# MATHEMATICAL MODELING OF IMMUNE RESPONSES TO VIRAL PATHOGENS

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# DECLARATION

This Research Project Report is my original work and has not been presented for an award of a degree or in any other university or any other award. I therefore make illegal any unauthorized replication of all or part of this proposal.

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We confirm that the work reported in this Research Project was carried out by the candidate under our supervision.

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# DEDICATION

This Thesis is dedicated to my family and my supervisors who pointed the way, helped me along, encouraged me and facilitated the success of my journey. Thank you for believing in me.

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I would like to thank God, my close friends and family for the inspiration support provided while writing this thesis and throughout my life. In particular, I'd like to thank Prof J.Kinyanjui of Karatina University, who has always supported me and perhaps fostered my love for knowledge and exploration. That said, I owe most of my gratitude to my supervisors, Dr. Titus Rotich of Moi University and Prof Francis Gatheri of Technical University of Kenya, without whom this thesis would not have been possible. They have been incredible mentors, and have invested much of their own personal time to help further my research. I'd also like to thank Dr Winnie Mutuku of Kenyatta University for inviting me to conferences and workshops, where I had the privilege of being exposed to a variety of interesting opportunities, research areas and people, many of whom are considered experts in their fields.

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# ABBREVIATIONS

| | |
|---|---|
| **AIDS** | Acquired Immune Deficiency Syndrome |
| **HIV** | Human Immunodeficiency Virus |
| **CTL** | Cytotoxic T Lymphocyte |
| **HCV** | Hepatitis C Virus |
| **SARS** | Severe Acute Respiratory Syndrome |
| **WMV** | West Nile Virus |
| **NK cells** | Natural Killer cells |
| **T Cells** | A type of Lymphocyte activated by thymus |
| **CD4+T** | Cells Mature T helper cells |
| **CD8+T** | Cells A type of a T cell that kills cancer cells |
| **APC** | Cells- Antigen Presenting Cell |
| **CMI** | Cell Mediated Immunity |
| **IFN $\gamma$** | interferon gamma |
| **ADCC** | Antibody-Dependent Cellular Cytotoxicity |
| **IgM** | Immunoglobulin M |
| **TNF** | Tumor necrosis factor |
| **IgE** | immunoglobulin E |
| **RNA** | Ribonucleic acid |
| **DNA** | Deoxyribonucleic acid |
| **HCC** | Hepatocellular carcinoma |
| **HCC** | Hepatocellular carcinoma |
| **MHC** | Major Histocompatibility Complex |
| $R_0$ | Basic Reproductive Ratio |
| **ALT** | Alanine Aminotransferase |
| **PBMC** | Peripheral blood mononuclear cell |

# ABSTRACT

The adaptive immune system, made up of a network of cells, tissues and organs, protect the body against infections and maintain overall health.Immunological research has identified two types of cell interactions: thymus(T) and bone marrow(B) derived cell interaction to explain immune responsiveness. Adaptive immune responses are highly specific to pathogens that induce them. Cell Mediated Immunity defend against intracellular pathogens such as viruses, intracellular bacteria and protozoa.The T cell function lies in the heart of an efficient cytotoxic response.The cells activation is highly regulated and is important to ensure that activation occurs in the right context to prevent development of harmful conditions. With some key processes of the immune system still poorly understood, construction of mathematical models of the immune responses provide the researchers and clinicians powerful tool for the simulation of immune system in order to increase its efficiency in the struggle against pathogens.The purpose of this study was to establish the interplay between the immune responses and viral pathogens.The study offers an innovative, analytical and methodological approach in elucidating key processes of the immune responses.The primary objectives ware: to develop a mathematical model that simulates immune system responses to viral pathogens, analyze the stability of the model in order to get some important ideas about the proliferation of the pathogen and to estimate the range of parameter combinations required to mount an immune response. To achieve this a mathematical model containing five variables: purely susceptible host cells, virus infected cells, free virions, antibody responses and cytotoxic T lymphocyte (CTL) response was formulated using differential equations. The simulations and analysis was done using Matlab & Mathematica softwares, available experimental data on Hepatitis C Virus is used to validate the analytical results. Stability analysis was carried out using the Routh -Hurwitz method and the theory of next generation matrix was used to determine the basic reproductive ratio as $R_0 = \frac{\beta\lambda\kappa}{\alpha\delta\omega}$. Threshold values of parameters that influence immune responsiveness were also determined using the same method. Three possible outcomes in the activation of immune responses were considered: CTL response is established & antibody response fail, antibody response is established & CTL response fail and both CTL & antibody responses become established. Critical bounds are established to determine the threshold requirement for establishment or failure of either CTL or antibody. Analytical results have shown that when antibody response is established and CTL response fail this will represent stable equilibrium while when both CTL and antibody responses are established it will represent an unstable equilibrium. The numerical simulations results showed that dominant CTL establishment is likely to clear a viral pathogen while dominant antibody response alone may not clear the pathogen. In the case that the virus is not cleared, viral evolution was considered, to examine how virus variation affects viral and host survival and to understand viral disease. It was found that that CTL-induced pathology is observed if the rate of viral replication is fast relative to the CTL responsiveness of the host and CTL activation at this stage is not beneficial to the host but can actually be harmful. In conclusion it is important for CTL and antibody responses mount at the right time and strength to reduce the chances of antigenic escape. It is recommended that the model be adopted as a tool to simulate different treatment protocols before administering them patients.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Infections are disorders caused by pathogenic agents such as bacteria, virus, fungi and protozoa.These are the major causes of morbidity and mortality mostly in low income nations and among children and the aged.This has elicited synergistic union of scientists in different disciplines to carry out research with the aim of understanding the spread of these infection causing pathogens in populations and also within the host. This would greatly help in the prevention and treatment of these infectious pathogens. The immune system is spread throughout the body and comprise of organs, tissues, cells and proteins that help the body fight these infectious agents and maintain the overall integrity of hosts health. Human beings are always at risk invasion by these infectious agents and have therefore evolved a system to eliminate these infective agents in the body, that is the immune system defense. The immune system is essential for the survival of the host with over 15% of genes in human genome being associated to immune function Saxena et al. (2007). Generally everyone's immune system has unique qualities different from another but in all hosts the immune system becomes stronger with age to some extent.This is partially because by the time of adulthood one will have encountered more pathogens and developed more immunity A distinguishing and unique feature of the immune system is in its ability to differentiate an un offending pathogen like embryo in a mother and an offending pathogen like a virus. It is also able to identify pathogens previously

encountered and those not previously encountered. This is a sophisticated process and is carried out by a host of cells each specialized in their functions in conjunction with biochemical substances such as enzymes and other proteins.

There are three distinct types of immunity in humans that are aimed at fighting pathogens: innate,specific/ adaptive and passive immunity. Innate immunity is present at birth, it is non specific and offers the first line of defense. It is activated when an offending pathogen is encountered and recognized because of its specific molecular pattern.It includes exterior barriers like the skin, mucous membrane and secretions. The adaptive immune system has two main branches that fight infectious agents, the Cell Mediated Immunity and the antibodies.

Cell mediated immunity are those specific immune responses in which antibody plays only a minor or subsidiary role.This immunity mainly involves the lytic activity of CTL to fight and eliminate intracellular pathogens

The CTL cells are produced in the bone marrow and matures in the thymus and are maintained in naive in secondary lymphoid organs. Cell mediated immunity is activated when a pathogen is presented by antigen presenting cells and identified as offending. This process leads to an immune response characterized by three phases: cellular expansion, contraction and memory cell generation, Papagno et al. (2004).

CTL cells mainly defend the host against virus in the intracellular phase and against intracellular bacteria and protozoa. The CTL cells detect pathogen driven groove of MHC class I as presented by the APCs, key among them the Dendritic cells.It has also the ability to examine inside the cell to establish its status, whether it is damaged or healthy.Normally cells can not examine what could be happening inside other cells.

By this cell-cell examination MHC class I provides a way of detecting cell normally allowing the immune system to expose the infected cells. Terry et al. (2012).

Passive immunity also called 'borrowed' immunity happens when immunity is passed from one source to another as it happens during breast feeding following birth or when the mother passes antibodies to an unborn child through the placenta. This immunity is short lived and is important in protection a new born in the early years. This study elucidates how principles from mathematical modeling can break down the complexity of immune system to smaller units that can be understood. Some of the key questions that would arise in such a study would be;

1. To what extent can a mathematical model represent an understanding of the immune systems?

2. What would be the measure of our supposed understanding of the key processes of the immune system?

3. Is such understanding sufficient to describe how the immune system behaves?

These concerns are pertinent and key in guiding a useful mathematical model in Biosciences. In response to the concerns careful attention must be given to the interrelationship any good model should have with:clinical data, experimental and the extent to which it estimates important parameters.This is the aim of theoretical mathematical modeling.

However, it must be understood that technically mathematical model description and explanations of a biological behaviour are not necessarily the explanations given by Biosciences. Mathematical modeling and analysis is critical and must be used if any

understanding will be converted from theoretical to predictive and quantitative science. The aim of mathematical modeling is not to develop a model that incorporates every aspect of the observed behaviour, if this was at all possible. If every detail was to be incorporated the resulting model would be too complex to give any meaning understanding of how crucial interactions within the system work. Rather it is to develop a model that incorporates important and critical interactions whose outcome cam be understood. Murray (2003).

## 1.2 Cells of the immune system

There are two major populations of cells in the blood composition. The red blood cells and the white blood cells. The red blood cells have their main physiological function of carrying oxygen to the tissues and organs. The white blood cells remove potentially harmful substances from the body.In this population therefore are the cells involved in immune defense.Several subpopulations have been identified which include:

**B lymphocytes,** They recognize antigen, proliferate and produce antibodies specific to antigens. They differentiate into plasma and memory. Plasma cells produce antibodies and memory cells keep the antibodies in supply in case of a second challenge with the same antigen.

**Helper T cells** These coordinate and amplify the immune responses. They communicate with some cells to stimulate B cells to produce antibodies and stimulate CTL to perform cytotoxic function. **Cytotoxic T lymphocytes,** these are the main immunological effectors involved in the elimination of intracellular pathogen infected

cells .

**Immuno-regulatory T lymphocytes,** which have the ability to down-regulate the immune.

**Antigen-presenting cells,** these include macrophages and macrophage-related cells and dendritic cells, their main function is to process the antigen and present it on the cell surface where it can interact with appropriate effector cells.

**Phagocytic cells,** They perform their protective role by a process called phagocytosis, that is ingesting the offending pathogen, damaged, dead or dying cells.

**Natural killer (NK)** They are part of the non specific immunity and defend the body against tumor cells and virus. They recognize antibody-coated cells and mediate ADCC.

**Cytokines** resulting in the expansion of CTL cells and the production of antigen-specific, Class I restricted, CTL cells. The cytokines produced during this stage of the response include IL-2, IFN -$\gamma$ , IL-4 and/or IL-10.

