Activation Level-Dependent Mutation Rates in Affinity Maturation of B-cells

Introduction

The Immune System

The immune system, a complex organization of molecules and subsystems, functions as a defense mechanism for the body. The molecules work collectively to identify and neutralize foreign pathogens after they have entered the body.

An essential aspect of an immune response is the ability to distinguish between "self" and "non-self" molecules. Immune cells make the distinction between self and non-self by the nature of peptides, which are presented on the surface of all cells. Self-molecules are those that present peptide fragments found accessibly throughout the body. The immune cells have been exposed to these peptides while developing thus assuring the absence of self-reactivity. (Janeway, 2001) The ability to discriminate between self and non-self is pertinent so the immune cells are not attacking healthy body cells. The immune system produces two general types of response: non-specific and specific.

Specific and Non-Specific Immune Responses

During the innate response, immune cells react to antigen in a non-specific manner. The primary cells involved with innate responses are phagocytes and granulocytes. Phagocytes destroy pathogens through lysis or phagocytosis; they are also important in specific responses. Granulocytes, which include eosinophils, basophils, and neutrophils, control particular aspects of the non-specific immune responses. For example, eosinophils remove parasites. (Janeway, 2001)

The specific response refers to the specialized response to each individual antigen. Humoral and cell-mediated are the two types of specific immune responses. Lymphocytes, T-cells and B-cells, are intimately involved with all specific immune responses. The humoral response involves the production of antibodies by B-cells. Cell-mediated refers to the use of killer T-cells to destroy the antigen and antigen infected cells.

The Humoral response, involving B-cells, refers to antibody-mediated immune response. Antibodies are molecules secreted by mature plasma B-cells. Antibody affinity has the range to with the ability to bind to any molecule existent in or out of body. Because of the large number of possible antigens extent in the world, the body must be able to create an astonishing number of molecules, capable of binding to every specificity of antigen. This corresponds to one hundred billion (10^{11}) antibodies. (Kuby, 2000)

Antibodies

Antibodies are y-shaped molecules. They consist of four chains: two heavy chains and two light chains. (See Figure 1 in Appendix) Each pair of heavy chains is identical as is each pair of light chains. Generally, each heavy chain is composed of one variable region and three constant regions. There is some variation in the number of constant regions. (Kuby, 2000) The light chains are composed of one variable region and one constant region. The mirror image sides of the y-shaped antibody are connected by disulfide bonds. The exact number of connecting disulfide bonds varies slightly dependent upon the type of antibody. (Kuby, 2000) The variable regions, aptly named, are the parts of the antibody molecule with the most variation. This area has the greatest association with the antigen. Each antibody has two identical antigen-binding sites.

In order to obtain the high diversity found among antibodies, the body uses three processes: VDJ recombination, somatic hypermutation, and class switching recombination. (Janeway, 2001) The first two processes provide major diversity in the variable regions, location of the antigen binding site on the antibodies. Variation in the variable region allows for many different antigens to be recognized. The third process creates minor variety in the heavy chain constant region of the antibodies. Other immune molecules, such as phagocytes, respond according the particular type of constant region.

VDJ Recombination

VDJ stands for variable, diversity and junction, the three parts of the antibody genome that are spliced together to form the variable region of the antibody. Each genome contains multiple variable, diversity and junction genes on different chromosomes. Only one of each kind, however, is spliced together to form the variable region. Because there are multiple forms of each of the VDJ genes, the recombination has several chances to form a functional antibody. (Janeway, 2001) A large assortment of antibodies is created merely with VDJ recombination. Both heavy and light variable regions undergo VDJ recombination. Recombination in the light chain only involves V and J segments. Otherwise, the processes in the two chains are similar. To provide more chances to create a functional antibody the rearrangements do not occur simultaneously; rearrangement in the heavy chain comes first. (Janeway, 2001)

Somatic Hypermutation

Somatic hypermutation, where point mutations are incorporated into the sites of the antibody molecule where the antigen will bind, increases the variability between antibodies. Once an antibody clone had been selected for its affinity to an invading antigen, the B-cell associated with the antibody migrates to the germinal center to undergo somatic hypermutation.

Certain areas of the variable region genes, named the hypervariable regions, receive point mutations at a much higher rate than the rest of the gene. (See Figure 2 in Appendix) These areas are most closely associated to the antigen binding site. Point mutation is more prevalent than the addition or deletion of nucleotides. This ensures that the reading frame is not disturbed and, therefore, a much higher probability of the antibody remaining functional. Additionally, transitions (purines replaced by purines or pyrimidines replaced by pyrimidines) are greatly preferred to transversions (purines replaced by pyrimidines and vice versa). (Kuby, 2000) Until the past year, little was known about the mechanism driving somatic hypermutation.

