are subdivided according to their function and their site of origin.

B. LYMPHOCYTES:

1. ORIGIN OF LYMPHOID CELLS

Lymphocytes originate from pluripotential stem cells in the bone marrow. As these stem cells differentiate along the lymphocytic lineage, they may either mature in the bone marrow, or they may migrate to and mature in the thymus. **Because the marrow and thymus are sites of lymphocyte production, they are termed *primary lymphoid organs*.**

Lymphocytes are constantly produced throughout the lifetime of an individual. Since there is a steady state of lymphocyte numbers within the body, it is clear that *turnover* occurs within the mature lymphocyte pool. The precise half life of unstimulated, or primary, lymphocytes is debated: estimates range from two weeks to two months or more. Regardless of the exact figure, this rate is relatively rapid compared to many other cell types. If stimulated by antigen, however, a lymphocyte and its clonal progeny may be very long lived - on the order of many years.

2. T- AND B- LYMPHOCYTES

Lymphocytes that mature in the bone marrow are known as B-lymphocytes, and those that mature in the thymus are called T-lymphocytes. The functions of the lymphocytes that develop in these distinct sites are quite different.

3. B-CELLS MAKE ANTIBODY

B-lymphocytes are the effectors of humoral immunity, i.e.; they produce antibody. Initially, antibody is produced and displayed as a membrane-bound surface molecule, and it is only after the antigen-driven activation of B-lymphocytes that the antibody is secreted. The membrane-bound form of antibody acts as an antigen-specific receptor for B-cell activation.

4. T-CELLS PERFORM VARIETY OF TASKS

T-CELL SUBSETS

T-lymphocytes may serve a variety of functions and are further subdivided accordingly. Three major “subsets” of T-lymphocytes exist.

a. CYTOTOXIC

These T-lymphocytes are the effectors of cell mediated immunity. When activated appropriately, T-lymphocytes of this subset can directly lyse other cells displaying foreign molecules on their surface. These cells are called cytotoxic T-lymphocytes, abbreviated Tc. Just as B-cells, Tc also display surface receptors for antigen on their surface, but these receptors are not antibody and they are not secreted in detectable quantity following activation.

b. REGULATORY T-CELL SUBSETS

The remaining subsets of T-lymphocytes regulate both the humoral and cellular arms of the immune response. Again, each of these cell types bear surface receptors which impart antigen specificity, but these receptors are not antibody and are not secreted upon activation.

i. HELPER

This regulatory subset of T-lymphocytes is <u>required</u> for the induction of most immune responses. These T-lymphocytes thus "help" immune responses and are accordingly called helper T-lymphocytes, abbreviated Th.

ii. SUPPRESSOR

The second set of regulatory T-lymphocytes acts to down-regulate immune responses and is appropriately termed suppressor T-lymphocytes, abbreviated Ts.

5. MACROPHAGES and DENDRITIC CELLS

Macrophages also arise in the bone marrow from pluripotential stem cells. Unlike lymphocytes, *macrophages bear no endogenously synthesized receptor for antigen*. Instead, the macrophage is able to phagocytose and pinocytose particulate or soluble materials in a relatively indiscriminate fashion. Macrophages and dendritic cells are of great importance throughout the immune response, and serve two major functions:

a. ANTIGEN "PRESENTATION"

Both macrophages and dendritic cells are able to "present" antigen to the lymphocytes (especially Th) in a fashion that leads to lymphocyte activation. In addition, macrophages secrete a variety of soluble mediators of lymphocyte activation. (These two properties are discussed in greater detail later). In particular, the follicular dendritic cells of the spleen are effective at presenting antigen during primary responses.

b. PHAGOCYTOSIS

Second, macrophages also act during the effector phase of the response by ingesting and destroying foreign materials, and are especially effective at ingesting materials coated with antibody.

6. THE CONCEPT OF "SURFACE MARKERS"

It's hard to tell mononuclear cells apart morphologically. Antibodies against cell surface molecules unique to particular cell types are a useful way to identify, separate, and study otherwise indistinguishable cells. Molecules recognized by such reagents are called cell surface "markers" [also "differentiation antigens" or "surface antigens"]. Many markers are defined which distinguish functional categories of lymphocytes. A table listing some of these markers is available in your text *but only memorize the three or four I tell you to in class*.

CLUSTER DESIGNATION ("CD") NOMENCLATURE

After it became common to use markers to distinguish cell types, a single nomenclature was decided upon (but not until the existing nomenclature was completely unintelligible, and if you read the old literature, you will find this out). The current nomenclature involves "*cluster designations*" - the idea being that particular clusters of markers can distinguish subsets of cells. Thus, most markers are preceded by "CD-" followed by a number. For example, a marker that is found an all T-cells happens to be called "CD3". I could thus say that if the cell is CD3 positive ($CD3^+$), it must be a T-cell.

ADVANCE ORGANIZER

The immune system consists of a variety of specialized organs and vessels which facilitate the "surveillance" role of the immune system. These organs are divided into PRIMARY and SECONDARY LYMPHOID ORGANS. Primary lymphoid organs are where lymphocytes are produced. Secondary lymphoid organs are areas to which lymphocytes migrate after differentiation and maturation. Lymphocytes are produced constantly, and recirculate at a relatively rapid rate. Most lymphocytes are relatively long-lived, in the range of months to probably years. The rapid production rate and the relative longevity of cells in the mature lymphocyte pool implies considerable cell loss among immature lymphocytes.

I. PRIMARY VERSUS SECONDARY LYMPHOID ORGANS

PRIMARY: MARROW
THYMUS

SECONDARY LYMPH NODES
SPLEEN
PEYERS PATCHES
GALT/BALT

II. RECIRCULATION AND TURNOVER

Most mature lymphocytes constantly recirculate through a system of vessels and organs called the reticuloendothelial system. This system includes the lymphatics, lymph nodes, and spleen. In contrast to the thymus and marrow, where lymphocytes are produced, these areas of lymphocyte recirculation and migration are called *secondary* lymphoid organs. The pattern of recirculation tends to be from blood to lymphatics to blood. The major areas of antigen contact and lymphocyte activation are the lymph nodes, although this may occur in the spleen and elsewhere as well. Both B-lymphocytes and T-lymphocytes recirculate, although at different rates. Further, the relative proportion of T- versus B- lymphocytes in these secondary lymphoid organs varies. The lymph nodes tend to be more rich in T-lymphocytes whereas the spleen has a larger proportion of B-lymphocytes.

ADVANCE ORGANIZER:

MULTIPLE CELLS ARE REQUIRED FOR IMMUNE RESPONSES.

Although specific receptors for antigen exist on lymphocytes, the simple interaction of these receptors with their ligand, though necessary, is usually not sufficient to produce activation and differentiation to effector function. Instead, the induction of immune responses involves the interaction of multiple cell types.

I. CELLULAR INTERACTIONS IN THE INDUCTION OF IMMUNE RESPONSES.

A. THREE CELL TYPES REQUISITE

The induction of either humoral or cell mediated immunity generally requires three cell types: macrophages, Th cells, and an appropriate effector cell (either B-cells or Tc cells). These three cell types interact with antigen, as well as with each other, in the induction of immune responses. This tripartite interaction involves not only specific recognition of antigen by lymphocyte surface receptor molecules, but the synthesis and secretion of other stimulatory molecules which lack any specificity for antigen. These 'nonspecific' molecules which are secreted by one leukocyte ("white blood cell") and affect another are collectively termed interleukins.

1. T-CELLS

A major cellular interaction in immune responses is mediated by Th cells and their products. Once the Th cell is activated by appropriately presented antigen, it will divide and secrete a variety of soluble molecules. These soluble factors do not have specificity for antigen, but are capable of participating in the activation of effector cells. In the case of B-cells, these mediators are a requisite second signal (the first being antigen binding by B-cell receptors) for division, differentiation, and antibody secretion. Similarly, the effectors of cell mediated immunity, Tc cells, also require soluble mediators produced by Th cells before they can divide and differentiate.

In addition to the activation of effector cells, activated Th cell products affect the macrophages. Most of these soluble products amplify the activities of macrophages so that they become even more effective at the presentation, ingestion, and destruction of antigen.

Thus, the activated Th cell and its products are central to the induction of both major effector arms of the immune response.

2. ANTIGEN PRESENTING CELLS

The pivotal cellular interaction in the induction of primary immune responses occurs between "antigen presenting" cells and Th cells. Macrophages, dendritic cells, langerhans cells, and a few others are able to ingest antigen and degrade it by a variety of proteolytic and oxidative mechanisms. Some intact antigen or fragments of antigen, however, are returned to the macrophage cell surface. Antigen presented on the surface of macrophages is required for recognition and activation of Th cells. The precise nature of this process is not entirely understood, although it is clear that specialized and precise intracellular trafficking mechanisms, as well as highly specialized molecules on the macrophage surface (discussed later in the course) are necessary for the fruitful interaction of Th cell receptors with antigen.

Macrophages also secrete a variety of soluble molecules when phagocytically active. Some of these soluble molecules are mitogenic for T-lymphocytes, provided the Th cell is also being activated by appropriately presented antigen.

Thus, the macrophage-Th interaction has two components: first, the processing and presentation of antigen in a suitable form, and second, the synthesis and secretion of soluble molecules that deliver signals to the Th cell.

THE CELLULAR BASIS FOR SPECIFICITY

ADVANCE ORGANIZER:

Clearly cells lie at the foundation of the mechanisms governing all immune responses. A unifying hypothesis to explain the cellular basis of the immune response is the clonal selection hypothesis of Burnet. This hypothesis is generally accepted and serves as a good framework within which to view the processes of immunity at a cellular level. The major tenet of this hypothesis is that specificities (receptors) are clonally distributed.

If specificities are clonally distributed, the breadth of antigens to which we can respond demands that the diversity of clonal specificities be enormous. The degree of diversity among B-cells has been estimated at greater than ten million. Although this explains the great specificity and heterogeneity of responses, it raises an intriguing genetic question; How can so many different specificities (in the case of B-cells, antibody variable regions) be encoded in the genome?

THE CELLULAR BASIS FOR SPECIFICITY

I. NOTION OF RECEPTOR DIVERSITY

It follows from our notions about the molecular and cellular bases for specificity that the array of receptors must be large. Otherwise, the immune system might fail to respond to many antigens. This perplexing problem of receptor diversity was studied extensively during the past decade; especially with respect to B-lymphocytes, where the chemical nature of the receptor was known. Indeed, the array of antibody specificities is very diverse. It is estimated that within a species, between ten- and one hundred-million possible combinations of heavy and light chain variable region pairs may be produced, with particular individuals expressing several million within their B-lymphocyte pool at any given time.

II. CLONAL DISTRIBUTION OF RECEPTORS

A. Specificity is achieved at the cellular level through the clonal distribution of antigen-specific receptors

Current thought about the cellular basis of immune responses is based upon the clonal selection hypothesis of Burnet. In general, this hypothesis explains the specificity of immune responses at the cellular level by requiring that the receptors for antigen be clonally distributed, and that the cells of the immune system become activated only following the interaction of these receptors with their corresponding antigen. Although originally formulated to explain the humoral response (i.e.; B-cell responses), the postulates are equally applicable to T-cell responses.

III. CLONAL SELECTION HYPOTHESIS

A. ***Lymphocytes express a receptor for antigen on their surface,*** and interaction of this receptor with ligand (antigen) is requisite for activation of the cell. Further, the specificity of the effector function (antibody specificity, or cytotoxic T-cell specificity) will be the same as the receptor's specificity. (RECEPTOR=PRODUCT)

B. ***At any time, a given cell expresses only one type of receptor*** (in terms of antigen specificity). Thus, each clone of lymphocytes is monospecific. (MONOSPECIFICITY)

C. Following activation, ***a cell and its clonal progeny will maintain the specificity originally expressed*** *in receptor form*. (CLONAL INTEGRITY)

A bit of reasoning will show that these tenets will account for the specificity and inducibility of the immune response at the cellular level. In addition, experimental evidence supports the tenets of this hypothesis to the extent that it is accepted nearly axiomatically.