## 1.3 Viruses

They are a bundle of genetic material (DNA or RNA) surrounded by a protein coat.It is still debatable among the scientists whether viruses are alive at all since when t not attached to an appropriate host cell it is of no effect.Unlike bacteria which perform all the necessary functions to be considered living, such as eat, grow, make waste and reproduce.Therefore when a virus is floating in the air or siting in the soil it is of no effect. When the same cell comes into contact with a suitable host cell it becomes active. Viruses do not eat but get their energy from the host cells they infect. They do

not grow in the sense of size but it does reproduce but not without the help of a living cell. They have various shapes and sizes,including multi-sided like diamond, others are shaped as sticks, oval with spikes or tiny sausages.

Virus need the reproductive machinery of a suitable living host cell in order to reproduce, but must first get inside the cell.This happens through a lock and key process.The cell membrane which is made up of protein molecules have specifically shaped receptors or landing sites where other molecules with matching shapes can land and lock. The pneumonia virus latches is capable of latching on to the lung cell, HCV latches on to the liver cell, HIV latches on to the human white blood cells.

## 1.4 The role of CMI in Host defense

Defense against extracellular pathogens such as protozoa extracellular bacteria fungi and virus in the extracellular phase is mediated by the humoral branch of immune system. CIM defends the body against intracellular pathogens most of which are viruses. This is done by killing the pathogen infected cell, which in many cases eliminate the pathogen as well. Viruses at the intracellular phase require the reproductive machinery of the cell in order to replicate. So destruction of the infected cell removes the virus source and this may lead to the resolution of the infection. CIM works together with other cells to recognize and destroy the virus. T helper cells recognize the antigen by MHC class II on APC such as dendritic cells and macrophages. The T helper produces IL-2 and IFN-$\gamma$ which stimulate the effector cells. The effector cells in CMI are called Cytotoxic T- Lymphocytes(CTL). The cytotoxic activity of the CTL depends on the specific antigen and also MHC. The

process of resolution of any pathogen by the CTL is carried out by the various ways given below:

1. **Perforin**,the CTL releases granules which when they come into contact with the target cell will puncture the membrane or cause formation of pores. These pores damage the cell membrane causing it to rapture, a process called lysis.

2. **Granzymes**,the granules may also release an enzyme into the target cell that impairs its internal mechanism.

3. **Cytokines**, mainly the IFN-$\gamma$ and TNF-$\alpha$ that induce metabolic changes in the target cell prompting it to initiate its own death , a process known as apoptosis.

4. **Fas and Fas Ligands**, these are surface molecules expressed by activates effector CTLs and they interact to induce apoptosis

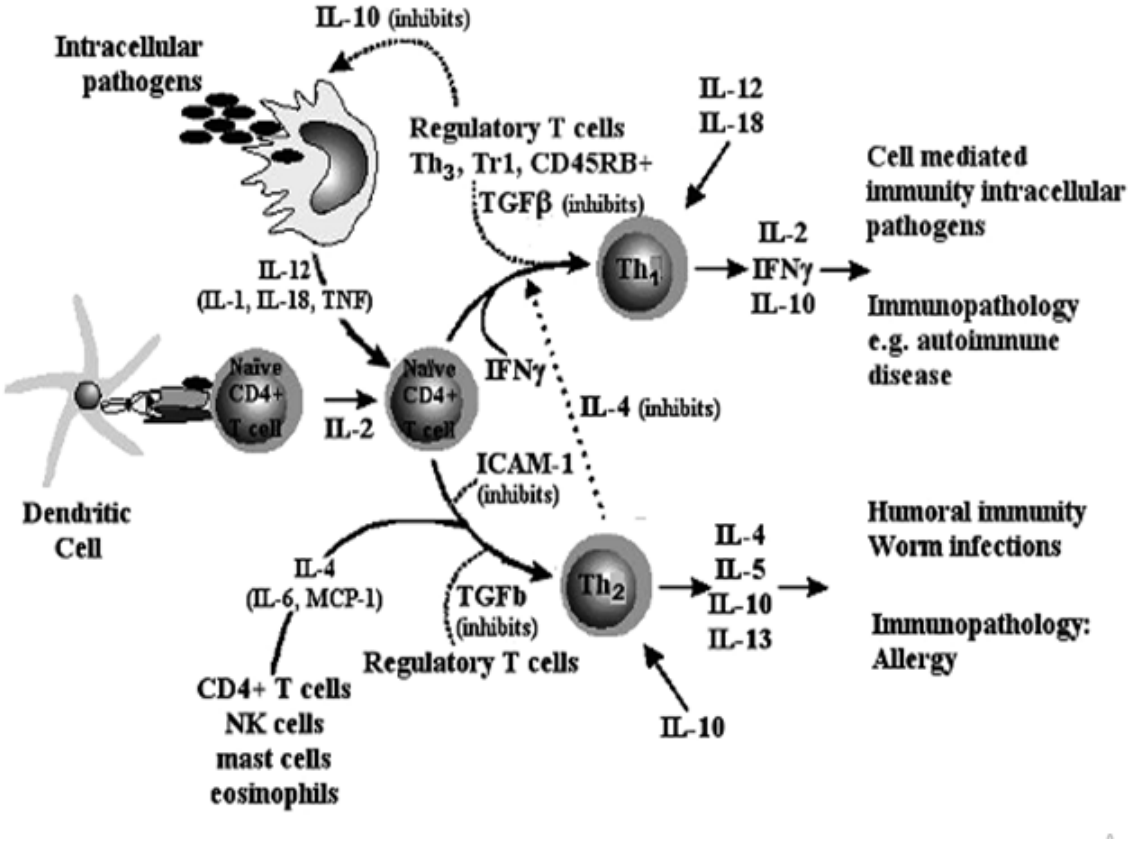


Figure 1.1: *schematic representation of regulation and outcome of Th1andTh2 responses. Source: Eales, L. J. 1997*

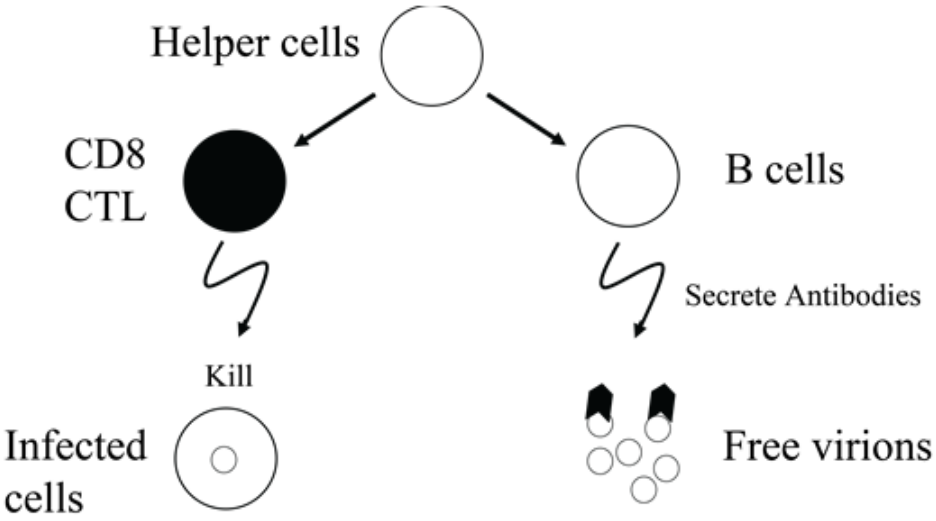


Figure 1.2: *Schematic representation of the adaptive immunity consisting of B and CTL: Source: author.*

## 1.5 General life cycle of a virus.

Though there are significant differences in the reproductive cycles of different virus, the basic processes that any virus must complete can be divided into six steps are as follows.

1. Attachment. This is the first encounter of the virus with the appropriate host cells. Virus attach to specific receptor sites of the host cell.The specificity of this interaction determines which hosts can be infected by the virus and also which cells within the host will be infected.

2. Penetration- They penetrate by either enveloped or non enveloped mechanism.For enveloped mechanism direct fusion or receptor mediated endocytosis is used to penetrate the cell, while for non enveloped mechanism receptor mediated endocytosis is used.

3. Viral un-coating. This process consists in the disorganization of the protective protein layers to release the viral nucleic acid inside the cellular cytoplasm.

4. Viral expression and replication of genetic information.All virus rely on the host translation machinery present in the host cell, ribosomes, for their protein synthesis.

5. Virus assembly and maturation. On production of a new protein there is assembly into new virions, After this the viral genome is inserted into the capsid to form a necleocapsid.

6. Exit/viral release that occur by lysis (rapturing the cell membrane) thus killing the cell or by budding through the cell membrane without necessarily killing the cell

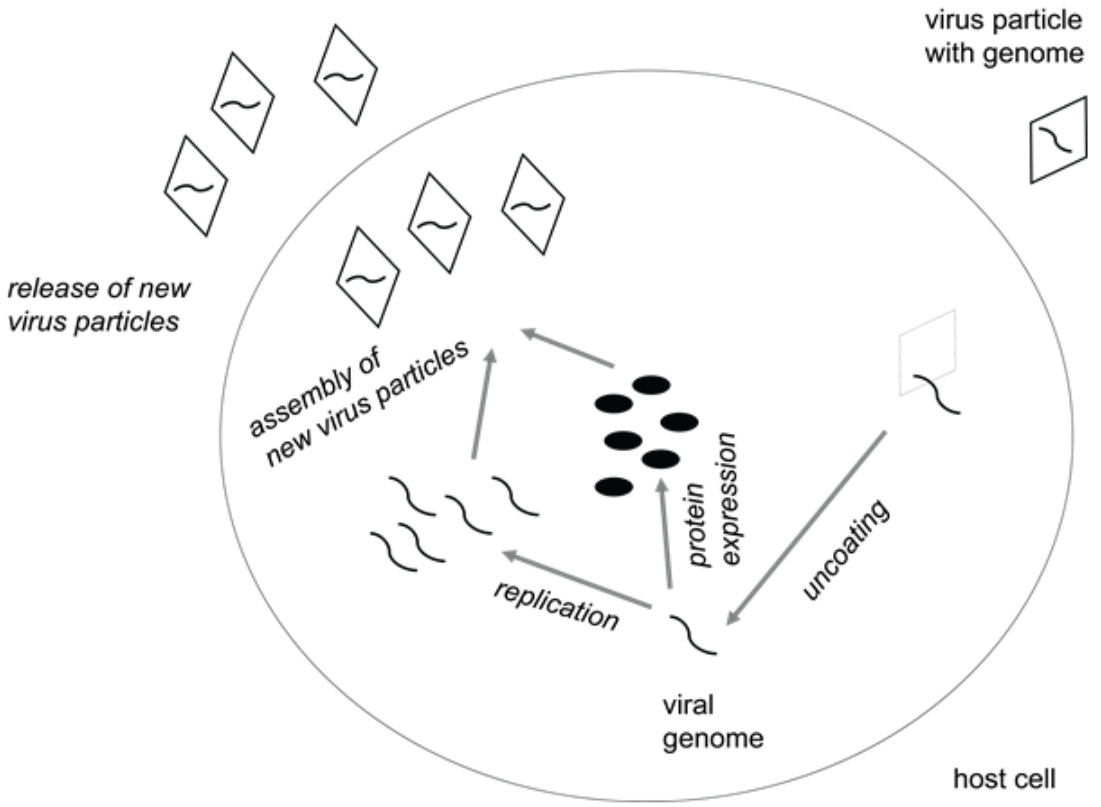


Figure 1.3: *Schematic representation of the general life cycle of a DNA virus.Source: author.*