Unlike VDJ recombination and somatic hypermutation, class switching recombination does not involve the variable region of the antibody. In the genome, beyond the variable, diversity, and junction genes, lay the genes for five different constant regions. These regions are found in consistent order among species. Initially each antibody receives first constant region, the μ region, corresponding to the IgM constant region. Later in the life of the B-cell, particularly if it has been selected for by antigen, different constant regions replace the mu region. Mature B-cells contain antibodies with IgG, IgE or IgA specificity. The final constant region, IgD, is seen only on immature antibodies; its function is not understood. (Kuby, 2000)

The Germinal Center Reaction

Once the humoral response has begun, B-cell move to germinal centers where their antibody variety greatly increases due to somatic hypermutation. This process is labeled the germinal center reaction. Germinal centers are transient structures that appear in the lymph nodes during a humoral immune response. (See Figure 3 in Appendix) They emerge several days into the infection and last until about three weeks following the initial exposure. (Kuby,

2000) Their purpose is the creation of B-cells with antibodies which have high specificity the attacking antigen.

Antigen in the system activates a small number of B-cell classes. Only the B-cells which higher than average specificity to the antigen, as determined by their particular VDJ segment, will become activated. The large majority of B-cell classes do not have high enough affinity with the antigen to be activated. Those B-cells that are activated migrate to the germinal centers and the germinal center reaction begins. The selection of only B-cells that respond to the antigen is known as clonal selection. (Kuby, 2000) Approximately three B-cells enter each germinal center, creating oligoclonal germinal centers.

Within the germinal there are several sectors that contain different cells and provide different functions. The dark zone and the light zone are the most important to our research. The dark zone is the area of the germinal center where the B-cells enter. B-cells characterized by their degradation of immunoglobulin expression as well as a high proliferation rate as labeled centroblasts. Centroblasts densely populate the dark zone. (Kuby, 2000) This area is believed to have a high rate of mutation. The high density of cells results from the rapid proliferation of cells. As the density in the dark zone increases, cells are pushed into the light zone.

The light zone, although less populated than the dark zone, still contains many dividing B-cells. B-cells become centrocytes, and their immunoglobulin expression rises dramatically. The light zone also contains a higher concentration of follicular dendritic cells than found in other parts of the germinal center. As their names implies, dendritic cells have long attachments similar to the dendrites of nerve cells. The follicular dendritic cells bind highly to antigen, and they function as antigen presenting cells. (Kuby, 2000) Through the interaction with the antigen held by follicular dendritic cells, B-cells are selected by their specificity to the antigen. Through

competition for antigen, only the B-cells with the highest affinity survive. The cells that cannot bind to sufficient antigen become apoptotic (a form of programmed cell death). This selection results in the light zone also containing a large number of dead or dying cells.

After leaving the light zone, B-cells enter one or two possible paths: cell maturation or further affinity maturation. (Janeway, 2001) If the antibody-antigen complex is very strong, demonstrating high affinity, the B-cells exit the germinal center and complete maturation. Maturation can either produce a plasma cell or a memory cell. Plasma cells, typically short-lived, are responsible for the secretion of antibodies. Memory cells keep the antigen-specific antibody to ward off further infection by the same antigen. These cells stay in the body for months and sometimes years. The second course for the B-cells is re-entrance into the germinal center to continue affinity maturation.

The majority of B-cells re-enter the germinal center. They again undergo mutation and selection. Those, which have acquired the highest affinity, exit the germinal center to mature. Multiple cycles through the germinal center was postulated for two reasons. Without such a strategy, the germinal centers could not maintain the B-cell population at the level that is observed. Within the germinal center many B-cells die so new cells must enter to replace those. Additionally, the re-entrance into the germinal center provides a phasic strategy of mutation. Phasic mutation has been proven mathematically to be the optimal approach for producing the largest number of favorable mutations in the shortest amount of time – eliminating the infection fastest. Thus most B-cells that ultimately emerge and complete maturation undergo mutation and proliferation numerous times until antibody affinity is extremely high. (Kepler & Perelson, 1992)

The Role of Activated Induced Deaminase (AID)