D. It is now evident that antigen-specific receptors on B-cells are antibody molecules, and the specificity of the secreted antibody product of each B-cell clone is equivalent to its receptor. Although changes in specificity may occur within a clone of activated B-cells by

point mutation within the genes that encode the antibody, the notion of clonal integrity is still generally valid.

E. T-cell receptors are clonally distributed as well, although a detailed discussion of this will wait until we have discussed certain aspects of T-Cell antigen recognition.

IV. RECEPTOR DIVERSITY

A. LIMITING DILUTION ANALYSES demonstrate enormous diversity in the repertoire of clonally distributed B-cell and T-cell receptors.

LECTURE 3

SESSION I

ADVANCE ORGANIZER

MOLECULAR GENETICS OF ANTIBODIES

Given this great degree of diversity, several intriguing biological problems were evident: First, how is so diverse an array of molecules maintained or generated in the genome of an individual? Second, how is it genetically possible to conserve the primary sequence of one end of a protein molecule while allowing the other end to vary so greatly?

The key to these questions lay in the discovery that the genes encoding a complete heavy or light chain do not occur as contiguous segments of DNA in the germ line genome. Instead, several gene segments, which are separated by considerable distance in the germ line DNA, are spliced together at the DNA level during the differentiation of B-cells. In this manner, many permutations of these different segments may be achieved even with a relatively small number of gene segments.

I. PROTEIN SEQUENCE ANALYSES PROVIDED CLUES TO THE GENETICS OF DIVERSITY

A. kappa light chains

B. lambda light chains

C. "germ-line" *versus* "somatic mutation"

II. THE NOTION OF MULTIPLE GENE SEGMENTS

A. A functional light chain gene is encoded in the germ line genome by three segments. One segment encodes the constant region, and there is one copy of each type of light chain constant region per haploid genome.

B. Each constant region gene segment is associated with two collections of additional gene segments which can encode the variable region of a light chain protein. These two sets are termed the light chain V-region genes (VL) and J-region (JL) genes.

C. There are 4 to 5 JL-region gene segments, each of which may encode the 14 amino acids amino terminal to the constant region.

D. There are approximately 100 VL-region genes that can each encode the remaining 96 positions amino-terminal to the J-region. The J-region was so named because it is the

segment which "Joins" the V- and C-region genes. The J-region genes are found fairly close (less than 2kb) from the C-region genes in the germ line genome. The J-region genes are separated from one another by introns of less than 2Kb as well. The V-region genes, however, are found clustered a considerable distance (at least 17kb) from the J-region genes. E. During differentiation, cells destined to become B-cells rearrange their DNA in this region and cause one of the V-region genes to be brought into association with a J-region gene. The resulting V-J-C combination is then transcribed, post-transcriptionally modified, and translated. The signals for appropriate joining are palindromic sequences found immediately 3' of each J-gene and immediately 5' of each V-gene. It is presently believed that any V- may be arranged with any J-. Thus, for kappa light chains this results in 400 V-J combinations. It is important to recall that the region encoded by the area of the V-J junction is located within one of the hypervariable regions, and will thus be likely to influence antigen specificity.

Therefore, from approximately 100 genes over 400 choices of kappa light chain specificities may be derived. A similar set of genes, although apparently not as extensive, appears to exist for lambda light chains. Kappa and Lambda genes are not linked and thus represent independent sets of V, J, and C genes (termed V, J, and C, and V, J, and C).

II. Heavy chains are produced by joining four gene segments

The strategy for generating diversity among heavy chains is similar to that of light chains, but instead of only two gene segments contributing to the formation of the variable region of the protein, three segments are involved. As among light chains, several gene segments exist just upstream of the heavy-chain constant region genes which encode a joining, or JH, region of the heavy chain. Also, a cluster of VH genes, which encode the amino terminal 96 amino acids of the heavy chain, are found a considerable distance 3' to the JH genes. Unlike light chains, a third group of gene segments exists between the VH and JH region genes. This third type of gene segment used in the formation of the heavy chain variable region is called the D region (for Diversity). Thus, during the formation of a functional heavy chain gene, a VH, D, and JH gene are juxtaposed using palindromic sequences which flank each segment as markers for splice sites, leading again to a large variety of permutations based on a small number of gene segments. In addition, it is not essential that a D gene be used (i.e.; some heavy chains are made up of only a VH-JH join) , so a further dimension in diversity generation is gained via the exclusion of D-regions in some heavy chains. It is presently felt that there are about 5 JH, 10 to 20 D, and 300 to 400 VH genes per haploid genome.

The arrangement of heavy chain constant region genes also differs from that observed for light chains. Although as in light chain genes, each possible heavy chain constant region gene (which will determine the isotype of the heavy chain produced) is represented once per haploid genome, they are arranged linearly on the same chromosome. Further, the three 110 amino-acid domains of each heavy chain constant region are encoded by exons separated by short introns and for each type of constant region there are two types of introns that can be used for the carboxy-terminal domain. This allows the carboxy-terminal domain to be either a trans-membrane form (which has an appropriately hydrophobic region commensurate with trans-membrane proteins) or a

secreted form, depending upon which intron is chosen during post-transcriptional modification of the primary transcript.

The order of heavy chain constant region genes is generally the same as the order in which the various isotypes are seen during cell differentiation and activation: the gene for C-mu is closest to the JH genes, followed by delta, the various gamma subclasses, alpha, and epsilon.

The exact molecular mechanism whereby the genetic rearrangements required for VL-JL or VH-D-JH joining, as well as those required for the switching of heavy chain isotypes during B-cell activation, are not yet understood.

IV. DIVERSITY IS GENERATED BY SEVERAL MECHANISMS

A. *Combinatorial association*

It is assumed presently that virtually any light chain may be paired with any heavy chain. The multiplication of all possible VLJL combinations by all possible VHDJH combinations shows that nearly 10^7 antibody specificities may be generated from a collection of about 500 gene segments.

B. *Junctional diversity*

In addition to the diversity inherent in the permutations of gene segments available, several additional diversity generating mechanisms exist. The first of these is called junctional diversity. During the rearrangement of VL to JL, and VH to D to JH, some mismatch will occur. As long as frameshift errors are not created, this means that the amino acids encoded in these junctional areas may vary, depending upon the exact codon created. This mechanism of junctional diversity has been clearly confirmed by comparing amino acid sequences of myeloma proteins to the genes available to encode them.

C. *Somatic variation*

Further diversity is generated by point mutations which occur during the lifetime of a B-cell clone. It has recently been estimated that the mutation rate in this region may be as high as one mutation per 103 base pairs per generation. This mechanism, assuming the B-cell clone is long-lived, could result in a considerable increase in diversity over that afforded by the simple shuffling of germ line gene V, D, and J segments.

V. T-CELL RECEPTOR GENES ARE ALSO SEGMENTED

The genes which encode T-cell receptors are similar to those that encode immunoglobulins in many respects. First, they occur as gene segments that require

rearrangement before a functional receptor gene is produced. In addition, several chains comprise the receptor in T helper cells (and possibly the other subsets as well). These different chains are, as in immunoglobulins, encoded by separate clusters of gene segments. Finally, palindromic sequences similar to those found among immunoglobulin gene segments appear to mediate the joining of the various segments. In contrast, the arrangement of the gene segments does not seem to be like that of the immunoglobulin genes.

SESSION II

ADVANCE ORGANIZER

REGULATION OF V(D)J RECOMBINATION

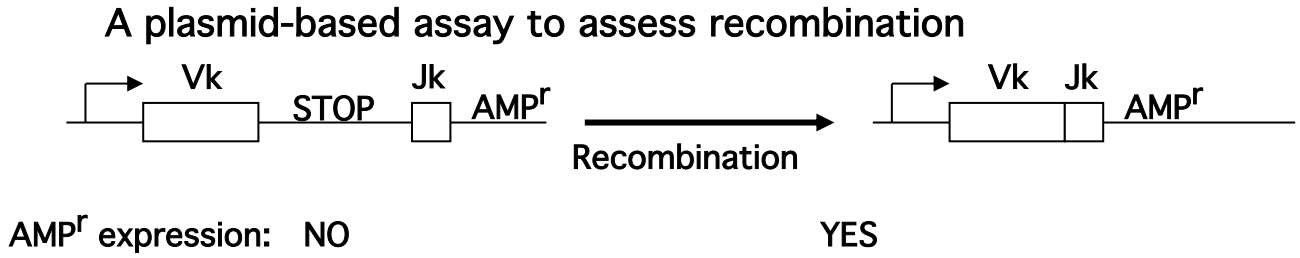
Although the discovery of V(D)J recombination solved a great puzzle in Immunology, its discovery also raised several new questions: First, how is this process turned on and off? Second, since different sets of genes recombine to form similar but unique receptors for B and T cells, how is recombination targeted to the appropriate loci? Third, given that all genes that are not sex-linked have two alleles, how do lymphocytes achieve monospecificity?

While the answers to these questions remain to be fully appreciated, a full understanding of this process requires consideration of its regulation at both the molecular and cellular level. Molecules that regulate V(D)J recombination include the recombinase activating genes 1 and 2 (**Rag-1/2**) which initiate V(D)J recombination by introducing double stranded breaks in antigen receptor loci, and the pre-B cell receptor (**pre-BCR**) which functions to deliver signals that regulate expression of the Rag genes. The activity of Rag-1/2 is as a result highly dynamic, resulting in the recombination of immunoglobulin heavy and light chain genes at separate stages of B cell development.

Recombinase activating genes 1 and 2 (Rag-1/2) promote V(D)J recombination in nonlymphoid cells.

David Schatz and Marjorie Ottinger designed a system to identify gene products that are sufficient to promote V(D)J recombination. This system involved introduction of two sources of exogeneous DNA into fibroblasts, a nonlymphoid cell type which does not recombine its antigen receptor genes under normal circumstances.

The first source of DNA was a plasmid designed to express an ampicilin resistance gene only as a result of a successful Vk-Jk recombination event. To accomplish this, Schatz and Ottinger inserted a STOP codon between the Vk and Jk segment. According to the models of V(D)J recombination put forth above, this STOP codon will be excised upon a Vk-Jk rearrangement.



The second set of DNA was a library consisting of large pieces of genomic DNA. These fragments of DNA were transfected into fibroblasts that already contained the plasmid recombinational substrate described above. The resulting cells were screened for expression of the AMP-resistance gene. Although most cells were negative, a few cells were positive and were used to harvest the fragment of genomic DNA responsible for inducing Vk-Jk recombination in a fibroblast. Remarkably, this piece of DNA had TWO intact genes which, when isolated and reintroduced into other plasmid-containing fibroblasts, again induced recombination. **These genes were given the names Recombination Associated Gene-1 (Rag-1) and Rag-2,** and were subsequently shown to be required for the recombination/assembly of all antigen receptor gene segments.

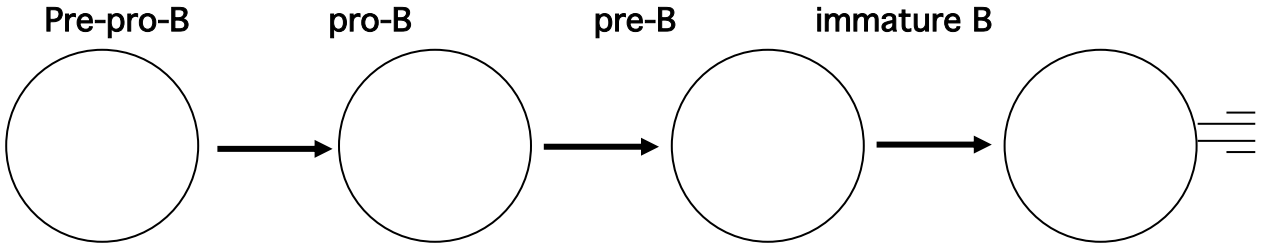
Although today the precise role of these genes in V(D)J recombination is a matter of intense study, it has recently been reported that they both function to bind to the conserved "heptamer-nonamer sequences" that flank all antigen receptor gene segments and initiate the DNA double stranded break reaction required for V(D)J recombination.