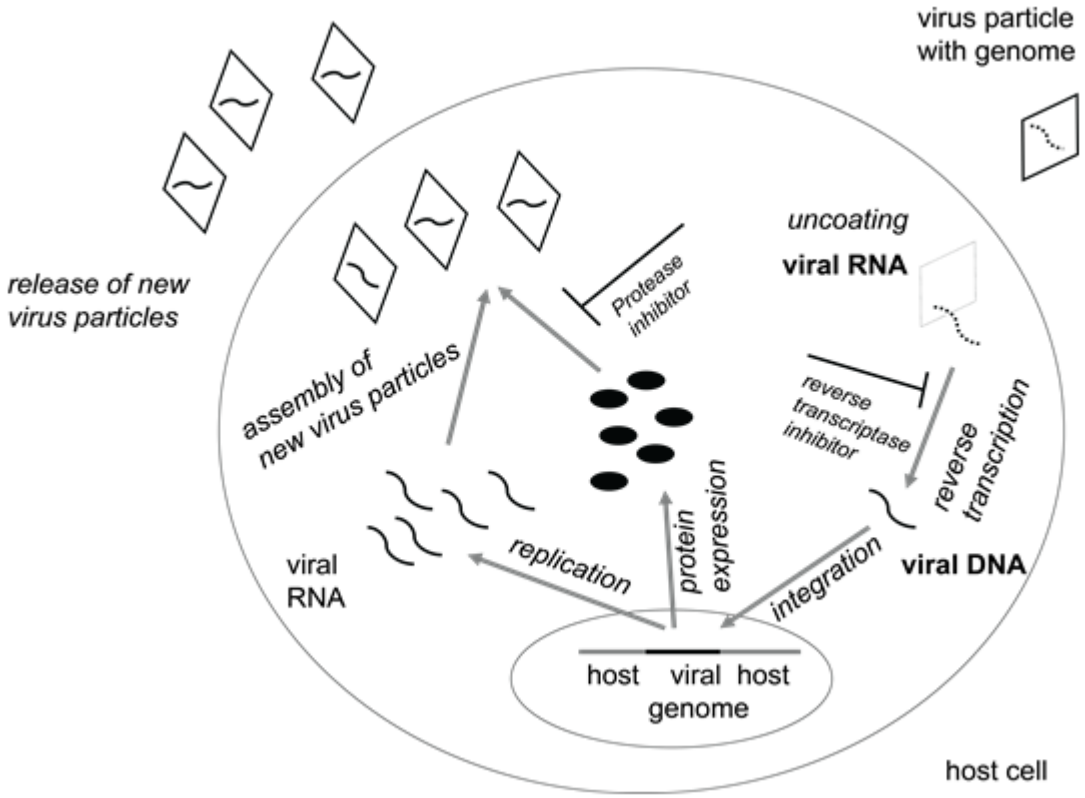


Figure 1.4: *Schematic representation of the general life cycle of RNA Virus Source: author* .

## 1.6 Definition of Terms

**Leucocyte/ Leukocyte:** Cells of the immune system that protect the body against contagious diseases and rid it of useless or toxic debris.

**Neutrophils** The most abundant Leucocyte in mammals, and the first to respond to inflammation; targets bacteria and fungi.

**Lymphocyte** A leukocyte present in the lymph.They are of two types, large and small lymphocytes Large are the Natural Killer cells and small are T and cells.

**Dendritic cell** A type of leucocyte that functions as antigen- presenting cell, activating T lymphocytes.

**Enzyme** A protein substance produced by living cells capable of speeding up chemical changes such as hydrolysis, oxidation or reduction but is is unaltered itself in the process.

**Cytokines** these are proteins important in signaling cells of immune system.

**Antibodies** protein produced in response to antigen stimulation with the capability to bind specifically the antigen.

**Antigen** a foreign molecule within the body that induces an immune response which induces the formation of antibodies.

**Phagocytic** cells any of various organisms or specialized cells that engulf and ingest other cells or particles.

**Lysis** rapture of the cell wall resulting in the dissolution of the cell.

**Necrosis** the death of most or all of the cells in an organ or tissue due to disease,

injury, or failure of the blood supply.

**Apoptosis** programmed cell death aimed at eliminating abnormal or unwanted cells.

## 1.7 Statement of the Problem

The adaptive immune responses are highly specific to pathogens that induce them. CMI is most effective in removing virus- infected cells and intracellular bacteria and protozoa. The T cells function lies in the heart of an efficient Cytotoxic response. T cells activation is highly regulated and is important to ensure that activation occurs in the right context to prevent development of harmful conditions.

This study establishes the interplay between the cell mediated immune response to viral pathogens.

## 1.8 Objectives Of The Study

1. Develop a Mathematical Model that simulates the Immune System response to Viral pathogens.

2. Analyze the stability of the model with respect to perturbation.

3. Estimate the range of parameters combinations required to mount an immune response.

## 1.9 Justification of The Study

Mathematics and other fields in science have always benefited from each other. Each interaction revitalizes and enhances the fields. For sustained, continued relevance and health of the subject, mathematicians must become involved with biology just as it has been involved and influenced physics and engineering.

The involvement of mathematics in biology is inevitable if at all there is hope to make biology qualitative, quantitative and predictive science.

The sophistication of Bio-science interactions makes multidisciplinary approach critical and essential. For a mathematician Biology offers an area of application for biologist, mathematical modeling offers another research tool commensurate with laboratory technique.

Well thought mathematical models have been critical in arousing counter intuitive understanding on how the immune system interact with the pathogens. This gives rise to interesting mathematical theories which form strong basis for designing experiments as well as forming questions which may direct future research.

The synergistic union of mathematician and biosciences researchers is necessary to bring the cost of health care down. Despite the government continuous increase in health care budgetary allocation a big portion of the Kenyan population still shy off from seeking medical care due to costs associated.

If the use of mathematical models stimulates experiments then the high cost of experiments in terms of resources (human & capital) and time can be minimized and this would considerably bring the cost of health care down.

## 1.10 Significance of the study

Gaining knowledge of the immune response mechanism provides a key to understanding disease processes and methods of effective medical treatment.

The adaptive immune system is astounding defense mechanism, it provides the means to make quick, specific and protective responses against multiple potentially harmful pathogenic microorganisms that inhabit the world today. Examples of severe immunodeficiency diseases genetically determined diseases, like sickle cell disease and in acquired immunodeficiency syndrome (AIDS), illustrates the critical role the immune system plays in protection against microbial infection.

The results of this study can be used to determine the possibility of viral clearance by the immune system responses only or whether intervention by treatment is necessary. The results can also be used to predicted the outcome of the infection, whether it will lead to chronicity or to CTL induced pathology. The optimal time for medical intervention can also be deduced from the study. It can not be overemphasized that some treatments that are becoming available to medics will become overwhelmingly useless unless a tool is found to simulate particular treatment protocols before applying them in practice. The models provides tools and platform through which such simulations can be performed. The study offers an innovative, analytical and methodological approach in elucidating key processes of the immune responses to viral pathogens.

## 1.11 Thesis outline.

Chapter one focuses on the background information of the immune system responses and in particular introduction of T cell mediated immune responses, definitions, problem statement objectives and justification of the study. These will be required for understanding the subsequent chapters.

In chapter two the literature review is given, here the various methods and approaches that have been used in modeling of biological systems and their outcomes are explored. A brief introduction of Hepatitis C virus, the pathogen whose data is used in numerical results is given.

Finally in this chapter is the research gap that this thesis seek to fill.

Chapter three presents the methodology employed to carry out the study.

Chapter four is divided into six sub-sections;

1. Analytical results
2. Simulated results.
3. Results and discussion
4. Limitation of the model
5. Experimental data on HCV
6. Model validation

In chapter five conclusion and recommendations are given.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

The immune system works in a complex, interrelated and interconnected network, This posses a challenge to experimental work in terms of resources that can carry out and give the intended results that can explain the observed behavior. The *in-vitro* experiments though providing useful insight on a few or several types of interactions are performed outside the natural setting and context of the immune system.On the other hand *in-vivo* experiments though done within the natural setting are unable to give information of the contributions of each component of the immune system. The information gap between the *in-vitro* and *in-vivo* experiments have given mathematical modelers a fruitful area of application of mathematical modeling. In the recent past many computational and mathematical models have been constructed to mimic and describe the immune defense system processes and its key features. The complexity of immune system has been recently investigated with the synergic union between high-thought experiments and computational modeling.

Several attempts have been made by different researchers to model different aspects of the immune system, innate and adaptive. Among the various approaches adopted to model the immune system are discussed below.

## 2.2 Ordinary Differential Equations,ODEs

This approach has extensively been used in modeling cancer tumor cells humoral responses, Natural killer responses,T cell dynamics and cells of immunological memory.The major advantage of this approach is that it is simpler relative to other approaches and computationally feasible, meaning that it allows for incorporation of several aspects of the immune system before the model can become computationally unfeasible. This approach has been used by Kim et al. (2009) to describe the role of Natural killer cells in surveillance and subsequent trigger of the T cells. In another model by Fouchet and Regoes (2008) considers the interactions of the T cells, APCs to describe the concept of self and non- self discrimination.In the model T cells differentiate to effector, T regulatory or memory cells. The model shows how the feed back control mechanism is important in causing the immune responses to commit to immunogenic or tolerogenic. Kim et al. (2009),also constructed ODE model of Influenza virus, that incorporated APCs, B cell memory , Treg dynamics and T cell responses.The immune environment considered in the model are the lungs and lymph nodes. Using the model it was demonstrated that antiviral therapy was optimal if administered with two days of exposure.

## 2.3 Delay Differential Equations,DDEs

While ODEs systems are finite dimensional systems DDEs are infinite dimensional. They require more computation capacity although they are similar in structure to ODEs.They are referred to as DDEs because they capture time delays observed in

most biological systems in response to a stimuli. The approach was used by Colijn and Mackey (2005)in the study of neutrophils population and the negative feedback involved in stabilizing neutrophils population. It was noted that long time delays could cause the neutrophils population to oscillate from abnormally high to abnormally low level. In another model by Kim et al. (2009) the natural regulation of T cell is studied. The model captures five subpopulations of the immune system cells: Treg cells, CD8+T cells, CD+4 T cells, APCs and antigen.The time delays considered include, time of cell division, time of simulation of CD+8 T cells to full activation and time lag after stimulation of CD+4 cells.

