MOLECULAR GENETICS OF ANTIBODIES: How to get a genetic bargain.

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 cut and then put next to each other 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 PROVIDE CLUES TO THE GENETICS OF DIVERSITY

A. kappa light chains

B. lambda light chains

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

This concept was first introduced by John Kappler in the tapes

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 2 kb as well. The V-region genes, however, are found clustered a considerable distance (at least 17 kb) 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 chain variable regions are produced by joining three 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 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, epsilon, and alpha.
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 to the 7th [that is, 10 million or 10,000,000] 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 10 to the 3rd [1000] kbase 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.

[The current work of undergraduates and graduates in the Durdik lab involves following the DNA cuts involved in developing lymphocytes from young and aging mice.]

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 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.

VI. B cell genes and Ig isotypes

On the other end, the C regions can also be cut up to change to a new isotype. This is termed isotype switching—or class switching. This happens later in the life of a B cell than VDJ recombination.

THE MHC AND T-CELL BIOLOGY

introduced by Pippa Marrack in the tapes

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 alloantigens. 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.

I. ISOGRAFTS SUCCEED, ALLOGRAFTS FAIL

In early skin graft experiments, it was observed that grafts between genetically identical individuals succeeded, whereas grafts between genetically different individuals failed.

\[ A \leftarrow \text{ACCEPTS} \rightarrow A \]

\[ A \leftarrow \text{REJECTS} \rightarrow B \]
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.

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. 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 3H-thymidine or MTT (an indicator of cellular metabolism)
4. Incubate
5. Spin in centrifuge
6. Count cell pellet

7. Higher counts [3H-Thy] or more color [MTT] = more proliferation

[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 51Chromium or Europium or LDH [an enzyme].
2. Mix with active Tc cells
3. Incubate
4. Spin in centrifuge.
5. Test supernate for counts [51Cr or Eu] or LDH enzyme activity
6. More = more lysis.

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

A. INBREEDING RESULTS IN HOMOZYGOSITY

1. Inbreeding for > 20 generations results in homozygosity at nearly all loci (<.05% chance of heterozygosity).

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

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

4. Used extensively in immunogenetic studies.

B. CONGENIC AND RECOMBINANT INBREDS

1. Congenic strains are constructs based upon two inbred parental strains. In congensics, 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 recombinants 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 either FOR Th or 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.

TISSUE DISTRIBUTION: ALL CELLS EXCEPT CENTRAL NERVOUS SYSTEM, and 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.

TISSUE DISTRIBUTION: THESE ARE MORE SELECTIVELY DISTRIBUTED. IN ALL SPECIES, MACROPHAGES AND B-CELLS EXPRESS CLASS II ANTIGENS. IN MAN, SOME ACTIVATED T-CELLS ALSO EXPRESS CLASS II LIKE ANTIGENS.

3. Class III genes

These are complement components (C2 and C4) whose importance we have already discussed.

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:

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.

2. The evolutionary reasons for this are debated.

3. This 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: congenics:

MHCA MHCB F1 A.B B.A

antigen x - + + + -

antigen y + - + - +

In this example the MHCB individual is a non-responder to the antigen "y", but responds normally to antigen "x", showing that the entire immune system of the MHCB 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 class II MHC GENES WERE LOCATED.

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 VACCINIA VIRUS

TARGETS:

| normal cells of: vaccinia infected | A | B | A | B |

Primed Tc:

A STRAIN Tc  - - + -
B STRAIN Tc  - - - +

*they won the Nobel prize in 1997 for these experiments.

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 Tc CELLS AND AS RESTRICTION ELEMENTS IN Tc 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. That is there is a dual requiremnt. This is easily explained by the structure that T cell recognize.

CLASS I I MHC GENE PRODUCTS ACT BOTH AS ALLOANTIGENS FOR Th CELLS AND AS RESTRICTION ELEMENTS IN Th RESPONSES TO [all] 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 reactive to one's own MHC 'sneak through' in the process - since you would lyse [kill your own cells!] 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

!
B. The T-cell receptor heterodimer is part of a multi-component trans-membrane signalling 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 through the 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 in the 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 in the 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 MHC class II molecules. This may result through co-internalization of surface Class II molecules during the phagocytic process.

See diagrams in text.

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 individual 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 this might be the explanation for so high a degree of polymorphism in the MHC. What do you think?

**A Joke:**

Three scientists go fishing 'cause there is nothing better to do.

While waiting for the fish to bite - one of them says "I have an idea - lets all tell what we think the most amazing phenomenon in the universe is and WHY"
They agree.

Physicist says weak forces - because it is in the end the basis for all matter and once it is understood we will understand the universe and its origins.

All nod sagely

The engineer says electrons - because they are the basis for interactions between matter and what holds atoms together and transfer of energy from one atom to another.

All nod sagely

Immunologist says "thermos jug"

All look a little embarrassed, and ask him why.

HE says - well you know, in the winter you can get up in the AM and put steaming hot coffee in it, then it can sit all day on the shelf at work, but when you open it it is still HOT.

They all look a little doubtful- "yes... go on....."

He says but in the summer you can put ice cold lemonade in it and then let it sit all day on the shelf and when you open it at 5pm it is still COLD!
Again embarrassment - and shrugs "so what do you find amazing about that?"

Immunologist says: "Well - how does the thermos jug KNOW if it is hot or it is cold?"

[CLEARLY THIS SCIENTIST WORKED ON TOLERANCE TOO LONG.]


INTERCELLULAR COMMUNICATION - LYMPHOKINES AND CYTOKINES

!
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. Macrophages, 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.

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

C. 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 activities

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 or differentiation 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 cells start DTH reactions

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 cells surrounding a chronic site - TB and fungal infections are good examples.
PHYSIOLOGY OF LYMPHOCYTE ACTIVATION

MOLECULAR BASIS FOR SPECIFICITY Ag-RECEPTOR SIGNALLING
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 that 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 an appropriate response. We will start with the B lymphocyte as an example, emphasize that most of the processes are common to T cells as well. Then discuss some interesting human mutants.

Molecular aspects of signal transduction and translation.

Make a diagram that 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:

![Diagram](image)

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

*Positive activation responses*

![Diagram](image)