Heavy and light chain rearrangements are ordered and highly regulated

Cell lines derived from B cell precursors exhibit a characteristic pattern of H and L chain rearrangements:

- Cells with L (kappa or lambda) chain rearrangements always have H chain rearrangements.
- Some cells with H chain rearrangements sometimes do NOT have L chain rearrangements.

These and other findings led to the model of V(D)J recombination vis a vis B cell development shown below:



Rearrangements on:

IgH: germ-line	Vh-Dh-Jh	completed VhDhJh	
IgL: germ-line	germ-line	VL-JL	completed VL-JL

LECTURE 4

SESSION I

THE B CELL RESPONSE

B cells function primarily to secrete specific antibodies to potentially dangerous pathogens. Although we've spent some time talking about the cellular and molecular basis for B cell specificity, these models do not explain how the resulting antibodies act to remove or effect the destruction of these pathogens. This lecture will deal with how different types of antigens induce B cells to secrete various antibody isotypes and how these different isotypes elicit one or more of the several unique effector activities of the immune system.

THREE CATEGORIES OF ANTIGENS FOR B CELL RESPONSES

T-cell independent type I - B cell mitogens such as lipopolysacharide (LPS).

These are not antigens in the sense that they do not bind to antigen-specific receptors on the surface of B cells. In fact, LPS induces B cell proliferation irrespective of the B cell specificity.

T-cell independent type II - Antigens that can effectively stimulate antibody secretion in B cells without interacting with T cells. These antigens are typically long, highly repetitive structures that can efficiently cross-link multiple B cell receptors on a given B cell.

T cell dependent - tend to be protein antigens to which B cells can bind through the B cell receptor but not effectively respond to without the addition of additional factors derived from T cells.

ISOTYPE SWITCHING and AFFINITY MATURATION

B cell responses to T-cell independent type II antigens tend to be predominated by IgM and, to a lesser extent, IgG3. In contrast, T-dependent antigens can elicit a wide array of antibody isotypes depending on a variety of factors that we will discuss in greater detail in the second half of the course.

In addition, in the 1970's it was first appreciated that antibody affinities for an immunizing antigen increase over time during a primary immune response to a T-dependent antigen. This phenomenon is now thought to be due to two distinct mechanisms, competition between low and high affinity clones (the high affinity ones win!) and a somatic hypermutation mechanism which introduces random point mutations in the V genes of antigen-reactive B cells. <u>The hypermutation mechanism is thought to be operative solely in germinal center B cells.</u>

ADVANCE ORGANIZER

Although antibodies show exquisite specificity for a particular antigen or determinant, they must also share many functions - particularly those that are designed to rid the body of the offending agent. These shared functions are collectively termed effector functions. The constant regions of immunoglobulins dictate their effector functions, and different isotypes mediate different effector mechanisms.

Some effector mechanisms are the direct effect of antibody interaction with antigen, such as NEUTRALIZATION.

Other effector functions, although initiated by antigen-antibody interaction, require other cells or molecules in order to proceed. Examples of this are OPSONIZATION and COMPLEMENT FIXATION.

THE VARIOUS FUNCTIONS OF THE IMMUNOGLOBULIN ISOTYPES

Heavy chain constant region isotype	**When in a whole molecule, the Ig is termed:**	**Major functional properties :**
μ	IgM(κ or λ)	First isotype made in primary responses (pentameric form), good complement fixation; Surface receptor for antigen on B- lymphocytes (monomeric form).
δ	IgD(κ or λ)	Surface receptor for antigen on mature primary B-lymphocytes. Not secreted.
γ1 γ2a γ2b γ3	IgG_1(κ or λ) IgG_{2a}(κ or λ) IgG_{2b}(κ or λ) IgG_3(κ or λ)	These IgG subclasses are the major isotype found in the serum during secondary responses and late primary responses. These are the isotypes generally associated with protection to pathogens, except for certain bacteria, or for pathogens that enter *via* mucosal routes
α	IgA(κ or λ)	Associated with mucosal sites (e.g.; peyer's patches); Transported across basement membranes
ε	IgE(κ or λ)	Binds to receptors on mast cells and mediates immediate hypersensitivity and anaphylaxis (types of allergy).

I. ANTIBODIES SHARE "EFFECTOR" FUNCTIONS

One generally assumes that the *raison d'etre* for the immune system is to detect foreign substances and then rid the organism of these substances. We've seen that the "detection" of these foreign substances is mediated by molecules of exquisite specificity - each molecule having a unique set of antigenic determinants with which it reacts. In contrast, it would be inefficient to be equally specific in the mechanisms of destroying and removing foreign materials, as this would require thousands of different mechanisms where one or a few might suffice.

Indeed, most of the mechanisms that serve this purpose are broadly shared. These shared functions are called **EFFECTOR MECHANISMS.**

MOST EFFECTOR MECHANISMS REQUIRE ANTIGEN-ANTIBODY BINDING TO BE ACTIVATED OR TO PROCEED.

Humoral effector functions are generally activated or mediated via immunoglobulin HEAVY CHAIN CONSTANT REGIONS. Thus, all antibodies with a particular heavy chain constant region will share the ability to induce a particular effector function, regardless of antigen specificity.

Obviously, since several different constant regions exist (the isotypes, remember?); the ability to mediate a particular effector mechanism will differ among the isotypes.

II. SOME EFFECTOR MECHANISMS ARE A RESULT OF ANTIBODY BINDING ANTIGEN. THIS IS TERMED <u>NEUTRALIZATION</u>

A. This process is particularly important in terms of toxins and viral infection.

1. NEUTRALIZATION OF TOXINS

a. Substances that cause severe pathology by binding to receptors and interfering with their normal activities (e.g.; tetanus toxin).

b. These may be *neutralized* if an antibody can bind and prevent the toxin from interacting with its target - this is usually simply a steric hindrance of the toxin's combining site for its target.

2. NEUTRALIZATION OF VIRUSES

a. The infection of cells with viruses can be prevented by antibody binding to the virus. Bound antibody may impede the virus by preventing any of the steps requisite for successful viral infection:

1. adsorption

2. penetration

3. "uncoating"

4. reduction of infectious units

III. SOME EFFECTOR MECHANISMS INVOLVE MAKING THE NORMAL PHAGOCYTIC PROCESSES OF MACROPHAGES AND NEUTROPHILS MORE EFFICIENT: OPSONIZATION

A. Anything that makes it easier for a phagocyte to ingest material is termed an OPSONIN (From the Greek opsonien - to prepare for eating).

1. Antibodies may act as *opsonins* in two ways:

a. Macrophages and neutrophils have receptors for some constant regions.

i. Increase avidity of interaction of the macrophage/neutrophil surface with the antibody-coated substance.

ii. High affinity receptors exist for several IgG subclasses (IgG2a is best in mouse, IgG1 in human although there are reports of receptors for nearly all isotypes - but some are remarkably low affinity and their in vivo significance is unclear).

b. The binding of antibody of any isotype will tend to neutralize the local surface charge - especially for surfaces which are otherwise very highly charged. Since macrophages do not phagocytose highly charged substances as well as neutral ones, this will result in making the material more easily ingested, whether receptors exist for the C-region or not.

IV. THE IgA ISOTYPE PROVIDES FOR IMMUNE ACTIVITY AT PARTICULAR ANATOMIC SITES: THE SECRETORY IMMUNE SYSTEM

A. IgA may be transported across basement membranes

1. Results in antibody in secreted fluids and is thus particularly important at mucosal surfaces:

a. gut - may restrict pathogens to gut, prevent systemic disease. Some pathogens can set up local infection but not produce disease, e.g.; polio.

b. lung mucosal surfaces - of great importance in protection from pulmonary infection, as well as restriction to portal of entry.

c. milk - role in neonatal protection debated, but possible. May also induce proliferation of particular clonotypes through idiotypic interactions, but this is also debated.

2. Mediated by IgA constant region site

3. Valuable in local immunity and restriction of infections to portal of entry.

THE COMPLEMENT SYSTEM

The complement system is a "cascade" of serum proteins which, when activated by antibody-antigen complexes, serves to lyse cells. This is accomplished by enzymatic activity that results in the creation of non-selective ion channels (i.e.; a hole) in the cell surface. Only certain isotypes can mediate complement fixation. Two major pathways of complement activation exist, termed the Classical pathway and the Alternate pathway. The complement system is tightly regulated by a complex system of positive and negative feedback loops, and interdigitates with other enzyme pathways important to inflammatory processes.

I. THE COMPLEMENT SYSTEM CONSISTS OF MANY MOLECULAR COMPONENTS

A. These are activated sequentially

1. many have enzyme activity

2. Each active form activates the next component in the cascade.

B. Components are numbered in order of DISCOVERY

The order of use is: 1 2 4 3 5 6 7 8 9

II. THE COMPLEMENT CASCADE MAY BE DIVIDED INTO TWO MAJOR STEPS:

A. ACTIVATION

1. Involves components 1,2,4, and 3

2. Ab Ag complexes activate C1

a. requires two Abs

b. IgM very good, since it is pentameric.

3. Activated C1 cleaves C2 and C4

4. C2b4b complex forms, binds cell.

a. not necessarily cell the antibody bound , so unwanted damage may occur.

5. C2b4b complex activates C3

B. LYTIC COMPLEX FORMATION

1. Involves components 5,6,7,8, and 9

2. C3a activates C5

3. C5b activates C6, etc, etc.

4. Results in a C56789 complex on the cell surface, which forms a hole in the membrane, causing the cell to lyse.

III. **THE COMPLEMENT SYSTEM IS VERY TIGHTLY REGULATED**

A. Negative regulators:

1. C1 inactivator

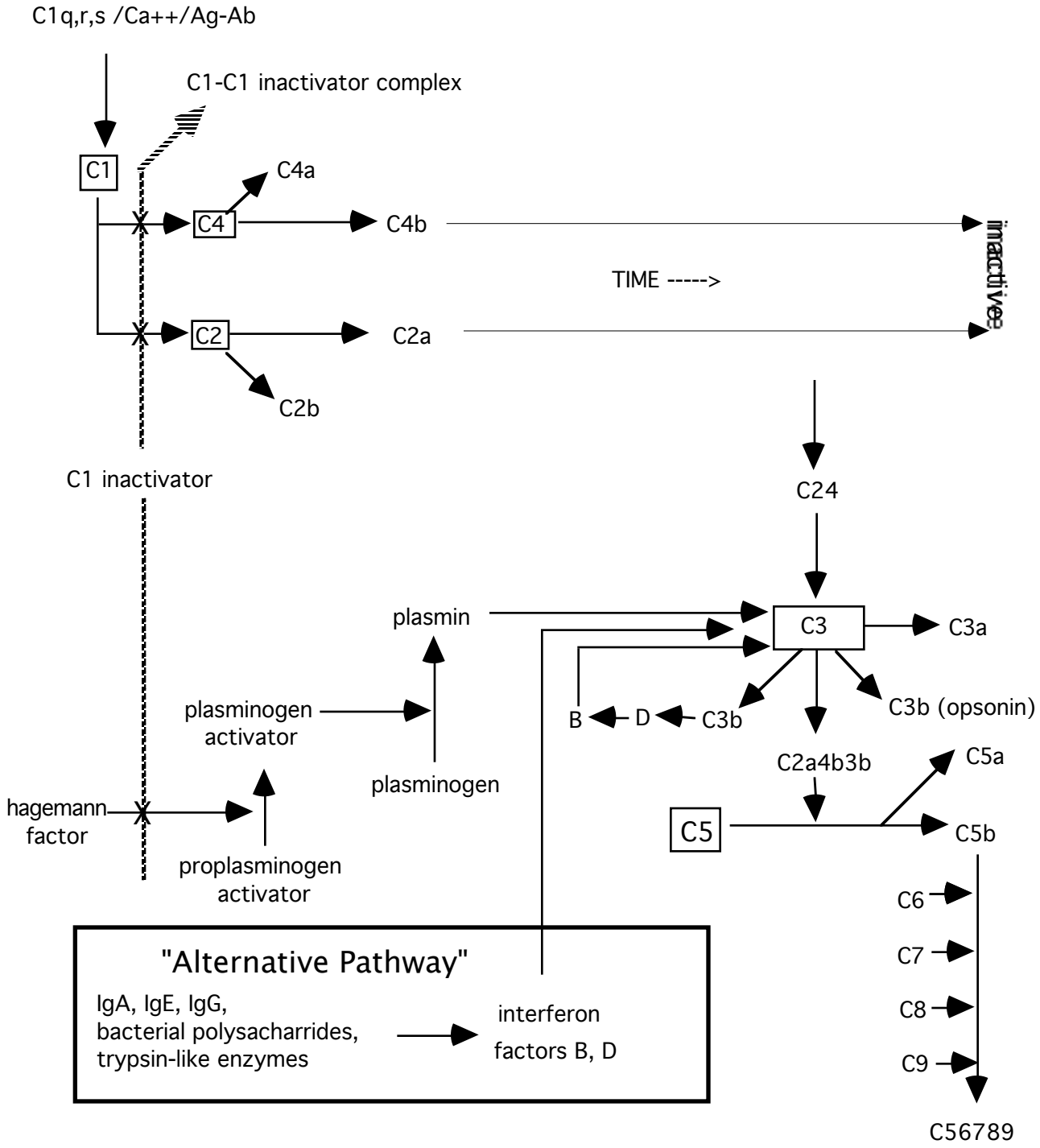
    a. also regulates clotting pathway.