## 2.4 Partial Differential Equations(PDE)

In this approach more details are captured relative to ODEs and DDEs. The approach is useful in age- structured and spatio- temporal models. Age-structured models deal with aspects that are affected by development level in a programmed manner, like maturity and cell division. These types of models are important when the spatial distribution of an infection in the organ of the host is important. The other modeling techniques assumes the concept of homogeneous mixing ie the infection is uniformly spread within the organ.Here the infection is thought to be spread in a planar region and considered according to its position with respect to fixed coordinates. With this more details and complexity is achieve in the other approaches.These models are popular in age-structured and spatio-temporal models. The age-structured model developed by Wherry et al. (2003) captured the the programmed proliferation of cytotoxic T lymphocyte.It was shown that there are times of expansion followed by

relative stabilization then contraction and finally re-stabilization.Using the model the effects of varying the scheduled program was studied and it was shown that T cell responses are governed by intracellular programs that will execute irrespective of wide rande of antigen stimulation. The age- structured models provide us with sufficiently good tool for studying interactions between internal and external regulatory mechanisms. On spatio-temporal model Onsum and Rao studied the migration of neutrophils to the sight of infection by chemotaxis. Chemotaxis is the directed movement of a chemical from a lower concentration to a higher concentration.Simulations were done on how chemotaxis influences chemical signaling to allow movement of neutrophils Onsum and Rao (2007)

## 2.5 Agent Based Modeling,ABM

Unlike differential equations models which deal with collective population of cells, ABM deal with discrete and distinguishable agents such as isolated molecules or specific group of individual cells. An example of this approach is by Catron *et al.* who constructed a model to simulate interactions of dendritic and T cell interactions in the lymph node.The study was to estimate the frequency of T- dendritic cell interactions and the duration of full stimulation of the T cell Souers et al. (2013) Another application of this approach is by Deutsch et al. (2005) who studied the competition for access to the binding sites on APCs. It was shown that T cell competition is dependent on antigen expression by APCs. Using the model it can be deuced that intracellular competition indirectly provides a means of T cell regulation. Another model is by DeCaprio et al. (1988) designed to study B cell migration to the

Lymph nodes. The model assumes random movement of B cells towards a chemoattractant. The model was able to resolve the paradox obtained in two-photon imaging data that B cells migration is initially chemotactical and then latter random. the model showed that chemotaxis must remain active throughout the B cell migration.

The advantage associated with this approach is that its able to account for the probabilistic uncertainty and stochasticity observed in biological interactions. However it has computational complexity as the main disadvantage.

## 2.6 Stochastic Differential Equations

This approach is a hybrid of differential equations and ABM.The formulation is similar to differential equations in that consider population collectively rather than unique individual groups but the variables involved are assumed to have random values. This approach is appropriate when aspects of noise, randomness and sporadic events are to be considered. These models have been extensively used in other disciplines like chemistry, physics and finance but they are yet to find extensive application in immunology. Figge *et al.*(2014 used this approach to model a genetic disorder that hindered B cell from producing immunoglobulin. The model was used to simulate exhaustion of immunoglobulin by natural means, and antigen consumption.The replenishment of B cells by therapy was also considered and found to be effective if administered at low levels at frequencies spread from one week to several weeks DeCaprio et al. (1988)

## 2.7 Hybrid Models

In this approach more than one modeling methods are incorporated to model one phenomena. The models discussed here incorporated ODE and stochastic approached in a single model. Ahmed et al. (2011),formulated a model to study the Hantavirus infection in rodents and in humans (the virus responsible for Hantavirus Pulmonary sydrome,HPS ans also haemorrhagic fever with renal sydrome,HFRS). The goal of the model and analyses thereafter was to demonstrate how competition dynamics between the antibodies and cytotoxic T-lymphocytes (CTLs) play out to eradicate the Hantavirus both in humans and rodents.It was demonstrated through formulation and analysis of systems of ordinary differential equations that the antibodies and CTL compete with each other to eradicate the virus.

Another mathematical model by de Pillis et al. (2005) studies tumor-immune system responses. The analysis provides framework in which to address specific questions relating to tumor-immune system interaction. The major focus of the model was the role played by natural killer cells,CTL in tumor surveillance with the aim of understanding dynamics of tumor rejection. Data from chromium release assays as well as in vivo tumor growth data ware used. Simulations of tumor growth using different levels of immune stimulating ligands, effector cells, and tumor challenge reproduced data from the published studies revealed that the variable to which the model is most sensitive is specific to patients. The variable sensitivity analysis suggested that the model can predict which patients may positively respond to treatment and those that may not. Gowal et al. (2007).

Crauste et al. (2015), developed a mathematical model describing the evolution of CD8 T cell counts and pathogen amount during an immune response. This model is characterized by nine parameters.The ability of the model to fit experimental data and to produce a CD8 T cell population mainly composed of memory cells at the end of the response, critical parameters were identified an valuable insights relating to influenza virus deduced. Among the parameters, two were related to the effector T cell mediated control and pathogen death. The parameter associated with memory cell death is shown to play insignificant role during the main phases of the CTL cell response, yet it becomes critical when predicting the outcome of a re-challenger of the infection several months after the initial infection.

Terry et al. (2012), developed a nonlinear mathematical model to describe the T CD8 immune response to a primary infection using three nonlinear ordinary differential equations and one nonlinear age-structured partial differential equation, to describe the evolution of CD8 T cell count and pathogen density.

## 2.8 The HCV infection belief overview

This introduction is considered necessary here because the data on the HCV is used to validate the model and the analytical results obtained in this study. This is the disease of the liver caused by the HCV.It is thought to be the major predisposing agent of liver cancer. The virus was the first virus to be identified by the methods of molecular biology in 1989, away from virological methods, using the blood plasma obtained from patients and chimpanzees that exhibited hepatitis non -A and non -B. It was discovered that most of those who suffered from hepatitis non-A, non-B ,alcoholic

liver cirrhosis or autoimmune hepatitis were actually hepatitis C patients. Alter et al. (1989). HCV can range from acute illness that may last a few weeks to chronic illness that may be life long.The major route in to the human system is through the contaminated blood , especially during blood transfusion,sharing needles and also during tattooing. Among the symptoms include but not limited to fatigue, nausea, loss of appetite,yellowing of the eyes and the skin.Though these are indicators medical tests must always be done to certain of rule out HCV infection The classification of this virus had tremendous effect on the medical intervention, therapy and prevention of liver cancer Hayashi and Takehara (2006). With more studies and understanding of the disease it was seen that HCV is a major predisposing factor of Hepatocellular carcinoma (HCC).

Unlike in the recent past few years when chronic HCV infection was incurable, now it is treatable albeit the treatment may take a long time with some severity of side effects. Available treatment therapies are by the use of antiviral medication The patients who clear the virus naturally are 30%. The bigger percent 70%, do not clear the virus by their immune system naturally and enter in a phase of persistence and chronicity. In this latter phase, medical intervention is necessary. Most of the patients in this latter phase of chronic hepatitis and develop cirrhosis in 20 to 30 years.Liver cirrhosis is a high predisposing factor of developing HCC , estimated at annual rate of 8%, while patients without liver cirrhosis do so at an annual rate of only 0.5%. In Japan it is estimated that about one million people suffer from HCV infection. World wide about 1.7 billion are infected with the virus. No data has been found for Africa but it is estimated be higher than in these developed countries .HCV infection poses a

serious challenge to the public health and economies because of the high cost of treatment and high mortality of those that develop HCC Vlad et al. (2004)

Since HCV replication is non-cytolytic, cell-mediated immune (CMI) responses to viral antigens are considered responsible for the clearance of virus from infected cells and also for the liver damage experienced in transient and persistent infections. This is presumed to occur via a direct, cytolytic effect of viral antigen-specific cytotoxic T lymphocytes (CTLs) on infected hepatocytes, or via the non-cytopathic action of inflammatory cytokines. In addition, neutralizing antibodies have been shown to prevent infection by blocking the ability of virus particles to bind to receptors on target cells Wodarz (2003).

## 2.9 Research Gap

Despite the extensive research and experiments that has been carried out by various multi-disciplinary agencies in the areas of Miro-Biology, Cell Biology, Clinical Immunology, and other areas of medical practice and health care, there still remains many aspects of a biological system that are scantly understood and whose behavior has not been explained.

In adaptive immune system for example it still remains unclear under what immunological factors combination would influence one branch of immune system (Humoral or Cell Mediated) to respond to an intracellular pathogen and the other one fail or when both will respond. A mathematical structure that mimics a functional immune system responses becomes an imperative tool to investigate and analyze their factors and there influence on the system. This would shed light and insight to other

researchers in their struggle to unravel the complexity of the immune system. The work presented here examine the immune system and its possible behaviors when a viral pathogen is introduced. The approach is innovative, analytical and methodological and has elucidated key processes of inter play between the immune system and viral pathogen.

# CHAPTER THREE

# METHODOLOGY

## 3.1 Mathematical Modeling in Biosciences

Mathematical models depict how incorporation of appropriate various elements in a biological system can lead to improved understanding of either the disease or the pathogen causing it. Models are used as predictive tools or a means of understanding complex fundamental immunological or epidemiological processes. With some simple models analytical results suffice to provide the required insight and understanding of the process. However,for more complex models computer simulations must be relied on to provide results.Intrinsic in every model is the aspect of purpose. Good model is therefore intended to purposefully represent certain aspects of reality. Another aspect that the model should capture is resources. In models resources could mean available knowledge in a particular field, computer application programs, time and so on.In models related to bio sciences a mathematician heavily relies on the current knowledge available in the area of interest. The third aspect resolution, this is the degree to which details are incorporated in a model. If the purpose of the model requires only a low resolution, then a low resolution model is acceptable even if resources would allow a higher resolution model and hence better results.The better results would not be more useful. For high resolution models, their low resolution state is still useful to get the ballpark estimate and successfully refine the model until the desired resolution is attained or resource limitation prevent one from moving further. The process of converting

knowledge in biosciences into a mathematical model is probably the most important step in the process of modeling. Since the problem that modelers need to solve is in the real world it is important to exclude details which are irrelevant to the purpose or which cannot be fixed together given the constrains. Therefore, using the concept of *Occam's razor* , that is excluding details that are irrelevant given the purpose or which cannot be handled given the constrains, the reality is cut into manageable size but with sensitivity to make sure that whatever is included is relevant to the purpose but also it should not make the model too difficult to solve. To do this simplifying assumptions are normally stated when reporting on the built model. The assumptions and model testing should come in handy in defense if any model. Formulating a mathematical model therefore should be able to take care of three important elements: accuracy, transparency and flexibility. Accuracy this is the ability of the model to reproduce to the desired extent the observed behavior and reliably predict the future outcomes. A qualitative fit is normally sufficient to gain insight on some important biological systems but it should also be useful in providing details of any future control policy. Accuracy is improved with improved resolution but care should always be taken not to compromise the computation power and mechanistic understanding of vital interactions. With this the accuracy of the model is always limited within acceptable range. Transparency relates to the understanding of how the various model components interact and their influence on the dynamics of the system. This can be achieved by sensitivity analysis of the components in the model. Flexibility is a measure of how well the model can be suited to other situations.This is important if the model will be useful in prediction future outcome in an ever changing

environment.The trade -off between these three often conflicting elements is important in the development of a model. Mathematical modeling is therefore a fast-growing and well recognized area. For those interested in application of mathematics in the field of biology this is a frontier application of mathematics.The variety of models developed help to understand and explain some complex biological phenomena. Application of mathematics will be inevitable in biology if there is hope to make biology more qualitative. This complexity of the biological sciences makes interdisciplinary involvement essential.