The relationship between affinity maturation and somatic hypermutation has been studied extensively for many years. The recent discovery of a molecule pertinent to somatic hypermutation, as well as to other processes in the development of B-cells and antibodies, has resumed high curiosity to these topics. The molecule activated induced deaminase, AID, has received lots of attention because of its compulsory role in both somatic hypermutation and class switching recombination. These processes are mechanistically unrelated, occur at different times, and generally thought to be isolated. There was little understanding about how AID was involved with somatic hypermutation and class switching recombination until Michael Neuberger proposed a mechanism. Now, it is believed that AID is involved with the opening of the DNA strand. Point mutation ensues or the type of constant region is switched, thus AID is to activate these processes. (Honjo, Kinoshita, & Muramatsu, 2002)

The Model

Our model describes the interaction of B-cells immunoglobulin and antigen found in the germinal centers of lymph nodes. As previously discussed, the germinal center is composed of several distinguishable areas including the dark and light zones. This model combines these areas into a single compartment. The reduction to a single compartment greatly simplifies the equations necessary for the model. In this compartment, B-cells undergo both mutation, proliferation and selection. Little accuracy is lost because the definitions between the dark zone and the light zone in the germinal center are not clearly defined except by the density of cells and the cell types.

Affinity-class B-cells

Only B-cells that have been stimulated by antigen grow. The initial absence of antigen stalls all B-cell population growth. The different classes of B-cells, which differ in their affinity

level towards the antigen, will grow at different rates in the presence of the same amount of antigen. A population with a higher affinity to the antigen uses the antigen more efficiently and thus out competes the B-cell classes with lower affinity. The populations with a higher affinity overtake the other populations.

An advantageous mutation creates a high affinity population. All non-advantageous mutations are lethal or silent mutations. The particular manner in which these deleterious mutations are expressed in the model will be discussed later. During each time step a stochastic probability determines whether or not a mutation will occur. As the probability is a random stochastic process, each time the model is run the results vary.

Parameters

The three classes of B-cells, distinguished by their differences in affinity, are labeled X_1 to X_3. Class three has a higher affinity to the antigen than class two. Similarly, class two has a higher affinity than X1. In the germinal center, there are many more than three B-cell classes but only three classes were used in our model because three classes provide sufficient diversity of affinity. Much diversity in affinity to antigen was unnecessary to answer questions of relation between activation level and affinity maturation, which we are attempting to answer.

Alpha represents antigen. The equation describing alpha is linear, not a differential equation. Mu represents the mutation rate. It appears in the linear and squared forms in the system of equations.

The probability of receiving a mutation between different classes of B-cells is expressed by the probability equations p_2 and p_3. A mutation producing a class two B-cell has a probability of p_2. As there is no backwards mutation, discussed late, only class one cells can mutate into class two B-cells. The probability p_3 is the probability of an advantageous mutation

from either a class one or a class two B-cell. Because the probabilities of obtaining a class three B-cell from class one and class two are not equal (justification for this will be provided later) the equation for probability p_3 is more complicated than the equation for p_2.

Constants

The many variables found in our model have been set with approximations based on known data and relationships. Gamma is the relationship between the proliferation constant kp and the death constant kd. These constants (kp and kd) are also known as "the time to divide," and seven is the average time for a proliferating cell in the germinal center to divide. Gamma is approximately equal to one since the proliferation constant and death constant are essentially the same; both equal one-seventh.

The constants r_i express relationships between reaction constants K_i. The reaction constants relate to the affinity of a particular B-cell class. K_1, for example, represents the affinity level of the class one B-cells. The ratio between affinities is very large allowing a clear distinction between the classes of B-cells. r_2 indicates a difference of one hundred between the first and second B-cell classes; r_3 indicates a difference of thousand between the first and third classes. Consequently, the third class uses antigen one thousand time more efficiently the first class.

We set epsilon, our proportion of advantageous mutants, to be one thousandth. The majority of mutations that occur are not advantageous but lethal or silent. Only mutants, which contain a desirable mutation as well as lack any deleterious mutations, are considered advantageous. Advantageous mutations constitute only a small portion of all mutations. In the gene encoding the antibody, there are approximately six hundred bases where a mutation could occur. At each location one of four bases, adenine, guanine, cytosine or thymine, could be

chosen. Consequently, there are approximately 2400 mutations that could occur. The probability that a single advantageous mutation occurs is $\frac{1}{2400}$, less than one thousandth. Previous studies show that approximations of order of magnitude are sufficient.