2. lability of activated C2 and C4

B. Positive regulators

1. C3 feedback loop

IV. **USE THE FOLLOWING TABLES AND FIGURES TO GET THE BIG PICTURE, DON'T GO FOR EXCESSIVE MEMORIZATION**.


C1q,r,s /Ca++/Ag-Ab
C1-C1 inactivator complex
C1
C4a
C4
C4b
TIME ----->
inactive
C2
C2a
C2b
C1 inactivator
C24
plasmin
C3
C3a
plasminogen
activator
B
D
C3b
C3b (opsonin)
C2a4b3b
C5a
plasminogen
hagemann
factor
C5
C5b
proplasminogen
activator
C6
C7
C8
C9
C56789
"Alternative Pathway"
IgA, IgE, IgG,
bacterial polysacharrides,
trypsin-like enzymes
interferon
factors B, D

Component	Activated by:	Active form	Acts on:	Regulated by: pos	neg	Other interaction	Other factors released
C1q C1r C1s	Ab-Ag Ca^{++}	C1qrs	C2 C4	C1 inactivator depletion by clotting activation	C1 inactivator	clotting system	-
C2	C1qrs	C2b4b	C3	-	lability	-	C2b
C4	C1qrs	C2b4b	C3	-	lability	-	C4a
C3	C2b4b	C2b4b3b	C5	plasmin, factors D, B of alt. pathway	C4 depletion		C3a xs C3b
C5	C2b4b3b	C5b	C6	-	-	-	-
C6	C5b	C56	C7	-	-	-	-
C7	C56	C567	C8	-	-	-	-
C8	C567	C5678	C9	-		-	-
C9	C5678	C56789	cells -> lysis -> tissue damage, inflammation				

LECTURE 6

THE MHC AND T-CELL BIOLOGY

Part 1

ADVANCE ORGANIZER

Early skin graft experiments provided the basis for two major areas of study in immunology: 1) the "cell mediated immune response, and 2) the elucidation of the genes which control this, as well as many other immunologic processes. T-lymphocytes are the central cell involved in these processes.

Graft rejection occurs when the host's immune system recognizes molecules on the grafted cells as foreign. When grafts are done within a species (e.g.; human ---> human), these antigens are merely ***allelic*** *forms of* ***normal*** *cell surface molecules*, and are called **allo**antigens. The immune response to alloantigens which results in graft rejection is largely mediated by T-cell, and both Th and Tc cells are involved. The activity of these Th and Tc cells can be measured in vitro by two tests: "Cell mediated lympholysis" assay (CML) measures the amount of killing done by Tc cells; and the "mixed lymphocyte culture" (MLR) measures the amount of proliferation by Th cells.

In all mammals studied to date, the strongest rejection responses are controlled by a group of alloantigens that are encoded by a tightly linked cluster of genes. This cluster is termed the Major Histocompatibility Complex, or MHC.

The gene products of the MHC are involved in the recognition of conventional antigens by T-lymphocytes. T-cells require the recognition of antigen in the context of these gene products. This dual recognition is termed 'MHC restriction".

I. ISOGRAFTS SUCCEED, ALLOGRAFTS FAIL

Early skin graft experiments showed that grafts between genetically identical individuals succeeded, whereas grafts between genetically different individuals failed.

A <--ACCEPTS-> A <---REJECTS----> B <--ACCEPTS---> B

These rejection phenomena showed all of the hallmarks of immunity:

They were specific and inducible.

They showed memory: After one rejection, the same type of graft is rejected more rapidly - a secondary response. (memory)

An individual does not reject his/her own tissue (non responsiveness to self)

II. GRAFT REJECTION IS MEDIATED BY CELLS, NOT BY ANTIBODY

Adoptive transfer experiments showed that serum from an immune animal (i.e.; one that had just rejected a graft) could not transfer the immunity to another, naive, animal. In contrast, lymphocytes from an immune animal could transfer the immunity - and allow a naive animal to reject grafts of a similar type as if they had been 'primed' already. The conclusion from such experiments was that this type of immunity was mediated by cells, not by antibody.

III. MAJOR GRAFT REJECTION PHENOMENA IS CONTROLLED BY A SINGLE GROUP OF TIGHTLY LINKED GENES

Through classical genetic analyses, the phenomenon of rejection can be shown to be controlled by genes or groups of genes. In experimental mice, all of the loci that can mediate graft rejection are called "H" loci; for "histocompatibility".

The rapidity of rejection differs widely among these loci, some taking as little as two weeks and some as long as months to cause rejection.

IN ALL SPECIES STUDIED TO DATE, A SINGLE, TIGHTLY CLUSTERED GROUP OF LOCI MEDIATE THE STRONGEST REJECTION PHENOMENA. THIS COMPLEX OF TIGHTLY LINKED LOCI IS CALLED THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC).

The MHC causes a primary rejection in 14 to 21 days, and a secondary ("second set") rejection in 7 to 14 days.

IV. THE ACTIVITIES OF T-CELLS CAN BE MEASURED IN VITRO

A. THE MIXED LYMPHOCYTE REACTION (MLR) MEASURES THE

PROLIFERATION OF Th CELLS

1. Mix two individual's lymphocytes
2. Incubate
3. Add ^{3}H-thymidine
4. Incubate
5. Spin in centrifuge
6. Count cell pellet
7. Higher counts = more proliferation

For example, if we used splenocytes from two MHC-disparate strains called Y and Z, the table of data would look something like this:

MLR (CPM ^{3}H-THYMIDINE INCORPORATED)

	STIMULATORS	
RESPONDERS	**Y**	**Z**
Y	**100**	**75,000**
Z	**75,000**	**100**

[N.B.: You can treat one population with agents that prevent division so that only the activation and proliferation of the other is measured (a "one-way MLR").]

B. THE CELL MEDIATED LYMPHOLYSIS (CML) ASSAY MEASURES THE LYSIS OF "TARGETS" BY Tc CELLS.

1. Label targets with ^{51}Cr.
2. Mix with active Tc cells
3. Incubate
4. Spin in centrifuge.
5. Count supernate
6. Higher counts = more lysis.

CML - (CPM ^{51}CR RELEASED)

	TARGETS	
KILLERS	**Y**	**Z**
Y	**100**	**55,000**
Z	**50,000**	**100**

V. INBRED, RECOMBINANT INBRED, AND CONGENIC MICE ARE USED TO STUDY AND SEPARATE THE LOCI WITHIN THE MHC

A. INBREEDING RESULTS IN HOMOZYGOSITY

1. Recall Valasquez' paintings of Spanish royalty.

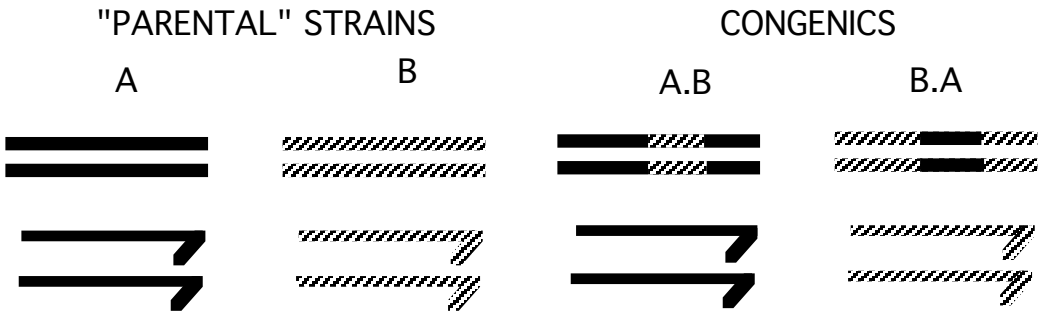
2. Inbreeding for > 20 generations results in homozygosity at nearly all loci

3. Inbred mice can be generated by successive backcross/intercross ([child x parent] crosses and/or [brother x sister] crosses) breeding schemes.

4. Results in useful genetic tools, since all individuals of a given *strain* are genetically identical.

B. CONGENIC AND RECOMBINANT INBREDS

CONGENIC strains are constructs based upon two inbred parental strains. In congenics, a segment of one chromosome from one parental strain has been placed upon the "background" of the other parent. This involves extensive breeding and phenotyping regimes - and in essence amounts to waiting for a double recombination event in F1 crosses, then breeding these recombinants back to the "background" parent while maintaining the "donor" parent's piece of chromosome. The upshot of the whole thing is a "new" inbred strain that is just like the 'background' parent, except for a little bit of chromosome - where it is like the donor parent. Thus, if a polymorphic trait is believed to be controlled by a locus in this area, the congenic provides a critical test of this notion.



2. *RECOMBINANT INBREDS* are inbred strains again produced from two parental inbreds. However, instead of going for a particular piece of chromosome, the parental inbreds are crossed to make an F1, then the F1's are crossed to make an F2. Note that in the F2's the parental chromosomes will independently assort, resulting in a lot of mice that are random mixtures of the parental chromosomes Now, if inbreeding is begun randomly among groups of F2's , the result will be a bunch of inbred lines, each of which is representative of some random mix of parental chromosomes Although this is a lot of work, it provides a set of mice that are akin to having a permanent F2 generation around, which is exactly what one does in gene mapping studies. Thus, if you have a new phenotype and want to map it, just drag out the RI strains and type them - which is a lot easier than doing all that breeding each time you have a locus you want to map.

VI. IN GENERAL, DIFFERENT TYPES OF LOCI IN THE MHC ACT AS ALLOANTIGENS FOR Th vs. Tc

C. THREE MAJOR CLASSES OF LOCI TYPES EXIST IN THE MHC

1. CLASS I GENES

These seem to act as the best alloantigens for Tc cells - i.e.; give a strong CML.

<u>TISSUE DISTRIBUTION</u>: ALL CELLS EXCEPT CENTRAL NERVOUS SYSTEM, MAYBE GERM CELLS. IN SOME SPECIES RED BLOOD CELLS HAVE CLASS I ANTIGENS, AND IN SOME THEY DON'T.

2. CLASS II GENES

These act as strong alloantigens for Th cells - as evidenced by good MLRs.

<u>TISSUE DISTRIBUTION</u>: THESE ARE MORE SELECTIVELY DISTRIBUTED. IN ALL SPECIES, MACROPHAGES AND B-CELLS EXPRESS CLASS II. IN MAN, SOME ACTIVATED T-CELLS ALSO EXPRESS CLASS II , BUT THIS IS NOT TRUE IN MOST OTHER SPECIES STUDIED.

3. Class III genes

These are complement components (C2 and C4) and are peripheral to today's discussion.