Biology give a mathematician a new branch of application while to the biologist mathematical models offers other research tools commensurate with laboratory techniques. A good model therefore should shows how a process works and then predict what may follow if certain changes occur. However,it must be understood that mathematical explanations of biological phenomena are not biological explanations.

The unifying aim of theoretical mathematical modeling and experimental investigation in the biosciences sciences is to understand and explain underlying biological processes that result in a particular observed phenomenon.

Mathematical models based on systems of ordinary differential equations (ODEs) are the most common of these types of models.The primary advantage of ODE modeling is that it has already been extensively applied in the study of reaction kinetics and other physical phenomena. In addition, the mathematical analysis of these systems is relatively simple compared to other types of models and their solutions can be computationally simulated with great efficiency. That is to say, these models can be made extremely complex, before becoming computationally unfeasible.

## 3.2 Immune System Network

To model the immune system network some basic knowledge on how it functions is important in order to understand the spread of the infectious pathogens qualitatively and quantitatively. Immune system is governed and regulated by a complex interrelated network of cells, cell products and molecules.This complexity of networks makes it difficult to understand it fully experimentally. On one hand *in vitro* experiments separate the immune cell from the natural context of large biological network, potentially leading to non-physiological behavior. On the other hand, *in vivo* observe the phenomenon in the physiological context but are incapable of resolving the contribution of individual regulatory network.

This disjoint in immunological knowledge forms a fruitful ground for mathematical modeling and scientific computation.

The immune system works by recognizing unusual molecules that are not usually found in the body (i.e. they are non-self). These chemicals may be complex (in the form of microorganisms) or simple (such as minor changes in molecules usually present in the body-altered-self). This ability to discriminate between what are sometimes very small differences in chemical structure is a property of the specific or adaptive immune system and is dependent upon the activity of a particular group of cells, the lymphocytes.

The immune system network is always in surveillance of the whole body in order to detect any offending pathogen. Before the immune cells encounter a pathogen, they are said to be naive. Upon encounter the naive cells produce IL-12 and other cells are

stimulated to produce cytokines that further influence the expansion of immune network activities.This development leads to development of effector type-1 or memory cells. These are the cells that eliminate the pathogen or kill the infected cells. The effector cells will secrete cytokines interferon gamma-IFN-$\gamma$ and tumour necrosis factor-TNF that stimulate a range of cells leading to cell-mediated immunity (CMI). Conversely, the presence of IL-10 will lead to the development of type-2 effector and memory cells. The effector cells secrete IL-4, IL-5, IL-6 and IL-13 which influence the humoral immune response and affect the class of antibody produced in response to the antigen (Figure 1.1). Both T helper and T cytotoxic cells develop these different cytokine secretion profiles.

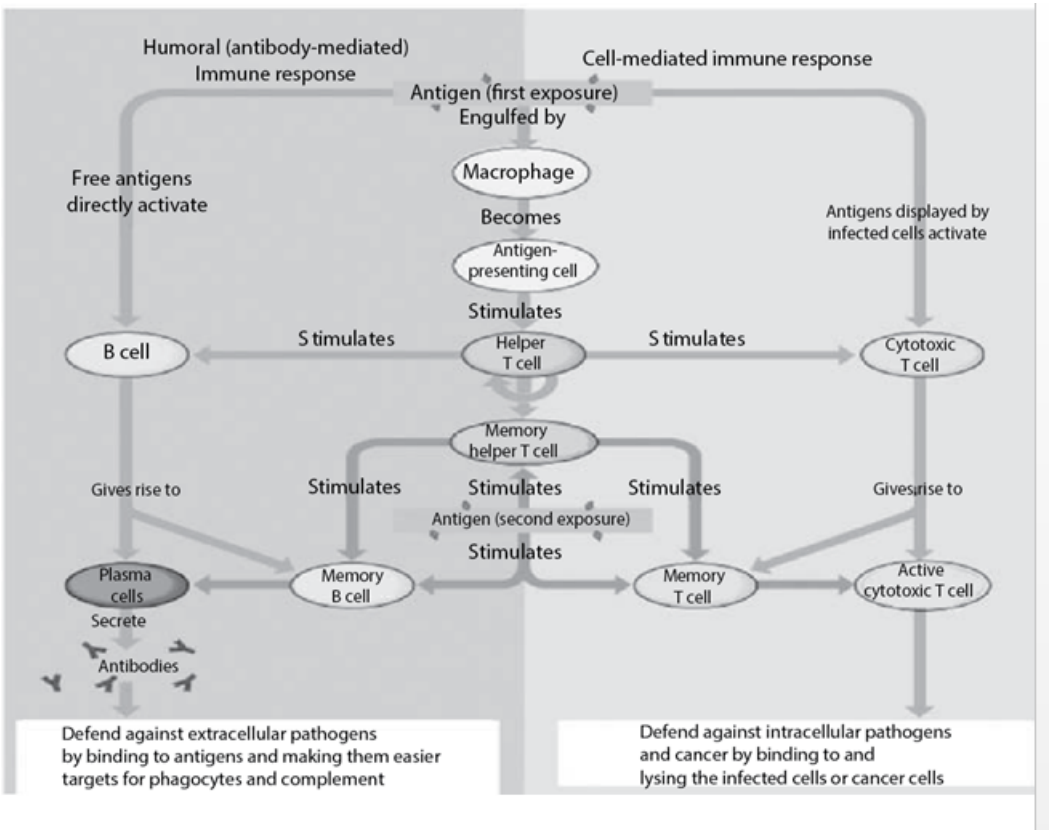


Figure 3.1: A simplified diagrammatic representation of adaptive immune system. Source Dasgupta D. and Nino, F. 2008

## 3.3 Ordinary Differential Equations

These equations are important in mathematics, in the field of research and in application in science and technology. The differential equation is of order $n$ if this is the order of the highest derivative in the equation.

**DEFINITION 3.1:** Let $S \subseteq \Re, V \subseteq \Re^K$ and $\alpha \subseteq \Re^j$ be open subset and suppose that $f : S \times V \times \alpha \to \Re^k$ is continuously differentiable function. An ordinary differential equation (ODE) is an equation of the form

$$\dot{x} = f(t, x, \lambda) \tag{3.1}$$

where dot denote differentiation with respect to the independent variable $t$(*usually measure of time*) and dependent variable $x$ is a parameter of state variable and $\lambda$ is a vector of parameters.

As convenient terminology, especially when one is concerned with the component of a vector differential equation, the equation (3.1) is known as a system of differential equations. Also if one is interested in changes with respect to parameters the differential equation is called a family of differential equations.

As an example, consider the forced van der Pol oscillator,

$$\dot{x}_1 = x_2$$

$$\dot{x}_2 = c(1 - {x_1}^2)x_2 - \lambda^2 x_1 + c\cos\lambda t$$

is a differential equation with $S = \Re, x = (x_1, x_2) \in V = \Re^2$

$$\alpha = \left\{(a, b, \lambda, \lambda) : (c, d) \in \Re^2, \lambda > 0, \lambda > 0\right\}$$

and $f : \Re \times \Re^2 \times \alpha \mapsto \Re^2$ defined in the components by

$$(t, x_1, x_2, c, d, \lambda, \lambda) \mapsto \left(x_2, d\left(1 - x^2{}_1\right) x_2 - \lambda^2 x_1 + a\cos\lambda t\right)$$

A differential equation with one component of the derivative of the unknown function **X**(t) that cannot be expressed as a linear (time varying or a constant) combination of the components of **X**(t) plus a given function of time is called non- linear differential equation. These equations can rarely be solved explicitly and therefore other techniques must be used.

In dealing with non-linear differential equations the matrix notation is sacrificed but preserve the convenience of vector notations for systems. The notation

$$\mathbf{X}' = \mathbf{f}(t, \mathbf{X}) \tag{3.2}$$

is a shorthand for the system of equations

$$\mathbf{x'_1} = \mathbf{f}(t\,, x_1\,, x_2\,, x_3 \ldots, x_n) = \mathbf{f_1}(t\,, \mathbf{x})$$

$$\mathbf{x'}_2 = \mathbf{f}(t\,, x_1\,, x_2\,, x_3 \ldots, x_n) = \mathbf{f}_2(t\,, \mathbf{x})$$

$$\mathbf{x'}_3 = \mathbf{f}(t\,, x_1\,, x_2\,, x_3 \ldots, x_n) = \mathbf{f}_3(t\,, \mathbf{x})$$

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$$\mathbf{x'}_n = \mathbf{f}(t\,, x_1\,, x_2\,, x_3 \ldots, x_n) = \mathbf{f}_n(t\,, \mathbf{x})$$

Where **f** is a vector whose entries are functions of the $n+1$ variables $t\,, x_1\,, x_2\,, x_3 \ldots, x_n$

**DEFINITION 3.2**: The system $\mathbf{X'} = \mathbf{f}(t\,, \mathbf{X})$ is called autonomous if **f** is independent of $t$.

It is always possible to rewrite a non- autonomous system in autonomous form by the introduction of a spurious variable. For example, in the non-autonomous equation

$$X' = t^2 x - e^t$$

let $x_1 = t\,, x_2 = x$

$$x_1' = 1\,, X' = {x_1}^2 x_2 - e^{x_1}$$

In vector form this pair of equations is

$$\mathbf{X}^1 = \begin{bmatrix} x_1 \\ \\ x_1 \end{bmatrix} = \begin{bmatrix} 1 \\ \\ x_1^2 x_2 - e^{x1} \end{bmatrix} \tag{3.3}$$

In general

$$X^1 = f(t,x), X = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ . \\ . \\ . \\ x_n \end{bmatrix}, f = \begin{bmatrix} f_1 \\ f_2 \\ f_3 \\ . \\ . \\ . \\ f_n \end{bmatrix} \tag{3.4}$$

the set

$$\mathbf{Y} = \begin{bmatrix} t \\ x_1 \\ x_2 \\ x_3 \\ . \\ . \\ . \\ x_n \end{bmatrix}, \mathbf{F} = \begin{bmatrix} 1 \\ f_1 \\ f_2 \\ f3 \\ . \\ . \\ . \\ f_n \end{bmatrix}, Y^1 = \begin{bmatrix} 1 \\ x_1^{'} \\ x_2^{'} \\ x_3^{'} \\ . \\ . \\ . \\ x_n^{'} \end{bmatrix} = \begin{bmatrix} 1 \\ f_1 \\ f_2 \\ f3 \\ . \\ . \\ . \\ f_n \end{bmatrix} = \mathbf{F(Y)} \tag{3.5}$$

is autonomous. System of differential equations often occurs in the population dynamics in which the dynamics are traced with respect to time. These equations will

also arise in naturally occurring situations

### 3.3.1 Fixed Points

**DEFINITION 3.3:** The point $\mathbf{x}^*$ is called a critical point of $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ if $\mathbf{f}(\mathbf{x}^*) = 0$

Such points are also called equilibrium points or fixed points.