The antigen limits the ability of the B-cells to grow without bound filling the germinal center. So the B-cells would not grow indefinitely our model needed to contain the total number of B-cells. The value sigma was created as the maximum number of B-cells in the germinal center. Initially, ten thousand was used as the maximum B-cell population as there are approximately ten thousand cells in the germinal cell. Sigma was lowered to one thousand because B-cells only comprise a portion of all cells found in a germinal center.

The division of a cell into two daughter cells is governed by the activation level of the cell. The B-cells in a single class has identical activation level in our model. The activation level is related to the affinity level (r) and to the amount of available antigen through $\left[\frac{r_i \square}{1 + r_i \square}\right]$. When the amount of antigen is small, the activation level is proportional to the affinity level times the amount of antigen, $r_i \square$. If the amount of antigen is large the activation level approaches one. All terms in the equations involve functions for the activation level. Terms which describe stimulation of the B-cell populations incorporate the activation level as $\left[\frac{r_i \square}{1 + r_i \square}\right]$. Those expressing the death of B-cells are one minus the stated activation level, $\left[\frac{1}{1 + r_i \square}\right]$. Therefore, as r becomes very large, the death terms become small. As the activation level of a cell increases, the chance of cell death decreases.

Mutation Rate

The mutation to form high affinity populations of B-cells determines the dynamics of the population growth. We used a stochastic probability, implemented using a Poisson distribution. The function incorporating the Poisson process depended upon the degree on the activation level. When $\mu(\alpha) = \alpha$, the influence of AID on the activation level was not taken into consideration. When alpha appears in the denominator of mu of alpha $\mu(\alpha) = \frac{\alpha}{1 + r_i \alpha}$, the activation level depends no AID. Our Poisson distribution differs from a traditional Poisson distribution because it combines two co-dependent processes. Separately, each of these processes is one-conditional.

Equations

Finally, each of these terms can be combined into the set of six equations, five of which are differential equations. (See Figure 3 in Appendix) The growth of each population of B-cells (class one, class two, class three) is governed by an individual differential equation. The first term in each equation describes the growth of the population. This includes parts for the activation level and the mutation rate. As the population of cells increases, the chance of gaining a mutation also increases. The second term, considered the "death term," includes one minus the activation level. The three B-cell equations, $\frac{dX_i}{d\tau} = \left[\frac{r_i \alpha}{1 + r_i \alpha}\right]\left(2e^{-\mu(\alpha)} - 1\right)X_i - \alpha\left[\frac{1}{1 + r_i \alpha}\right]X_i$ differ in their value for r which increases from population one to population two and again from population two to population three. Apart from the value of r, the B-cell equations are very similar.

The population of antigen in the germinal center is determined by the fourth equation $\alpha = \frac{\sigma}{1 + \sum_i r_i X_i}$. The presence of B-cells limits the population of antigen. This is related to sigma, the maximum population of B-cells in the germinal center. As the populations of B-cells

increase, the denominator of the equation increases, lowering the amount of antigen. Anatomically, as the B-cell populations increase there are more B-cells to uptake antigen so the antigen population decreases as exhibited by this equation. To determine the amount of antigen uptake, the total of each population is multiplied by its affinity. The later populations of B-cells take up more antigen per B-cell.

The probability of the second and third B-cell populations arising is determined by the final two equations. As probability equations, these are required to be bounded between zero and one. This is ensured by the $(1-p)$ in the equation. The activation level of the B-cell is incorporated in the Poisson process term. The inclusion of the Poisson process function, $2\sum_{j=0}^{i}\left[\frac{(\cdot(\cdot))^{j}e^{-\cdot(\cdot)}}{j!}\left[\frac{r_j\cdot}{1+r_j\cdot}\right]X_j\right]$ determines stochastically when an advantageous mutation will occur. Two types of mutation can occur: one that improves affinity by a single level, or one that improves by multiple affinity levels. Mutations advancing the cell one affinity class have a much higher overall probability than those advancing two affinity classes as determined by the terms of the Poisson process function.

Assumptions

In formulating these equations, several mathematically and theoretically accurate assumptions were made. A class one, lowest affinity, B-cell will mutate to a class two B-cell much more frequently than to a class three B-cell. As previously mentioned, mutations to either class are possible but are not equally likely. The probability of gaining multiple advantageous mutations during a single mutation process is much lower than the probability of gaining a single advantageous mutant. Advantageous mutations occur much less frequently than lethal mutations, as discussed with the choice of epsilon.