D. THE MHC HAS BEEN MAPPED AND MULTIPLE CLASS I AND II LOCI EXIST.

1. MOUSE:

map distance <---------0.5 centimorgans--------->

LOCUS NAME -- K ----- I-A -- I-E ----- S ------- D --

LOCUS CLASS I II II III I

E. THE GENERAL STRUCTURES OF MHC CLASS I AND II GENE PRODUCTS ARE KNOWN:

(DRAW AND LABEL A DIAGRAM OF MHC CLASS I AND CLASS II MOLECULES HERE)

VII. AN UNUSUALLY HIGH DEGREE OF POLYMORPHISM CHARACTERIZES THE CLASS I AND CLASS II GENES OF THE MHC

A. EACH LOCUS IN THE MHC HAS MANY ALLELES

1. The degree of polymorphism in the MHC is one of the greatest known in mammalian genetics. The evolutionary reasons for this are debated, but may be a function of the MHC gene product's roles in normal immune responses.

VIII. HUMORAL RESPONSES TO SOME ANTIGENS ARE MEDIATED BY GENES THAT MAP WITHIN THE MHC - IMMUNE RESPONSE GENES

A. Early experiments with simple antigenic compounds showed that responder vs. non-responder phenotypes mapped to the MHC.

GENERAL EXAMPLE

	PARENTAL STRAINS		F1	CONGENICS	
	MHC^A	MHC^B		A.B	B.A
ANTIGEN X	+	+	+	+	+
ANTIGEN Y	+	-	+	-	+

In this example the MHC^B individual is a non-responder to the antigen "y", but responds normally to antigen "x", showing that the entire immune system of the MHC^B individual isn't faulty. The F1 shows that responsiveness is dominant, and the congenics show that the non-responder/responder phenotypes are controlled by genes within the MHC (since the A.B has the MHC of B but the background of the "A" individual, whereas the B.A has the MHC of the A and the background of B).

B. How could this be explained?

1. Further experiments with congenics and recombinants were able to show that these IMMUNE RESPONSE GENES MAPPED WITHIN THE AREA WHERE THE I-A AND I-E GENES (class II) ARE LOCATED.

Part 2

ADVANCE ORGANIZER

MHC RESTRICTION AND IMPLICATIONS

Normal B- and Tc responses require the activity of macrophages and Th cells. The interaction of Th cells and macrophages include not only the recognition of antigen, but the recognition of MHC class II gene products on the macrophage that are the same as those of the environment in which the T-cells matured. This required "dual recognition" has been termed MHC restriction. It is the basis for Immune response genes (they are the same as Class II genes: I-A and I-E). In addition, this may explain the selective pressure for a high degree of polymorphism among MHC genes.

Similarly, the recognition of conventional antigens (such as viral proteins, as contrasted to alloantigens) on target cell surfaces by Tc cells requires the concomitant recognition of Class I MHC gene products that are the same as those in the environment where the Tc cell matured. This is Class I MHC restriction of Tc cell activity.

Although this explains some earlier observations, and gives us a nice way to explain away polymorphism in the MHC, it also raises the rather sticky question of how the T-cell repertoire of specificities is "instructed" so as to have this restricted recognition.

I. CLASSIC EXPERIMENTS OF ZINKERNAGEL AND DOUGHERTY SHOWED THAT CYTOTOXIC T-CELLS COULD ONLY KILL TARGETS OF THEIR OWN MHC TYPE:

A. USED Tc RESPONSE TO VACCINNIA VIRUS

	TARGETS:			
Primed Tc:	normal cells of:		vaccinia infected	
	A	B	A	B
A STRAIN Tc	-	-	+	-
B STRAIN Tc	-	-	-	+

B. TERMED THE PHENOMENON "MHC RESTRICTION"

In many subsequent experiments, this need for Tc cells to not only "see" the foreign antigen on the target but to see "self" MHC Class I gene products as well, has been confirmed.

CLASS I MHC GENE PRODUCTS ACT BOTH AS ALLOANTIGENS FOR $CD8^+$ T CELLS AND AS RESTRICTION ELEMENTS IN $CD8^+$ T CELL RESPONSES TO "CONVENTIONAL" ANTIGENS

II. Th cells can only be activated by antigen presented by macrophages or B-cells that are of the same MHC type.

CLASS I I MHC GENE PRODUCTS ACT BOTH AS ALLOANTIGENS FOR $CD4^+$ T CELLS AND AS RESTRICTION ELEMENTS IN $CD4^+$ T CELL RESPONSES TO "CONVENTIONAL" ANTIGENS

III. THE NOTION OF "DUAL RECOGNITION":

This "dual recognition raises several interesting points. First, the T-cell receptor for antigen must be somewhat different than what we are used to in B-cells, since no such restriction seems to be placed upon antigen antibody interactions.

Second, it raises the rather intriguing issue of just where a T-cell "learns" what is self MHC. This is an important issue to grasp - the MHC gene products are NOT the T-cell receptor, they are *recognized by it*.. Thus, either the receptor genes for a given MHC haplotype must be so tightly linked to the MHC genes that they never get separated (otherwise you couldn't make your T-cells work); or the T-cell repertoire must have the potential to work with any MHC haplotype, in which case the actual repertoire of T-cell receptors must be selected from this potential array. This is OK, but one then also must make sure that no T-cells that are alloreactive to one's own MHC 'sneak through' in the process - since you would lyse yourself.

Finally, the restriction of Th cells to class II raises some interesting ideas about tolerance, that we'll talk about later.

IV. HEREDITY VS. ENVIRONMENT IN ESTABLISHING THE RESTRICTION OF T-CELLS: ENVIRONMENT WINS.

A. *Chimeric* mice and thymus transplants indicate that restriction is "learned" - probably in the thymus.

1. *Chimeras* are animals whose own stem cell and lymphoid compartment has been destroyed and replaced with another's. This is usually done by irradiation and then injection of bone marrow cells.

2. In these animals, the restriction phenotype is that of the host, not the donor.

3. If the host thymus is replaced as well, however, the restriction phenotype will be that of the thymic environment.

V. THE NATURE OF THE RECEPTOR ON T-CELLS

A. T-cell receptors may be one of two types of Heterodimers

α–β

δ–γ

B. The T-cell receptor heterodimer is part of a multi component trans-membrane signaling complex. This complex *in toto* is termed the CD3 complex (or just CD3). The CD3 complex consists of the TcR heterodimer, plus multiple integral trans-membrane components important in delivering signals when the receptor is occupied. The CD3 components are necessary to get the heterodimer to the surface of the cell, so all functional T-cells are CD3-positive.

C. The TcR appears to have co-evolved structurally to provide a complementary fit for peptides within the groove of MHC molecules, and thymic selection appears responsible for selecting an appropriate repertoire of receptors in any given individual (more on this later).

D. Diversity among TcR heterodimers is produced throught he same paradigm as are Ig heavy and light chain V regions, although the organization of the genes varies somewhat. Consult your text for these.

E. TcRs are closely associated with accessory molecules that “stabilize” interactions with MHC molecules.

CD4 appears to interact with non-polymorphic regions of MHC class II molecules, making CD4 positive cells most likely to interact fruitfully with when antigen is presented int he context of MHC class II.

CD8 appears to interact with non-polymorphic regions of MHC class I molecules, making CD8 positive cells most likely to interact fruitfully with when antigen is presented int he context of MHC class I.

VI. Antigen processing and presentation occurs through one of two intracellular trafficking pathways.

- Endogenous processing pathway - Class I

Proteins synthesized within the cell are degraded and the resulting peptides associated with Class I molecules, presumably during transit through the golgi

- Exogenous pathway - Class II

Proteins ingested from the extracellular environment are digested and processed through the lysosomal system, which results in selective association of resulting peptides with MHCclass II molecules. This may result through co-internalization of surface Class Ii molecules during the phagocytic process.

VII. IMPLICATIONS FOR SELECTION FOR HIGH DEGREE OF POLYMORPHISM - IS THIS TO AVOID CATASTROPHIC DISEASES WITHIN THE POPULATION AS A WHOLE?

1. Since the ability to respond to antigens hinges upon the MHC molecules, it is conceivable that an organism might manage to evolve to a form which could not be perceived by a particular individuals with a particular MHC. Clearly, the more alleles that the population had at each locus, the less likely this would become. Some people think that his might be the explanation for so high a degree of polymorphism in the MHC.

LECTURE 8

LYMPHOCYTE DEVELOPMENT AND TOLERANCE

ADVANCE ORGANIZER

The immune system's development is a complex and intriguing problem. Think about it: The system has to generate several interdigitating systems of receptors; must avoid producing receptors that will react with self while at the same time insuring that it can see enough foreign structures to be protected from disease; must constantly be interacting with the environment and tailoring the existing system to new environmental assaults, and must control the proliferation and activities of the cells involved in immune responses. Development of both T- and B-cells can be described by using surface markers or functional properties indicative of various maturational stages. Lymphocytes are produced and turnover constantly, although the rates are debated. Labeling experiments and experiments which use genetically engineered mice have been used to try and get at this issue. The driving forces underlying lymphocyte generation and development are unclear, although several models are popular.

B-cell specificities seem to emerge in a patterned fashion, as shown by experiments which look at the emergence of particular specificities or Vh segment use during early development.

The problem of self/non-self discrimination remains unresolved, although it has been probed extensively for both T- and B-cells.

I. THE PRODUCTION OF LYMPHOCYTES IS A CONSTANT PROCESS

A. Estimates of lymphocyte production and turnover

1. B-cells
   a. fetal liver
   b. marrow
   c. half-life estimates of different pools indicate rapid turnover among newly formed cells (both marrow and periphery), but a long-lived peripheral pool.

2. T-cells

   a. Thymic migration
   b. Cell death in the thymus
   c. Thymic egress

II. THE ACQUISITION OF RECEPTORS BY B-CELLS

A. Experiments to describe the emergence of B-cell clones

1. The appearance of B-cell specificities may not be random

In these experiments, the appearance of responses to particular antigens was monitored. There was an order to what responses appeared. In subsequent experiments, the "clonotypes", measured by various criteria, within a given response were monitored for their time of appearance.

CONCLUSION - THERE IS A PATTERN TO THE WAY IN WHICH B-CELL SPECIFICITIES APPEAR DURING EARLY DEVELOPMENT

4. Do these patterns reflect programmed gene expression?
   a. Yancopoulous and Baltimore
      i. First rearrangement may always be most proximal gene family to IgCh.
      ii. doesn't explain later life.

III. ACQUISITION OF RECEPTORS BY T-CELLS

1. Receptor gene expression in the thymus

2. Order of expression of Tcr, receptor complex, and accessory molecules

IV. MODELS OF TOLERANCE

A. CLONAL DELETION MODELS OF TOLERANCE

1. Clonal deletion models for B-cells

a. Nossal and Pike experiments

In these experiments, the responsiveness of mature and immature B-cells were compared to each other after they had been subjected to interaction with ligand under non-stimulatory conditions. RESULT- THE IMMATURE CELLS BECAME REFRACTORY (ANERGIC) TO STIMULATION AFTER SUCH TREATMENT. CONCLUSION: IF IMMATURE B-CELLS SEE THEIR DETERMINANT, THEY BECOME NON-RESPONSIVE.

Tolerance versus activation may reflect "uncoupling " of intracellular signals leading to programmed cell death (apoptosis)

c. Longevity of tolerance

Subsequent experiments have monitored the return of responsiveness after tolerogenic treatment. In these, the B-cell responses were observed to return after 40 to 60 days. This may reflect replenishment with newly formed cells, or may indicate that the tolerance is transitory, and doesn't actually get rid of the cells.

2. **Transgenic mice** have been used to ascertain general principles of tolerance. In particular, models developed by D. Nemazee *et al* and by C. Goodnow *et al* have yielded insight.

Clonal deletion *versus* anergy

Ligand density may dictate deletion *versus* anergy

Can B cells escape tolerance through "editing" of their receptors?

CLONAL DELETION AMONG T-CELLS

a. does thymus negatively or positively select - or both?