**THEOREM 3.1:** The point $\mathbf{x}^*$ is a critical point of $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ if and only if the constant function $\mathbf{x}(\mathbf{t}) = \mathbf{x}^*$ is a solution of $\mathbf{x}' = \mathbf{f}(\mathbf{x})$

**PROOF :** If $\mathbf{x}(\mathbf{t}) = \mathbf{x}^*$ is a solution of

$$\mathbf{x}' = \mathbf{f}(\mathbf{x}), \frac{d\mathbf{x}^*}{dt} = 0 = \mathbf{f}(\mathbf{x}^*) \tag{3.6}$$

which implies that $\mathbf{x}^*$ is a critical point of $\mathbf{x}' = \mathbf{f}(\mathbf{x})$. Conversely, $\mathbf{f}(\mathbf{x}^*) = 0$ then since

$$\frac{d\mathbf{x}^*}{dt} = 0, \mathbf{X}(\mathbf{t}) = \mathbf{x}^*$$

is a solution of $\mathbf{X}' = \mathbf{f}(\mathbf{x})$

### 3.3.2 The Jacobian Matrix

To study the variation of $\mathbf{f}(\mathbf{x})$ it is useful to introduce the Jacobian Matrix of $\mathbf{f}(\mathbf{x})$, namely,

$$\mathbf{JF(x)} = \begin{bmatrix} \frac{\partial f_1}{\partial x_1}\mathbf{x} \frac{\partial f_1}{\partial x_2}\mathbf{x} \frac{\partial f_1}{\partial x_3}\mathbf{x} \ldots \frac{\partial f_1}{\partial x_n}\mathbf{x} \\ \\ \frac{\partial f_2}{\partial x_1}\mathbf{x} \frac{\partial f_2}{\partial x_2}\mathbf{x} \frac{\partial f_2}{\partial x_3}\mathbf{x} \ldots \frac{\partial f_2}{\partial x_n}\mathbf{x} \\ \\ \frac{\partial f_3}{\partial x_1}\mathbf{x} \frac{\partial f_3}{\partial x_2}\mathbf{x} \frac{\partial f_3}{\partial x_3}\mathbf{x} \ldots \frac{\partial f_3}{\partial x_n}\mathbf{x} \\ \\ \vdots \\ \\ \frac{\partial f_n}{\partial x_1}\mathbf{x} \frac{\partial f_n}{\partial x_2}\mathbf{x} \frac{\partial f_n}{\partial x_3}\mathbf{x} \ldots \frac{\partial f_n}{\partial x_n}\mathbf{x} \end{bmatrix} \tag{3.7}$$

In vector form $\mathbf{J} = \frac{\partial \mathbf{f}(\mathbf{X})}{\partial \mathbf{X}}$

Note that the Jacobian Matrix depends on both **f** and **x**. Its components representing the variation of each of the components of **f** with respect to each of the components of **x**.

For a given **f**, $\mathbf{Jf}(\mathbf{x})$ is a function of **x**. If each of the partial derivatives $\frac{\partial \mathbf{f}_i}{\partial \mathbf{x}_j}, i = 1,2,\ldots n \;\; j = 1,2,\ldots n$ varies continuously for values of **x** near some fixed $\mathbf{x}_0$ then the Jacobian Matrix may be used to approximate the change in $\mathbf{f}(\mathbf{x}), \mathbf{f}(\mathbf{x}) - \mathbf{f}(\mathbf{x_0})$,

as follows:

$$\mathbf{f}(\mathbf{x}) - \mathbf{f}(\mathbf{x_0}) = \mathbf{Jf}(\mathbf{x})(\mathbf{x} - \mathbf{x_0}) + \mathbf{E}(\mathbf{x} - \mathbf{x_0}) \tag{3.8}$$

where the "error" term $\mathbf{E}(\mathbf{x} - \mathbf{x_0})$ is "small" compared to $(\mathbf{x} - \mathbf{x_0})$ in the sense that $\frac{\|\mathbf{E}(\mathbf{x}-\mathbf{x_0})\|}{\|\mathbf{x}-\mathbf{x}_0\|} \to 0$ as $\mathbf{x} \to \mathbf{x_0}$.

This is expressed as

$$\mathbf{f}(\mathbf{x}) - \mathbf{f}(\mathbf{x_0}) \approx \mathbf{Jf}(\mathbf{x})(\mathbf{x} - \mathbf{x_0}) \tag{3.9}$$

### 3.3.3 Steady State Analysis

Stability of equilibrium/fixed points can be local or global. Local stability concerns itself on the behaviour of the solution near the equilibrium point $x^*$.

Let $\mathbf{J} = \frac{\partial \mathbf{f}^* \mathbf{x}}{\partial \mathbf{x}}$ where $\mathbf{f}^*$ refers to $f_i(x_{1,}x_2, x_3 ... x_n)$ calculated at equilibrium i.e $f_i(x^*{}_{1,}x^*{}_2, x^*{}_3 ... x^*{}_n)$ be the Jacobian matrix. The Eigenvalues $\eta_i (i = 1,2,3...n)$ are solutions of $\det(\mathbf{J} - \eta \mathbf{I}) = 0$ where $I$ is the identity matrix. This will give rise to a polynomial in $\eta$ of degree $n$. This is called the characteristic polynomial which when set to zero and solved give rise to Eigenvalues $(\eta_1, \eta_{2,}, \eta_3 ... \eta_n)$ .

The fixed point $x^*$ is considered to be locally stable if all the Eigenvalues of the Jacobian matrix determined $x^*$ are negative. The equilibrium point is unstable if at least one of the eigenvalues has a positive real part.

**DEFINITION 3.4** A stable critical point is called asymptotically stable if there exists $\delta_0 > 0$ such that $\|\mathbf{x}_0\| < \delta_0$ implies that $\lim_{t \to +\infty} \mathbf{x}(t; \mathbf{x}_o) = 0$

Unstable critical points are never asymptotically stable.

However there may cases where characteristic equations which are analytically infeasible in such cases Routh Hurwitz Stability Criterion will be used. Clark (1992) . Using the characteristic equation a number of Hurwitz determinants is calculated in order to eventually determine the stability of the system.The system's characteristic equation is defined as;

$$f_n(S) = c_0 g^n + b_1 g^{n-1} + b_2 g^{n-2} + ... + c_{n-1} g + c_n \tag{3.10}$$

Now the number of determinants are $n$ of $n^{th}$ order.

The chronological procedure for $n^{th}$ order characteristic equation is as given below:

**First determinant :** This is given by $|c_1|$ where $c_1$ is the coefficient of $g^{n-1}$ in the characteristic equation.

**Second determinant :** Its value is determined as follows,

$$\begin{vmatrix} c_1 & c_3 \\ c_0 & 2 \end{vmatrix}$$

Row one is made up of the first two odd coefficients and row two of the first even coefficients of the characteristic equation.

**Third determinant :** Its value is given by,

$$\begin{vmatrix} c_1 & c_3 & c_5 \\ c_0 & c_2 & c_4 \\ 0 & c_1 & c_3 \end{vmatrix}$$

Now the first row will consist the first three odd coefficients, second row first three even coefficients and in the third row first element zero and rest of two elements are given by the first two odd coefficients.

**Fourth determinant:** likewise it is calculated as given below,

$$\begin{vmatrix} c_1 & c_3 & c_5 & c_7 \\ c_0 & c_2 & c_4 & c_6 \\ 0 & c_1 & c_3 & c_5 \\ 0 & c_0 & c_2 & c_4 \end{vmatrix}$$

Likewise the first row is the first four odd coefficients, row two the first four even coefficients, row three the first element as zero & rest of three elements as first three odd coefficients and fourth row the first element as zero & rest of three elements as first three even coefficients.

By using a similar procedure the generalized determinant formation is given below.

$$\begin{vmatrix} c_1 & c_3 & c_5 & . & . & . & c_{2n-1} \\ c_0 & c_2 & c_4 & . & . & . & c_{2n-2} \\ 0 & c_1 & c_2 & . & . & . & c_{2n-3} \\ 0 & c_0 & c_2 & . & . & . & c_{2n-4} \\ . & . & . & . & . & . & . \\ . & . & . & . & . & . & . \\ 0 & 0 & 0 & 0 & 0 & 0 & c_{2n} \end{vmatrix}$$

So in order to establish the stability of the above system, calculation of each

determinant is made. The necessary condition for the system to be stable is that the value of each of the determinants should be greater than zero. Otherwise the system will be unstable.

However the Modified Hurwitz Criterion of stability of the system gives the necessary and sufficient condition for the stability of a system.

**One:** (Necessity condition): In this two conditions are given below:

1. In the characteristic equation there should not be any value of coefficient that is less zero.

2. The characteristic equation should not have a zero as a coefficient.

**Two :**(Sufficiency for stability)The establishment is began by construction of Routh Hurwitz array as explained below.

The first row of the array is given by the even terms of the characteristic equation when arranged from the first even term to the last even term. Symbolically the first row is as written below:

$$m_0, m_2, m_4, \ldots$$

Likewise the second row will consist of the odd coefficients of the first row of the characteristic equation when they are arranged from the first to the last. Symbolically it is as given below.

$$m_1, m_3, m_5, ..$$

The third row of the array is found as follows Now the elements of third row are calculated as follows:

Element one : Multiply $m_0$ with $m_3$ in other words,the element in the opposite diagonal of the following column, it is then subtracted from the product $m_1$ and $m_2$ and then divided by $m_1$. Symbolically written as follows,

$$n_1 = \frac{m_1 m_2 - m_0 m_3}{m_1} \tag{3.11}$$

Element two: $m_0$ is multiplied with $m_5$, the element in the opposite diagonal of the next column then subtracted from the product, $m_1$ and $m_4$ and finally divided by $m_1$. Symbolically written as follows;

$$n_2 = \frac{m_1 m_4 - m_0 m_5}{m_1} \tag{3.12}$$

In a similar manner all the elements of the third row are calculated.

The procedure for calculating the elements of fourth row is given below.