Mutations increasing the affinity of the B-cell are the only mutations which are modeled. Lethal mutations are not accounted for specifically but through the presence of epsilon. There is no ability, in our model, for a B-cell in the second or third class to mutate to back to a class of lower affinity. Although such mutations are observed in the germinal center, these mutations would not affect the total B-cell population significantly enough to include. For example, once the second B-cell population has begun to grow, a mutation back to the first class of B-cell only increases the first B-cell population by a single cell. The second B-cell population is growing at a rate one hundred times the first population much faster than can be compensated for by single cell mutations to the first population.

Mutations always change the affinity class as they are either advantageous or lethal. B-cells either move to a higher affinity class or die. Within the body, silent mutations can also occur. Silent mutations alter a single nucleotide but do not change the amino acid it encodes, thus generating the same protein. Silent mutations represent such a small proportion of all mutations, it is reasonable to disregard them in our model.

The number of mutations found in the antibodies of B-cells is much higher than the amount of mutations found in replication of the same quantity of germline DNA. The large number of mutations is attributed to somatic hypermutation. Three specific areas in the variable region of each antibody molecule are particular nucleotides sites prone to mutation. Surrounding nucleotides, less involved with antigen binding, do not experience such a high incidence of mutation. The mechanism for somatic hypermutation is believed to require activation involving AID. Our model attempts to associate AID with mutation rate via its involvement with activation level.

Three populations of B-cells with different levels of affinity for the antigen are represented in our model. Three populations were sufficient because we were only interested in comparing global dynamics between populations. Multiple populations, as exist in the germinal center, should not change the overall dynamics as described by our model. Additionally, the classes were given fixed, discrete affinity levels. In the germinal center a continuum of affinity levels is observed. Although all germinal center B-cells will have different affinity levels, those with similar affinity will bind antigen at comparable rates. B-cells with similar antigen affinity can categorized into discrete affinity levels. Only when a B-cell is produced with vastly superior antigen association will a significant affinity gain be seen in the germinal center. The use of affinity classes in our model exhibits the discrete affinity levels.

Results

The simulation was run beginning with two B-cells of class one and a large antigen population. Initially there was low competition for antigen, so the class one B-cells proliferated rapidly. The B-cell population continued to grow rapidly until equilibrium between the number of B-cells and the amount of antigen formed or until a mutation to a higher class of B-cell occurred. Once a single class two B-cell was created, the class two B-cell population rose rapidly and the class one B-cell population declined. This dynamic occurred because of the much higher affinity of the second class of B-cell to the antigen. This B-cell population uses antigen more efficiently, out competing the class one B-cells. The equilibrium established between the second class of B-cell and antigen is distinct from the first equilibrium established. Similarly, as soon as the third class of B-cells is populated by a single cell, it dominates in population growth. (See Figure 5 in Appendix) The equilibrium for the third class of B-cells reaches another level than

either of the first two equilibriums established. At this point in the simulation, the level of class one and of class two B-cells is essentially zero.

The results between individual trials produced similar results. Each population of B-cell, typically grew to an equilibrium before the mutation to the next class occurred. (See Figure 5 in Appendix) Occasionally, the B-cell population and antigen population would not reach equilibrium before the mutation to the next B-cell class transpired. A mutation directly from the first class of B-cells to the third class, bypassing the second B-cell class did not take place frequently. In these cases the second B-cell class never began to grow since there is no backwards mutation once the third population begins to grow.

A data point was collected for each trial when the third class of B-cells was populated with a cell. The creation of the third B-cell class signified the creation of a B-cell with suitable affinity to the antigen. This corresponds to the point in the germinal center response where B-cell would leave the germinal center ready to recognize and destroy the antigen. The germinal center reaction typically lasts one to three weeks.

One-thousand trials of the antigen dependent and the antigen independent were averaged at each mutation rate. An average was needed to properly account for the randomness, provided by the stochastic probability. The antigen independent data, where $\square(\square)=\square$, were compared with the antigen dependent data where $\square(\square)=\frac{\square}{1+r_i\square}$. Antigen independent data could only be collected between mutation rates of zero and approximately 0.69, where there was a vertical asymptote. (See Figure 6 in Appendix)

Antigen dependent trials consistently required less time to create the highest affinity B-cells. The data of the antigen dependent trials reliably fell below the antigen independent data. Only at low mutation rates (below μ=0.2) did the antigen independent trails show a comparable

time to the mutation to class three B-cells. The absolute minimum for the antigen dependent trials (approximately 19) was only about 2.5 times steps below the minimum time (approximately 21.5) for the antigen independent trials. (See Figure 6 in Appendix) Although this disparity was significant, it was not as absolute as projected.