Chimera and transgenic experiments of P. Marrak *et al*; H. VonBoehmenr *et al*

TOLERANCE 2
MECHANISMS OF PERIPHERAL TOLERANCE

ADVANCE ORGANIZER

The ability to tolerize/remove/delete recently generated lymphocytes bearing specificities to self antigens before such cells exit primary lymphoid organs is clearly an important mechanism of self-tolerance. However, tolerance models such as this do not account for potential self-reactivity to self-antigens not present in the primary lymphoid organs. Thus, many researchers have proposed that additional peripheral tolerance mechanisms must exist. There are two general models for peripheral tolerance. Each is founded in the two signal model for lymphocyte activation/tolerance. This model predicts that a signal through the lymphocyte receptor for antigen (signal 1) without a concomitant costimulatory signal (signal 2) will result in tolerance rather that activation. Thus, the regulation of signal 2 becomes particularly important in determining whether an antigen is viewed as foreign versus self.

I. FAS-FAS LIGAND AND PERIPHERAL TOLERANCE

This model predicts that signal one results in upregulation of a death receptor termed fas. When fas interacts with its ligand [fas ligand or fasL], a series of intracellular enzymes termed caspases are activated which results in the ultimate degradation of the cellular genome and cell death. This type of cell death is termed apoptosis. Presumably, a costimlation signal (signal 2) will somehow interfere with fas derived signals and thus prevent signal 1 induced apoptosis.

II. CTLA-4, A NEGATIVE REGULATOR OF COSTIMULATION

CTLA-4 and CD28 are both receptors for B7.1 and B7.2. However, unlike CD28 which sends a positive regulatory signal to the stimulated T cell, CTLA-4 sends a negative signal that dampens the T cell response. The critical importance of CTLA-4 is best illustrated by CTLA-4 knock out mice. These mice display uncontrolled T cell proliferation and die of autoimmunity by 6 weeks of age.

III. SUPPRESSOR CELLS IN TOLERANCE

A. Some think that suppressor T cells are the major mechanism of non-responsivenss to self.

III. THE PROBLEM OF "PERIPHERAL" TOLERANCE (is it a problem?)

A. What about autoantigens expressed exclusively in the periphery?

B. Is class II restriction and limited tissue distribution a means of attaining peripheral tolerance?

1. Since many self antigens are tissue-specific (i.e.; they don't run around in solution, but are displayed on particular cell surfaces, it is possible that they simply never get presented in the context of Class II. Recall that Class II gene products have a rather narrow tissue distribution. If this is true, then you could have receptors that see these determinants, but never respond to them anyway, since you could never get help for such responses.

2. Is the requirement for "professional APC's" by naive T helper cells a means of reducing the risk of autoreactivity to peripheral autoantigens?

C. Is peripheral tolerance more a matter of "bad versus good" instead of "self versus non self?"

1. The "danger hypothesis."
See reviews by Polly Matzinger

LECTURE 7

FACTORS CONTROLLING THE T-CELL RESPONSES

ADVANCE ORGANIZER:

The immune system must respond to and eradicate pathogens anywhere in the body in a highly regulated and controlled manner. To achieve this, pathogens are picked up and processed by Class II+ antigen presenting cells such as dendritic cells or activated B cells that subsequently migrate to secondary lymphoid organs. Dendritic cells and activated B cells are highly effective at stimulating resting Ag-specific T cells. Initial activation of resting T and B cells takes place in the specialized microenvironments of the spleen and lymph nodes. Once activated, CD4+ T-helper cells assist in the differentiation of B cells into plasma cells or germinal center B cells or the activation of effector CD8+ cytotoxic T cells, or regulate other inflammatory responses through . Activated effector CD4+ and CD8+ T cells subsequently reenter the recirculating lymphocyte pool and may also leave the blood/lymphatic system and migrate into pathogenic sites.

I. Architecture of secondary lymphoid organs.

Secondary lymphoid organs such as spleen and lymph node are unique microenvironments which provide factors necessary for keeping B and T cells alive. These microenvironments are also required for the initial priming/activation of resting T and B cells.

The spleen is comprised of two major compartments, the red pulp containing erythrocytes and the white pulp containing leukocytes such as lymphocytes, macrophages, and dendritic cells. The white pulp can be divided into two areas: surrounding a central arteriole is a T cell rich area termed the periarteriole lymphoid sheath or PALS. All leukocytes are thought to enter the white pulp through the central arteriole. Adjacent to the PALS are 2 or 3 follicles which contains mainly resting B cells. The border between the follicles of the white pulp and the red pulp is called the marginal sinus.

In a lymph node resting T cells are found in the cortex which surrounds B cell follicles.

II. Antigen-reactive T and B cells in the splenic microenvironment.

Much has been learned about T-B interactions in vivo by tracking Ag-specific B cells in the spleen following immunization of inbred mice. These experiments involve staining frozen sections of spleen with antibodies to B and T cells and antigen, all coupled to enzymes or flourochromes that allow the subsequent visualization of these cells.

One day after immunization antigen-reactive T and B cells can be found in close proximity to one another in the PALS near the PALS-follicle border. AT day 2-3 a cluster of antigen-reactive B cells referred to collectively as primary foci can be seen. These cells

are thought to give rise to antibody secreting plasma cells. By day 8 a unique structure termed a germinal center begins to form in the follicle. Germinal centers are comprised largely of activated B cells but also contain activated T cells. Germinal center B cells are thought to leave the germinal center as memory B cells.

SESSION II

LYMPHOKINES/CYTOKINES/COSTIMULATION

ADVANCED ORGANIZER:

Cells can communicate *via* soluble molecules that they secrete. Such substances are generically termed cytokines. Cytokines that are made by lymphocytes are termed lymphokines. Further, lymphokines that give signals to other lymphocytes are called interleukins. (Got that straight?) Lymphokines and Cytokines are important in the communication of activation and regulatory signals in immune responses. Antigen-presenting cells, T-cells, and B-cells make a variety of these substances, each with characteristic activities. It is clear that all of the interleukins and their activities have yet to be described satisfactorily. We will concentrate on only those whose activities are well proven and central to basic immune response and regulation.

In addition to antigen presentation and recognition, additional interactions may occur between APC's, TH cells, and effector cells. In particular, so-called engagement of *co-stimulatory* molecules may be required (in addition to antigen binding and interleukins) for a helper cell to be activated. Currently, two pairs of costimulatory molecules have been clearly established in the activation of T-cells. Naive T-cells appear to have an absolute requisite for this costimulation, whereas primed or activated T-cells seem to have less stringent requirements.

I. SOLUBLE MOLECULES CAN ACT AS MESSENGERS BETWEEN CELLS

A. Do not have antigen specificity themselves, but may be only produced or exert activity following a specific stimulus

B. Lymphokines are important in all immune responses.

C. Provide signals between lymphocytes.

II. ANTIGEN PRESENTING CELLS MAKE INTERLEUKIN 1

A. Interleukin 1 (Il-1) is required for Th activation

B. Implies a receptor for Il-1 on Th cells.

C. Activated macrophages make more Il-1.

1. Activated = phagocytically active
2. Other factors (see below) can also cause macrophages to become more active.

``

III. HELPER T CELLS MAKE IL-2 WHEN ACTIVATED.

A. IL-2 is necessary for Tc activation

B. IL-2 also can further activate Th cells

1. Th cells up-regulate and alter their IL-2 receptor when they make and bind IL-2. This self-stimulation is termed "autocrine" stimulation.

IV. Th CELLS ALSO MAKE FACTORS THAT AFFECT MACROPHAGE ACTIVITY

A. gamma interferon

1. Makes macrophages "angry"
   a. increased phagocytosis
   b. increased class II
   c. increases enzyme activity

B. Migration Inhibition Factor

1. Attracts and keeps macrophages in the area.

V. Th CELLS MAKE FACTORS THAT AFFECT B-CELLS

A. B-cell growth factors produced by T-helpers include IL 4, 5, 6, and 7.

B. These vary in effect, and include division, differentiation, and isotype switching (see diagrams).

C. The differentiation state of B-cells may influence whether they are responsive to a particular interleukin.

VI. HELPER T CELLS MAY BE FURTHER SUB-DIVIDED ACCORDING TO THE LYMPHOKINES THEY SECRETE.

Th 1: produce gamma-interferon, which promotes cytotoxic T cell activity

Th 2: produce IL-4, which promoted germinal center responses and Ig class switching

Th17: Produce IL-17, which promotes inflammatory responses

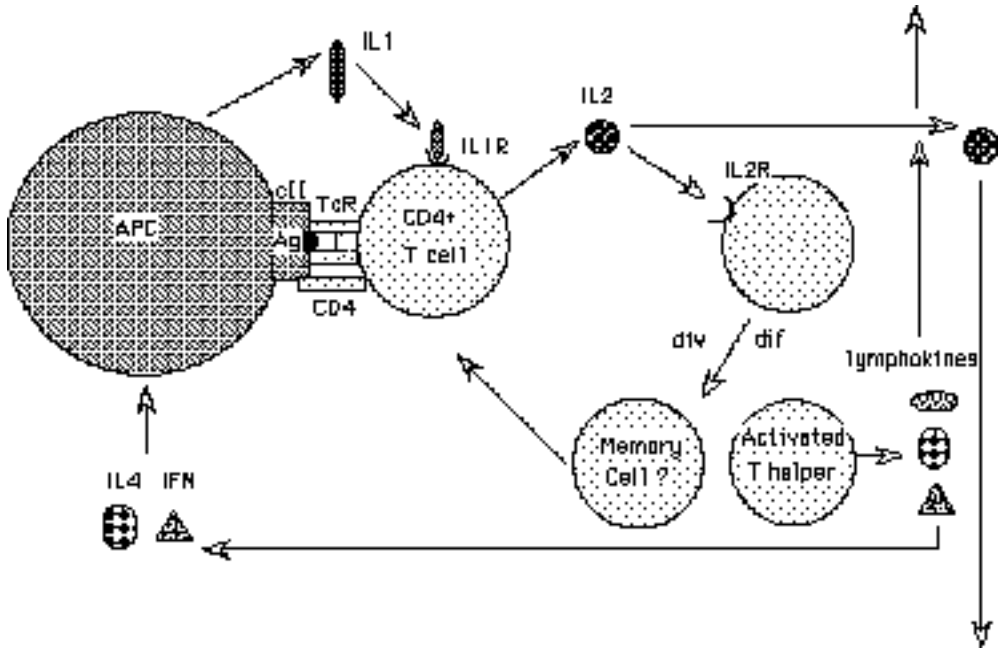
Whether these are separate lineages of T-cells whose spectrum of lymphokines is fixed, or instead simply reflects different states of differentiation or activation of a single lineage of T-cells is debated. Further, these definitions are largely operational, and some latitude in these interleukin profiles is likely to be the case.