Element one : $n_1$ is Multiplied with $a_3$ and subtracted from the product of $m_3$ and $n_2$. Symbolically written as

$$p_1 = \frac{n_1 m_3 - m_1 n_2}{m_1} \tag{3.13}$$

Element two : get the product of $n_1$ with $m_5$, then subtract this from the product of $m_1$ and $n_3 b$, then divide by $m_1$. Symbolically written as

$$p_2 = \frac{n_1 m_5 - m_1 n_3}{n_1} \tag{3.14}$$

Similarly,

$$p_3 = \frac{n_1 m_7 - m_1 n_4}{n_1} \tag{3.15}$$

This procedure can be followed to generate all the elements of the fourth row, and similarly all the elements of all the rows.

The system will be stable if all the elements of the first column are greater than zero. If however anyone of them is negative the system will be unstable.

### 3.3.4 Basic Reproduction Number ($R_0$)

The basic reproductive number $R_0$ is defined as the expected number of secondary infections arising from a single individual infected cell during its entire infectious period, in a population of purely susceptible cells Diekmann et al. (1990) and Van den Driessche and Watmough (2002). This number measures the potentiality for the virus to spread within a population of cells. This concept is fundamental to the study of immunity and within host pathogen dynamics since it serves as a threshold parameter that predicts whether the pathogen will spread or not.

By the definition then if $R_0 < 1$, means that each infected cell produces, on average, less than one new infected cell, thus failing to replace itself, and therefore the infection will be cleared from the population, or the viral materials will be cleared from the individual. If, on the other hand, $R_0 > 1$, then the number of infected cells will increase with each generation and the infection will spread or the pathogen is able to establish itself in the susceptible host.

### 3.3.5 Computation of $R_0$

This is computed using the next-generation matrix approach as outlined by Diekmann et al. (1990) and Van den Driessche and Watmough (2002) .

Using this approach two viral replication classes of the model are considered Y and V. $f$ is defined as the matrix whose elements represents the rate of change of new viral materials, or the rate of appearance of new viruses but does not include terms which describe the transfer of infected cells from one compartment to another. Also let the matrix $h$ denote the rate of change of cell populations through other means,like natural death i.e. the elements of $h$ denotes the rate of transfer of individuals by other means in epidemiology. Then the difference $f-h$ gives the total rate of change of cell populations in the two compartments.

The next generation matrix $FH^{-1}$ is formed from evaluating the partial derivatives of $f$ and $h$ at the fixed points (DFE), that is,

$$F = \frac{\partial f_i(x_0)}{\partial x_j} \tag{3.16}$$

$$H = \frac{\partial h_i(x_0)}{\partial x_j} \tag{3.17}$$

The entries of $FH^{-}1$ gives the rate at which infected cells produce new infective viruses times the average length of time a cell spends in a single visit to the compartment. $R_0$ is given by the spectral radius (dominant eigenvalue) of the matrix $FH^{-}1$

Using this approach, basic reproductive ratio defined as $R_0 = \rho(FH^{-1})$ is computed

### 3.3.6 Sensitivity Analysis

The aim of this is to find out the contribution of each parameter relative to $R_0$ in the persistence and spread of the pathogen. This quatity reveals parameters that may need to be controlled in oder to control the pathogen or the disease. Sensitivity analysis is carried out on all parameters by relating each parameter with the basic reproduction number $R_0$. The sensitivity for a parameter say $\alpha$ is given by the equation

$$S_\alpha{}^{R_0} = \frac{\partial R_0}{\partial \alpha}\frac{\alpha}{R_0} \tag{3.18}$$

as given by Fiacco et al. (1983).

### 3.3.7 Numerical simulations

To make the analytical solutions clearer,an illustration with specific numerical example is given, specifically using the Hepatitis C virus infection. The model developed here with a complete list of parameters and their estimated values is used. The Runge Kutta method of order 4 & 5 inbuilt in Matlab software is used to simulate different scenarios in the repertoire of immune responses to HCV.

## 3.4 Intracellular Pathogens

Among the many pathogen types, viral pathogens live inside cells but can also have and extracellular phase. The majority of those are viruses.Immune system responses

against viral pathogens give a better representation of both humoral and cellular responses during an infection. In the intracellular stage, these pathogens are relatively well shielded from humoral immunity. During this phase, the microbial proteins are processed and peptides presented in the context of Major Histocompatibility Complex (MHC) molecules, thus promoting activation of T-lymphocytes. Ancient (but still existent) as well as newly emerging diseases caused by intracellular bacteria that are of paramount significance for humans are Mycobacterium tuberculosis, Mycobacterium leprae, Salmonella enterica serovar Typhi, and Chlamydia trachomatis, the etiologic agents of tuberculosis, leprosy, typhoid, and trachoma, respectively, which, together, afflict more than 600 million people, Kaufmann et al. (1993).

Intracellular parasites like nearly all protozoa are thought to be susceptible mainly to cell-mediated immune effector mechanisms. Nevertheless, during their initial host invasion as well as they transit to new cells they are potential targets for antibody-mediated attack. Cell-mediated immunity against intracellular protozoa also involves the CTL responses.

## 3.5 The Law of Mass Action

The law of mass action says that the rate of chemical reaction is proportional to the product of concentrations of the reactants. This law has been used to model the interactions between the immune system and the viral pathogens.

## 3.6 Model Development

This model is motivated by the predator-prey model (Lotka-Volterra Model). Naturally,species compete, evolve and disperse in order to seek resources to sustain their struggle for their very existence. Depending on their specific settings of applications, they can take the forms of resource-consumer, plant-herbivore, parasite-host, pathogen-immune system, susceptible-infectious interactions, etc. This will generally be loss-win, coexistence or competition interactions. When seemingly competitive interactions are carefully examined, they are often in fact some forms of predator-prey interaction in disguise.There is evidence of competition of the humoral and CMI branches of immunity documented by Van den Driessche and Watmough (2002). Facts accumulated in ecology can be harnessed, applied and extrapolated beyond land mass ecosystems. Hence,here the immune system is considered as a miniature of an ecosystem consisting of five possible variables: purely susceptible host cells, virus infected host cells, free virus particles, antibodies responses and T Cell Cytotoxic responses.

### 3.6.1 Model Assumptions

The specific biological assumptions taken into account when developing the model equations are based on accepted knowledge of immune system function. The assumptions are;

1. T and B lymphocytes, which are the precursors of the immunocompnent are produced in the bone marrow.

2. Cytotoxic T Lymphocyte (CTL) can kill infected cells or shut down virus replication in the cell

3. Anti bodies will fight (neutralize) free virus released by infected cells.

4. Pathogen-specific CTL and antibody-specific responses are initiated once the virus is present.

5. Immune responses will decay after some number of encounters with virus or upon virus eradication.

6. The CTL response and antibody response are independent.

7. The immune system can recognize pathogens that have been encountered before and those encountered for the first time.

8. The immune system is able to differentiate between self and non-self substances that are non-offending from those that are offending.

9. viruses that attack the immune system are excluded

### 3.6.2 Model Flow Chart

Using the assumptions enumerated above from , a system as five coupled differential equations is developed, where each equation gives the rate of change of the particular cell population in terms of growth, death, cell-cell kill, cell recruitment, and cell decay.

In the equations the five populations are denoted by;

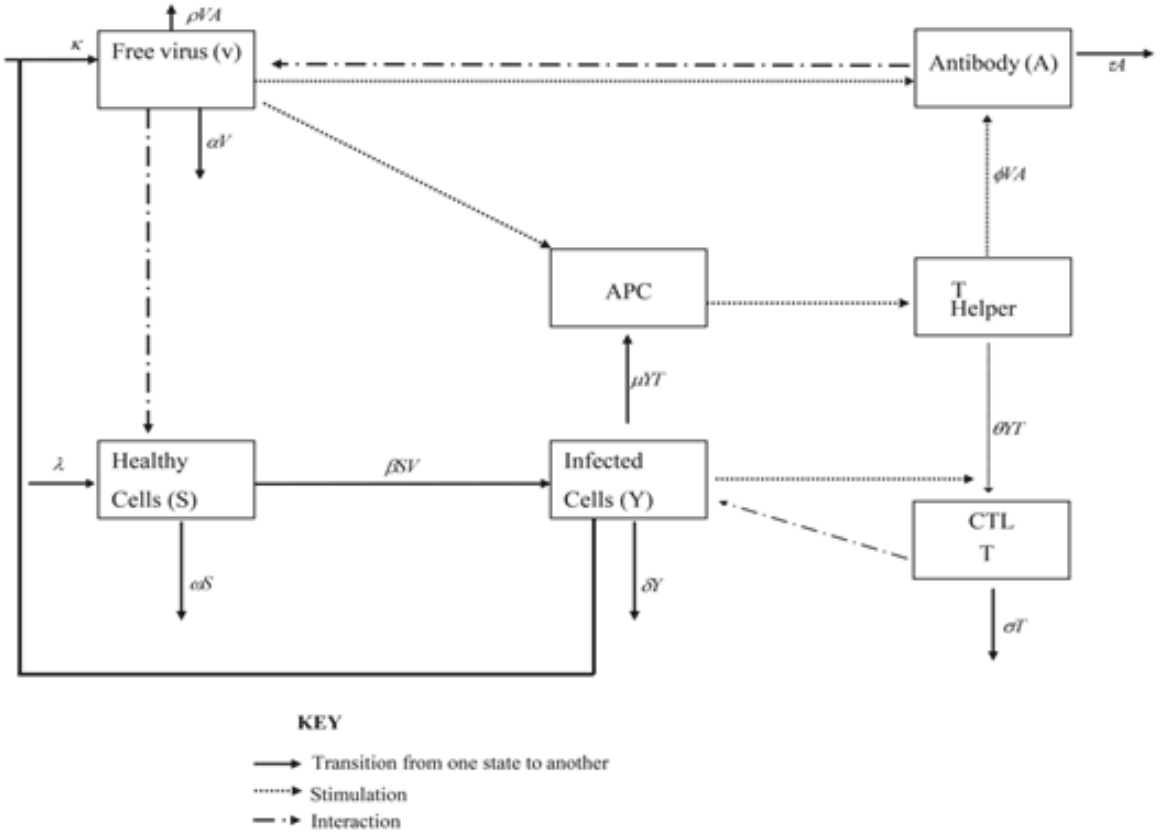


Figure 3.2: *Schematic representation of virus clearance process.Source:Author*

i) $S(t)$ susceptible host cells at any time $t$.

ii) $Y(t)$ infected host cells at any time $t$.

iii) $V(t)$ free virus in the host at any time $t$.

iv) $A(t)$ antibodies at any time $t$.

v)$T(t)$ CTL at any time $t$.

Inputting the particular mathematical expressions for each growth,death and decay give the system of equations given below.

$$\dot{S(t)} = \lambda - \omega S - \beta SV \tag{3.19}$$

$$\dot{Y(t)} = \beta SV - \delta Y - \mu YT \tag{3.20}$$

$$\dot{V(t)} = kY - \alpha V - \rho VA \tag{3.21}$$

$$\dot{A(t)} = \varepsilon A + \phi VA - \tau A \tag{3.22}$$

$$\dot{T(t)} = \upsilon T + \theta YT - \sigma T \tag{3.23}$$

### 3.6.3 Model Description

In the model $\lambda$, is the rate at which purely susceptible host cells are produced in the hosts body, these cells die naturally at the rate $\omega S$ the rate at which the virus infect susceptible healthy cell is $\beta SV$, this term is derived using the law of mass action.