Antigen dependent data was relatively independent of the mutation rate. At a mutation rate of exceeding one, the time to mutation remained below twenty time steps through and beyond a mutation rate of six. In contrast, the antigen independent data varied greatly conditional on mutation rate. Above a mutation rate of approximately 0.69 the antigen independent data goes off to infinitely, preventing mutation to class three B-cells at high mutation rates.

Overall, antigen dependent populations received a mutation to the third class (highest affinity) B-cell sooner than the antigen independent populations. Additionally, antigen dependent populations were largely unconnected to the mutation rate.

Appendix

Figure 1 – Antibody Molecule

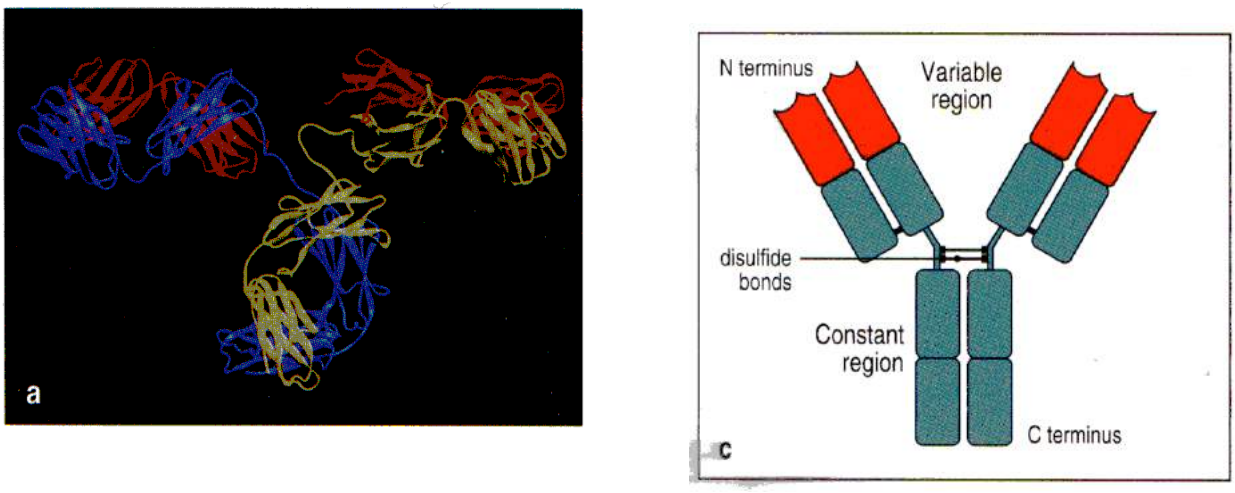


Fig 3.1 pg 94 (Janeway, Charles, et al. Immunobiology.New York, NY: Garland Pub., 2001.)

Figure 2 – Hypervariable Regions

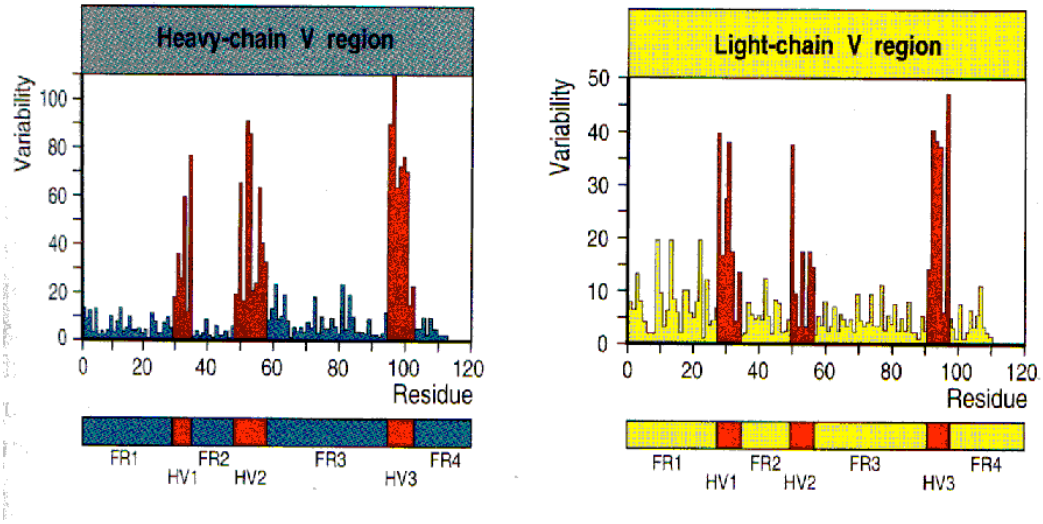


Fig 3.6 pg 101 (Janeway, Charles, et al. Immunobiology.New York, NY: Garland Pub., 2001.)