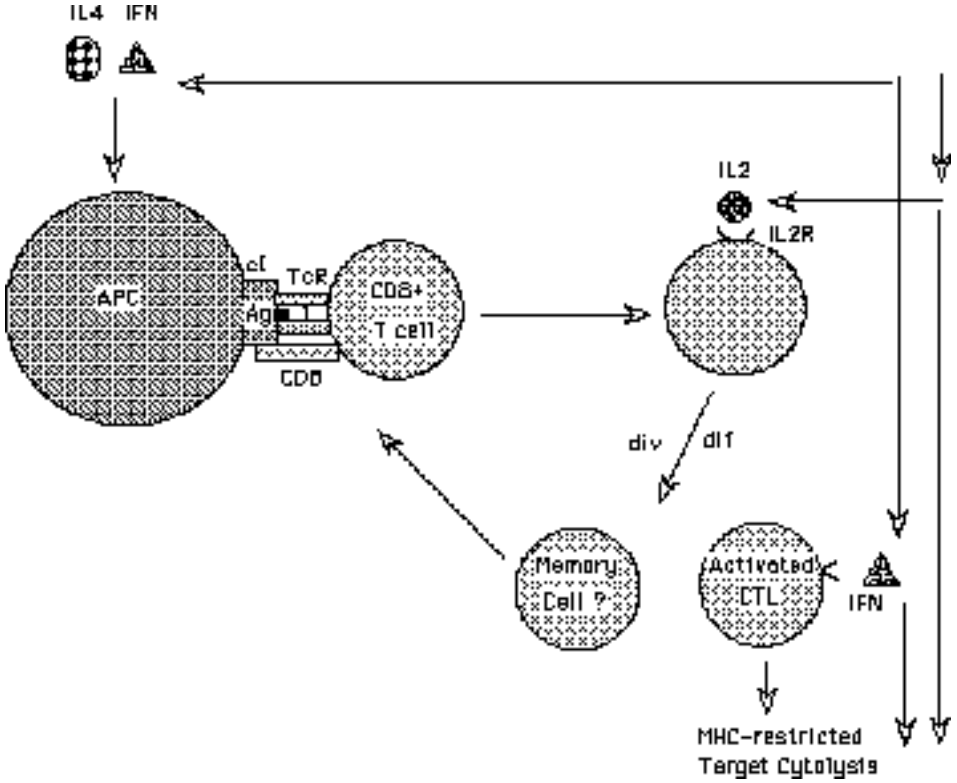
VI. DELAYED HYPERSENSITIVITY (DTH) IS AN EFFECTOR FUNCTION RESULTING FROM THE CHRONIC PRODUCTION OF CYTOKINES

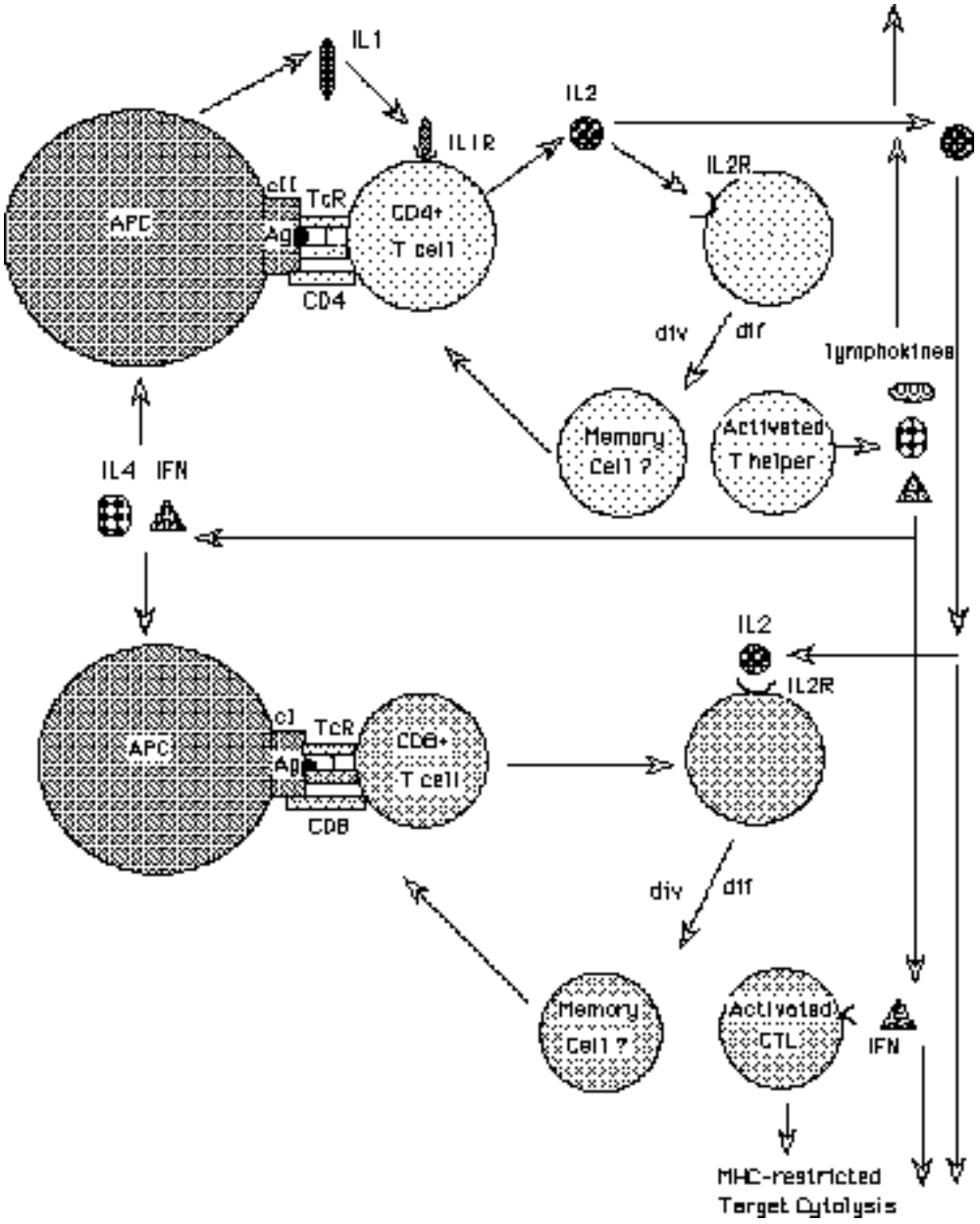
A. Chronic stimulus of T-cells leads to chronic production of some lymphokines

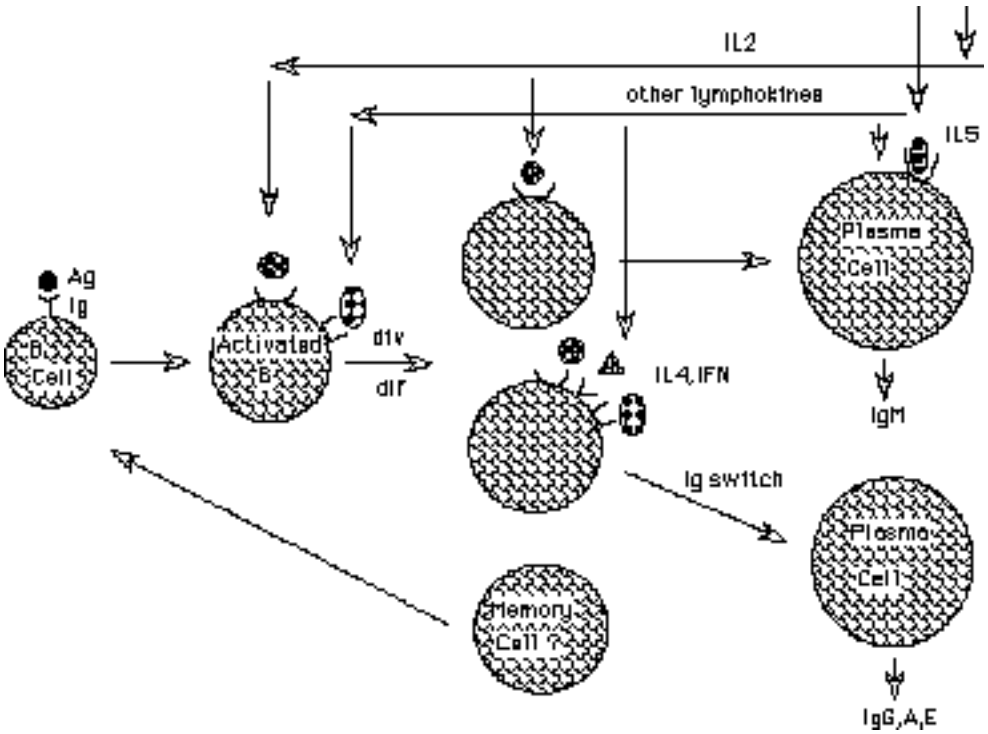
B. Results in chronic macrophage influx and activity, as well as chronic T-cell activation..

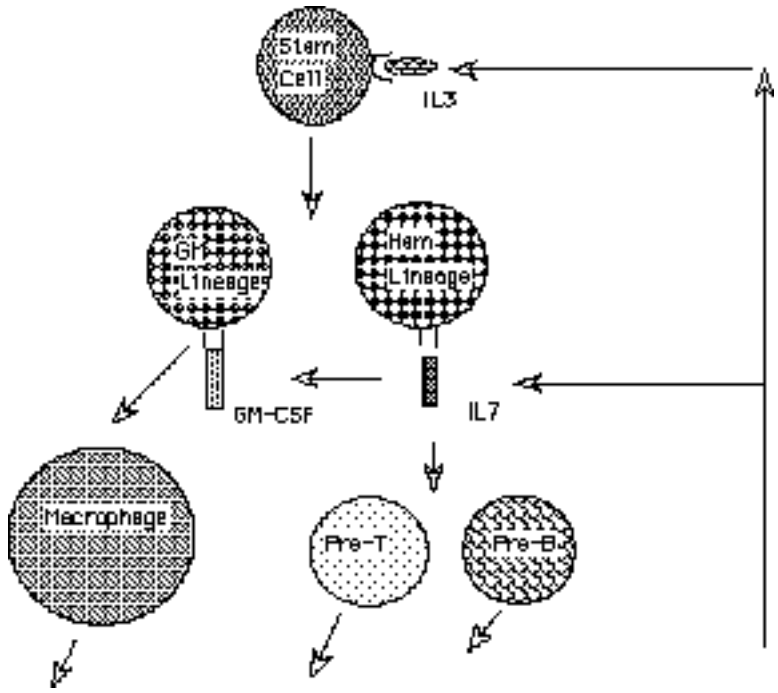
C. Results in masses of such sells surrounding a chronic site - TB and fungal infections are good examples.


IL1
IL1R
IL2
IL2R
APC
MHC
Ag
TcR
CD4
CD4+
T cell
div
dif
lymphokines
Memory
Cell ?
Activated
T helper
IL4
IFN


IL4
IFN
IL2
IL2R
APC
cl
TcR
Ag
CD8+
T cell
CD8
div
dif
Memory
Cell ?
Activated
CTL
IFN
MHC-restricted
Target Cytolysis


IL1
IL1R
IL2
IL2R
APC
cll
TcR
Ag
CD4
CD4+
T cell
div
dif
lymphokines
Memory
Cell ?
Activated
T helper
IL4
IFN
IL2
IL2R
APC
cl
TcR
Ag
CD8
CD8+
T cell
div
dif
Memory
Cell ?
Activated
CTL
IFN
MHC-restricted
Target Cytolysis


IL2
other lymphokines
IL5
Ag
Ig
B Cells
Activated B
div
dif
IL4,IFN
Plasma Cell
IgM
Ig switch
Memory Cell ?
Plasma Cell
IgG,A,E


Stem Cell
IL3
GM Lineage
Hem Lineage
GM-CSF
IL7
Macrophage
Pre-T
Pre-B

Costimulation molecules - Antigen is not enough

Much debate has surrounded the question of what types of MHC Class II+ cells are capable of presenting antigen to resting previously unstimulated T cells (sometimes referred to as naive or virgin T cells). Experiments using B cell tumor cell lines showed that when pulsed with antigen these B cells could readily stimulate T cells, leading to the notion that B cells were the most efficient Ag-presenting cell because B cells specific for a particular Ag would bind and internalize this Ag via its BCR and thus display increased numbers of Class II-specific peptide complecxes. In this model non-B cells (lacking a BCR) would pick up Ag less efficiently relative to a B cell and therefore would serve a secondary role in Ag presentation in vivo. This idea was latter challenged by the following experiment:

Highly purified T cells from a TCR transgenic mouse carrying the α and β chain genes specific for pigeon cytochrome c PCC)/H-2^b (nearly every T cell is specific for PCC) were incubated with the following sources of Ag presenting cells (APC) and the amount of Ag-induced proliferation determined.

Table 1

APC (all H-2^b)	Antigen	Proliferation (CPM)
Whole splenocytes	None	100
Whole splenocytes	PCC	10,000
Very pure resting B cells	None	100
Very pure resting B cells	PCC	1000
Very pure B cells stimulated with LPS 48 hours earlier	None	100
Very pure B cells stimulated with LPS 48 hours earlier	PCC	100,000
Dendritic cells	None	100
Dendritic cells	PCC	100,000

This led immunologists to conclude that B cells are rather inefficient APCs unless first activated and that dendritic cells are naturally highly efficient APC. The term professional APC was coined to describe cells such as dendritic cells which can readily prime resting/virgin T cells.

The molecular basis for these data was subsequently determined following the definition of CD28, a cell surface molecule constitutively expressed by all T cells. The crosslinking experiment performed with highly purified T cells and antibodies to TCR and CD28 shown in table 2 provided evidence that CD28 played a key role as a costimulation molecule:

Table 2

Antibody	Proliferation (CPM)
anti-TCR	100
anti-CD28	100
anti-TCR plus anti-CD28	100,000

T cells also express another costimulation molecule named CTLA4. Unlike the constituitive expression of CD28, CTLA4 expression is induced by Ag-MHC complexes. CTLA4 knockout mice contain large numbers of activated autoreactive T cells, lending to the idea that CTLA4 is a negative regulator of T cell activation.

The natural ligands for CD28 and CTLA4 are two cell surface molecules termed B7-1 and B7-2. Interestingly, B7-1 and B7-2 are found on activated but not resting B cells but are always found on dendritic cells, providing a correlation between B7-1/2 expression and the ability of a given cell to prime resting/virgin T cells. The transfection experiment in Table 3 in which genes for B7-1 or B7-2 are introduced into MHC Class II+ B7-1/2-negative cell lines provide strong evidence that B7-1 and B7-2 play a key role in T cell priming. (The T cells used in Table 3 are the same as in Table 1.)

Table 3

APC (all H-2b)	Antigen	Proliferation (CPM)
Whole splenocytes	None	100
Whole splenocytes	PCC	10,000
B71/2-negative B cell line	None	100
B71/2-negative B cell line	PCC	100
B71/2-negative B cell line transfected with the gene for B7-1	None	100
B71/2-negative B cell line transfected with the gene for B7-1	PCC	100,000
B71/2-negative B cell line transfected with the gene for B7-2	None	100
B71/2-negative B cell line transfected with the gene for B7-2	PCC	100,000

SESSION III

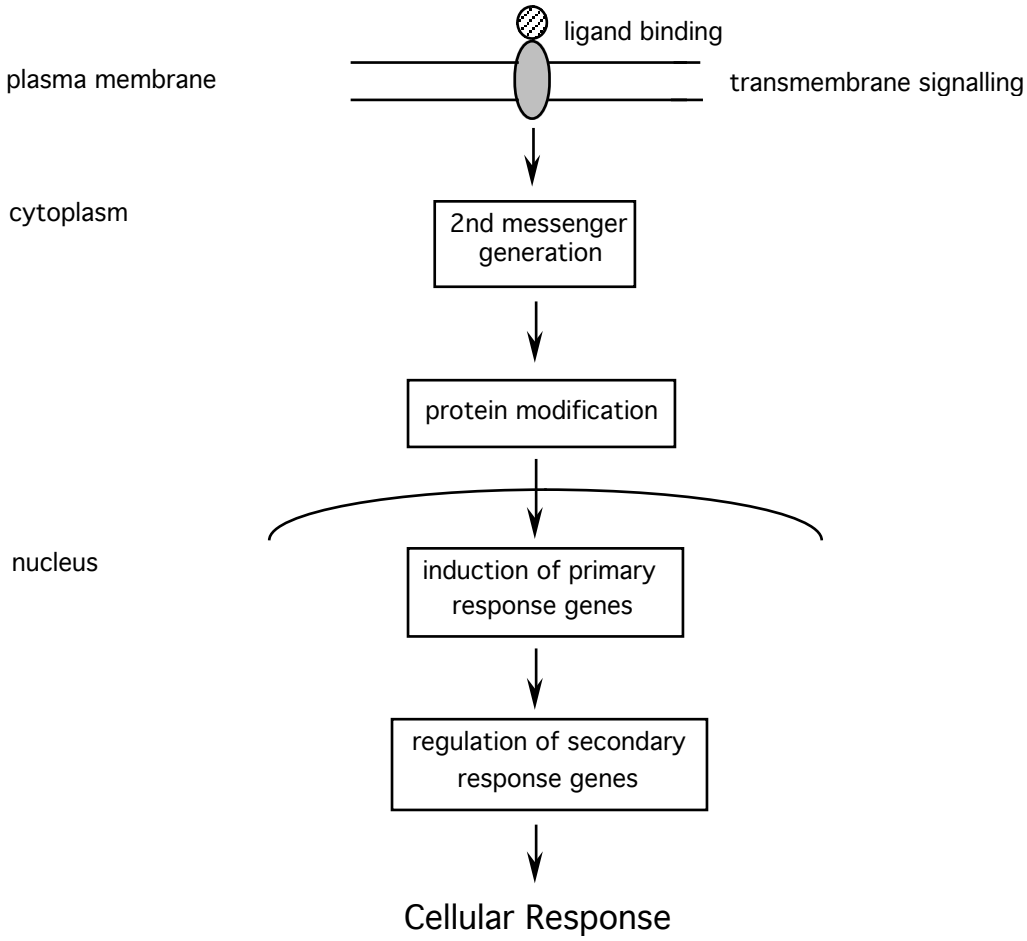
PHYSIOLOGY OF LYMPHOCYTE ACTIVATION

ADVANCE ORGANIZER:

Cells, including those of the immune system, interact with and change in response to their environment. These interactions are mediated by proteins on their surface called receptors. When a receptor binds its ligand, a biochemical signal is generated and transduced across the plasma membrane into the cytoplasm. You are familiar with one type of receptor system, that of the antigen receptor on B and T lymphocytes. In this case, the ligand is antigen (together with class I or II for T cells). Antigen stimulation initiates a series of biochemical processes which ultimately lead to B and T lymphocyte responses. In this lecture we will discuss how these signals are generated and processed by the cell into a appropriate response. Primarily, we will use the B lymphocyte as an example even though most of the processes are common to T cells as well.

Molecular aspects of signal transduction and translation.

The following diagram illustrates the principle components operative in the transduction of receptor-generated signals into specific cellular responses.



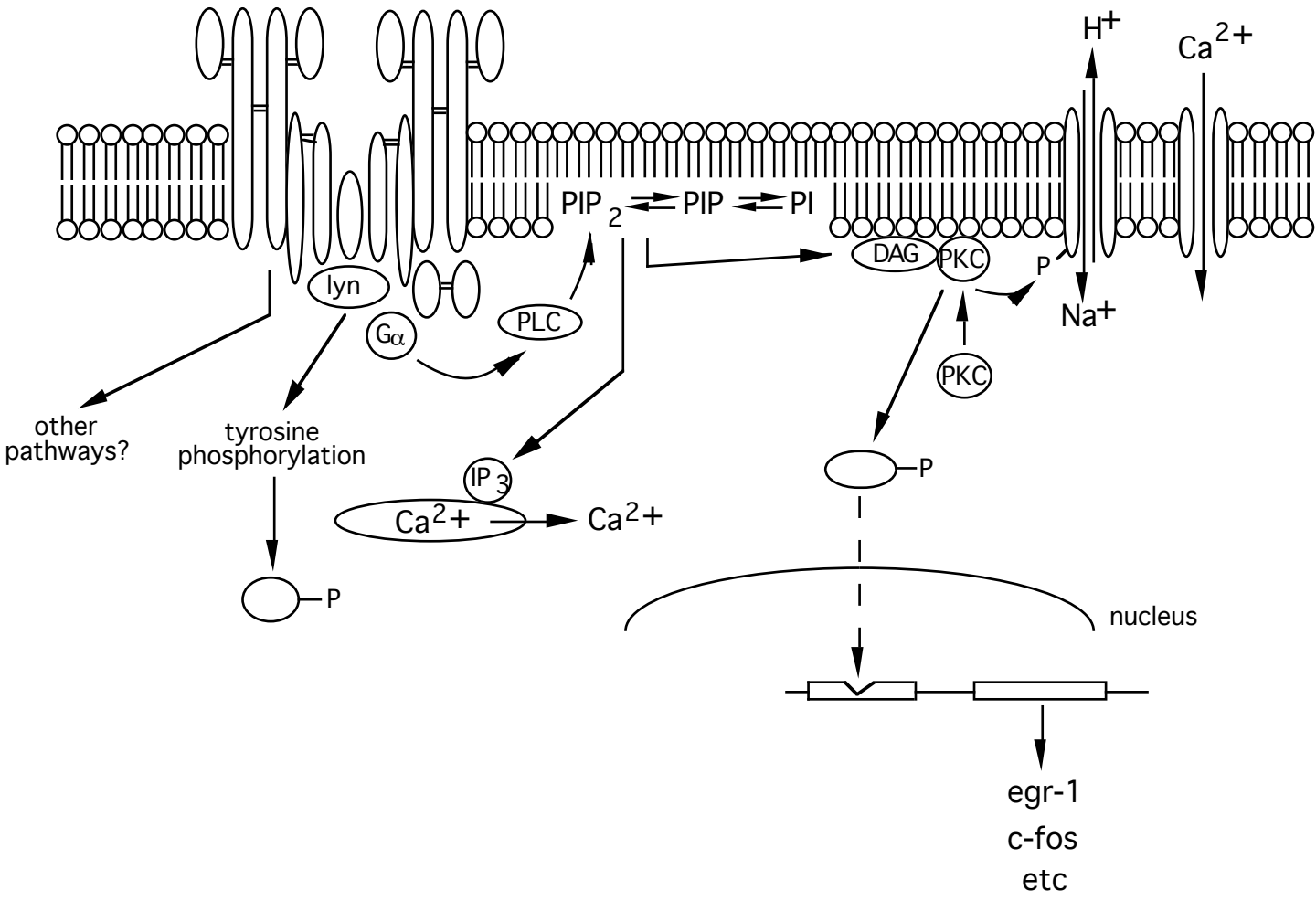
Ligand binding. Signals are generated as a consequence of ligand (in this case antigen) binding to the receptor. B cells use a membrane form of immunoglobulin as their antigen receptor. The T cell antigen receptor is unique. For the B and T cell antigen receptors, conformational changes consequent to this interaction do not appear to be as important as antigen induced crosslinking of multiple receptors for generating activation signals. Thus, antigen must be presented in a multivalent form.

Transmembrane signaling. The receptor-generated signal must be transmitted into the interior of the cell in order for the activation process to be propagated. In T and B cells, this process involves receptor-associated proteins which span the plasma membrane and some of which are localized to the inner surface of the plasma membrane. In the T cell, these proteins comprised the CD3 complex. CD3-like molecules are probably also present in the B cell, but are much less well characterized. The B cell antigen receptor is also associated with a GTP binding protein which facilitates the transduction of its antigen receptor-generated signals.

Second messenger generation: Second messengers and second messenger pathways couple the receptor to specific changes in the cell. They are the biochemical events which occur within the cytosol as a consequence of receptor-ligand interaction. Examples of second messenger systems include: inositol phospholipid hydrolysis, tyrosine kinase activation, cyclic nucleotide changes, etc.

Changes in early and intermediate gene expression: Second messenger signals must be translated by the lymphocyte into specific cellular responses. Examples of these responses are lymphocyte proliferation, antibody secretion, lymphokine production, and tolerance. The process by which the cell knows what to do when a particular second messenger pathway is initiated involves a process of signal translation. Second messenger pathways lead to the modification of pre-existing proteins in the cytoplasm which then activates these molecules. These modified proteins then enter the nucleus where they turn on a class of genes called primary response genes or immediate/early type genes. These genes encode proteins which are transcriptional regulatory molecules which then coordinately up and down regulate whole sets of secondary response genes. The particular set of secondary genes expressed, then dictate the exact nature of the cellular response. Thus, the primary response genes act as intracellular "third messengers" to differentially translate receptor signals into specific cellular responses by determining the profile of secondary (intermediate) gene expression.

This process for the B cell antigen receptor is diagrammed below.


H+
Ca2+
PIP2
PIP
PI
DAG
PKC
P
lyn
Gα
PLC
Na+
PKC
other
pathways?
tyrosine
phosphorylation
IP3
Ca2+
Ca2+
P
P
nucleus
egr-1
c-fos
etc

Antigen-induced B cell responses: *A relevant example of signal transduction at work.*

Positive activation responses