Appendix (continued)

Figure 3 – Germinal Center

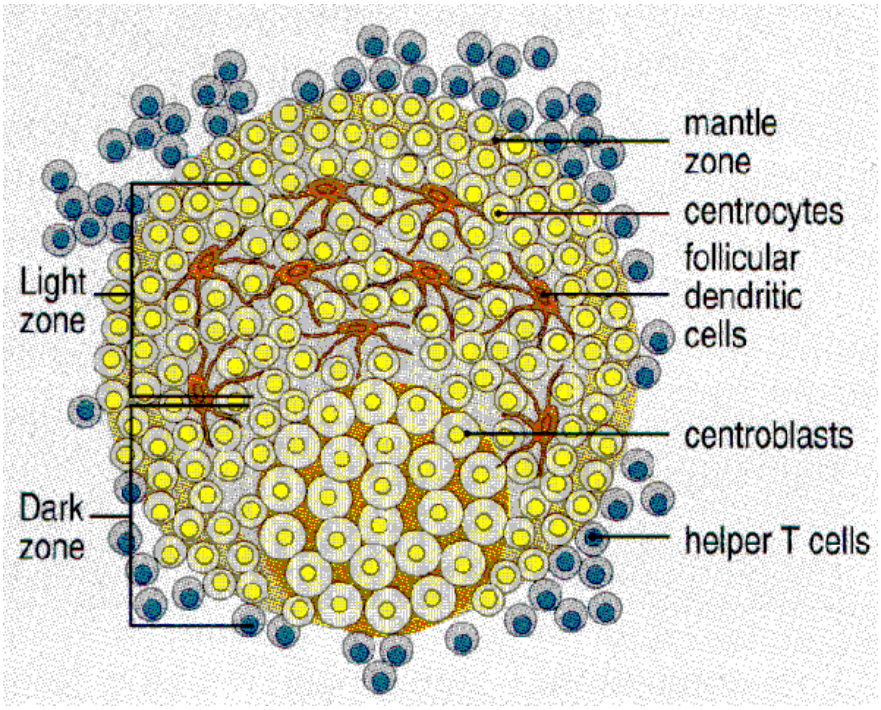


Fig 9.12 pg 352 (Janeway, Charles, et al. Immunobiology.New York, NY: Garland Pub., 2001.)

Figure 4 - Equations

B-cell Equations:

$$\frac{dX_i}{d\tau} = \left[\frac{r_i\tau}{1+r_i\tau}\right]\left(2e^{\alpha\tau(\tau)} - 1\right)X_i - \tau\left[\frac{1}{1+r_i\tau}\right]X_i$$

Antigen Equation:

$$\tau = \frac{\tau}{1+\sum_i r_i X_i}$$

Probability Equations:

$$\frac{dp_i}{d\tau} = (1-p_i)2\sum_{j=0}^{i}\left[\frac{(\alpha\tau(\tau))^j e^{-\alpha\tau(\tau)}}{j!}\left[\frac{r_j\tau}{1+r_j\tau}\right]X_j\right]$$

Appendix (continued)

Figure 5 – Results of Individual Trial

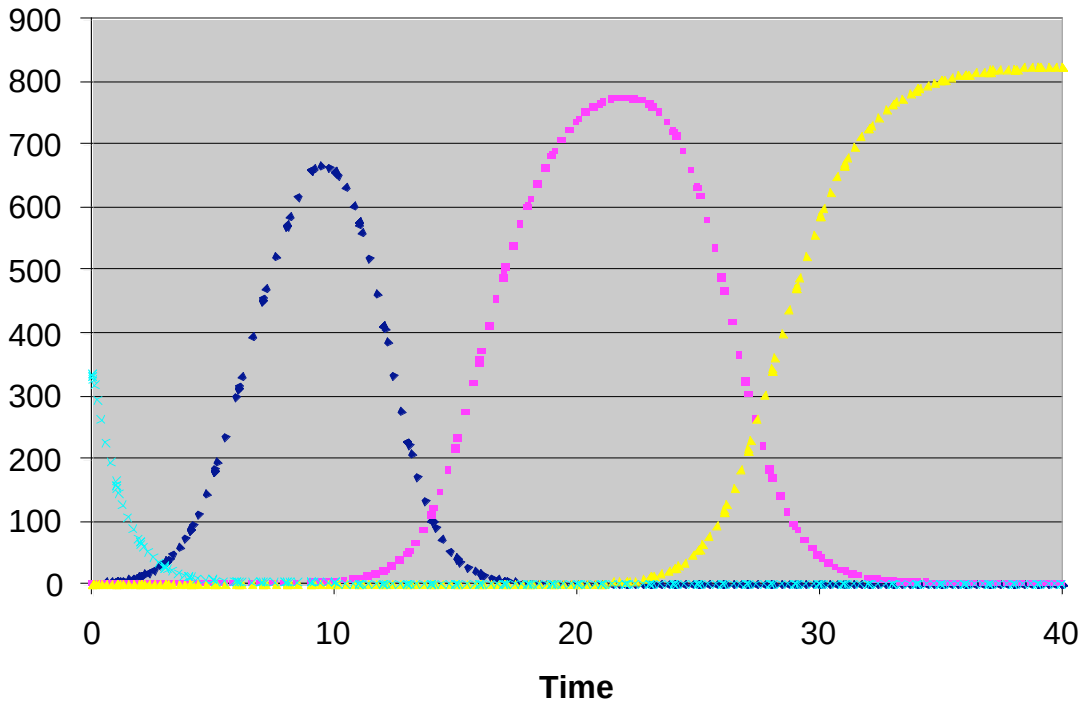
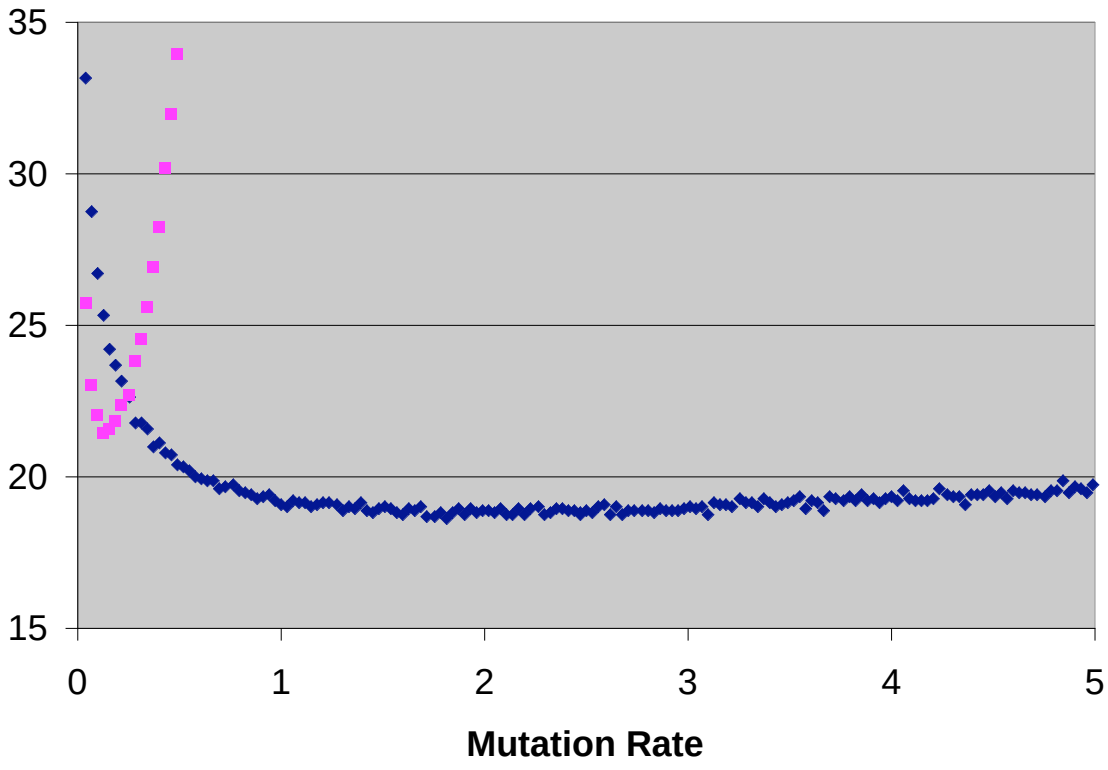


Figure 6 – Results of Multiple Trials



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