Infected cells die naturally and this rate of death is denoted by $\delta Y$. Upon CTL activation the rate of lytic activity is $\mu YT$.

Upon successful infection, the cells release virions at the rate $k$Y, the free virions decay at a rate $\alpha V$. Upon the activation of the antibodies by the free virus, there is neutralization of the virus at the rate $\rho VA$.

The virus specific antibodies of immunological memory , if the immune system had encountered the viral particle before, are lost at rate $\varepsilon$, the antibody responses against the free virus are mounted at the rate $\phi VA$ and will naturally decay at a rate $\tau$A.

Virus specific CTL of immunological memory, if the viral particle had been encountered before, is lost at the rate $\upsilon$, CTL mount upon activation by the virus from the infected cells at the rate $\theta$YT, and decay naturally when the stimulation is absent at the rate $\sigma$T.

$\varepsilon$V and $\upsilon$T indicate that even in the absence of pathogen there is possibility of typically small standing stock of both antibodies and CTL ready to fight an attack is the immune system had encounters the pathogen before. Without this standing stock immune response system would take longer to respond to pathogen. For primary infections $\varepsilon \approx \upsilon \cong 0$, The average duration of the antibodies and CTL memory cells can be represented as E = $\frac{1}{\varepsilon}$, G = $\frac{1}{\upsilon}$ respectively.

Despite its simplicity, the model (3.19) to (3.23) cannot be solved explicitly. That is, an exact analytical expression for the dynamics of S, Y, V, A and T through time is unfeasible; instead the model has to be solved numerically. Nevertheless, it is invaluable for highlighting very important qualitative immunological principles.

In the MATLAB environment, the mathematical model developed from equations (3.19), (3.20), (3.21), (3.22) and (3.23) are used to perform simulations for Cell Mediated Immune Response to HCV.

MATHEMATICA software has also been used to simplify and manipulate complex Algebraic expressions arising from the analytical analysis. The existing experimental data on HCV is used to justify the validity of the model and analytical results.

# CHAPTER FOUR

# RESULTS

## 4.1 Analytical Results

This is the use of algebraic and / or numeric methods as the main technique for solving a mathematical problem. Analytical results give a general description of system for any value of parameters.

### 4.1.1 Basic Reproduction Number

Without the establishment of the pathogen ;

$$S_{e0} = \frac{\lambda}{\omega}, \; Y_{e0} = 0, \; V_{e0} = 0, \; A_{e0} = 0, \; T_{e0} = 0 \tag{4.1}$$

Using the method described in section 3.4.5 and the model described by equations (3.19) through (3.23) the virus exists in two compartments only. That is equations (3.20) and (3.21), either in the infected cell or as free virus. Hence letting,

$$f = \begin{bmatrix} \beta SV \\ 0 \end{bmatrix} \tag{4.2}$$

$$h = \begin{bmatrix} \delta Y \\ \kappa Y + \alpha V \end{bmatrix} \tag{4.3}$$

$$F = \begin{bmatrix} 0 & \beta S \\ 0 & 0 \end{bmatrix} \tag{4.4}$$

$$H = \begin{bmatrix} \delta & 0 \\ -\kappa & \alpha \end{bmatrix} \tag{4.5}$$

$$H^{-1} = \begin{bmatrix} \frac{1}{\delta} & 0 \\ \frac{\kappa}{\alpha\delta} & \frac{1}{\alpha} \end{bmatrix} \tag{4.6}$$

the next generation matrix is ;

$$FH^{-1} = \begin{bmatrix} \frac{\beta S\kappa}{\alpha\delta} & \frac{\beta S}{\alpha} \\ 0 & 0 \end{bmatrix} \tag{4.7}$$

The Eigenvalues are

$$\begin{bmatrix} 0 \\ \frac{\beta S\kappa}{\alpha\delta} \end{bmatrix} \tag{4.8}$$

The basic reproductive ration is $\frac{\beta S\kappa}{\alpha\delta}$ at $S_{e0} = \frac{\lambda}{\omega}$. Therefore

$$R_0 = \frac{\beta\lambda\kappa}{\alpha\delta\omega} \tag{4.9}$$

It is required that this quantity be greater than one for establishment of any infection.

### 4.1.2 Immunity free equilibrium and its stability

Without any immune responses, both CTL and Antibody responses are not mounted. This may correspond the full blown AIDS stage when the immune system is highly compromised or any other infection that substantially impairs the CD4 T helper cells and hence not able to respond to pathogen invasion. The model equations will then involve equations (3.19) through (3.21). The system settles down to the following equilibrium points;

$$S_{e1} = \frac{\alpha\delta}{\beta\kappa}, Y_{e1} = \frac{\beta\lambda\kappa - \alpha\delta\omega}{\beta\delta\kappa}, V_{e1} = \frac{\beta\lambda\kappa - \alpha\delta\omega}{\alpha\beta\delta}, A_{e1} = 0, T_{e1} = 0,$$

$$V_{e1} = \frac{\kappa}{\alpha} Y_{e1} \tag{4.10}$$

In terms of the basic reproductive ratio the equilibrium points are

$$S_{e1} = \frac{\lambda}{\omega}\frac{1}{R_0},\ Y_{e1} = \frac{\alpha\omega}{\beta\kappa}(R_0 - 1), V_{e1} = \frac{\omega}{\beta}(R_0 - 1), A_{e1} = 0, T_{e1} = 0 \tag{4.11}$$

From the system of model equations, if no immunity is mounted equations (3.22) and (3.23), CTL killing rate term and antibody neutralizing term are redundant. The

Jacobian of the resulting matrix is

$$J = \begin{pmatrix} -\omega - \beta V_{e1} & 0 & -\beta S_{e1} \\ \beta V_{e1} & -\delta & \beta S_{e1} \\ 0 & \kappa & -\alpha \end{pmatrix} \tag{4.12}$$

To obtain a polynomial of Eigenvalues subtract $\Lambda$ from the diagonal elements of $j$ and work out the determinant of the Jacobian i.e. $\det|J - \Lambda I| = 0$ where $I$ is a $3\times3$ identity matrix. The following characteristic equation is obtained

$$\frac{\alpha\delta\beta^2\kappa^2(\beta\lambda\kappa - \alpha\delta\omega) - \beta\kappa(\alpha+\eta)(\delta+\eta)(\beta\kappa(\beta\lambda\kappa - \alpha\delta\omega) + \beta\delta\kappa\delta(\eta+\omega))}{\beta\delta\kappa\beta\kappa\delta} = 0 \tag{4.13}$$

The characteristic equation is not computationally feasible. Using Routh's stability criterion discussed in section 3.4.3

$$a_0 = -1 \tag{4.14}$$

$$a_1 = \frac{-\alpha\beta\delta\kappa\delta + \beta\kappa(\alpha\delta\omega - \beta\lambda\kappa) - \beta\delta\kappa\delta(\delta+\omega)}{\beta\delta\kappa\delta} \tag{4.15}$$

$$a_2 = -\frac{\alpha(\beta\kappa(\beta\lambda\kappa - \alpha\delta\omega) + \beta\delta\kappa\delta(\delta+\omega)) + \delta(-\alpha\delta\omega\beta\kappa + \beta\beta\lambda\kappa\kappa + \beta\delta\kappa\delta\omega)}{\beta\delta\kappa\delta} \tag{4.16}$$

$$a_3 = \frac{\alpha\delta\beta^2\kappa^2(\beta\lambda\kappa - \alpha\delta\omega) - \alpha\beta\kappa\delta(-\alpha\delta\omega\beta\kappa + \beta\beta\lambda\kappa\kappa + \beta\delta\kappa\delta\omega)}{\beta\delta\kappa\beta\kappa\delta} \tag{4.17}$$

This equilibrium is unstable since the coefficients of the characteristic polynomial are not all positive as required in the Routh Criterion .

For the immune responses to develop the conditions

$\varepsilon + \phi V(0) > \tau$ and $\upsilon + \theta Y(0) > \sigma$ must be met. With this, three possible cases arise as discussed below. Only the first time encounter cases with the virus are considered.

### 4.1.3 Case 0ne: Strong CTL Only Immune Response

The CTL responses dominate strongly and drive the antibody responses to extinction.In this scenario the strong CTL lowers the viral load below the threshold requirement for the stimulation of the antibody responses. For the analysis of this case therefore only equations (3.19), (3.20), (3.21) (without the neutralizing term by antibodies) and (3.23) are considered. The following equilibrium points are obtained.

$$S_{e2} = \frac{\alpha\lambda\theta}{\alpha\theta\omega + \beta\kappa(\sigma - \upsilon)}, Y_{e2} = \frac{\sigma - \upsilon}{\theta}, V_{e2} = k\left(\frac{(\sigma - \upsilon)}{\theta}\right), A_{e2} = 0,$$

$$T_{e2} = \frac{\beta\lambda\kappa(\sigma - \upsilon))}{\alpha\omega\theta + \beta\kappa(\sigma - \upsilon)} \tag{4.18}$$

The equations in $V_{e2}$ and $Y_{e2}$ can be expressed as $V_{e2} = kY_{e2}$ and

$$T_{e2} = \frac{\beta S_{e2} V_{e2} - \delta Y_{e2}}{\mu Y_{e2}}$$

respectively.

This tells us that virus population is a function of the infected cells and CTL immune response is a function of healthy & infected cells and free virus.

For primary infection $\varepsilon \approx \upsilon \approx 0$, hence

$$S_{e2} = \frac{\alpha\lambda\theta}{\alpha\theta\omega + \beta\kappa\sigma}, Y_{e2} = \frac{\sigma}{\theta},\ V_{e2} = \frac{\kappa\sigma}{\theta} = \frac{\kappa}{\alpha} Y_{e2}, A_{e2} = 0, \tag{4.19}$$

$$T_{e2} = \frac{\beta\lambda\kappa\sigma}{\alpha\omega\theta + \beta\kappa\sigma} = \frac{\beta S_{e2} V_{e2} - \delta Y_{e2}}{\mu Y_{e2}} = \frac{\beta\kappa S_{e2} - \delta}{\mu} \tag{4.20}$$

Therefore, the behavior of the system at equilibrium can be determined by the Jacobian matrix of the system

$$J = \begin{pmatrix} -\omega - \beta V_{e2} & 0 & -\beta S_{e2} & 0 \\ \beta V_{e2} & -\delta - \mu T_{e2} & 0 & -\mu Y_{e2} \\ 0 & \kappa & -\alpha & 0 \\ 0 & \theta T_{e2} & 0 & \theta Y_{e2} - \sigma \end{pmatrix} \tag{4.21}$$

The determinant $\det|J - \Lambda I| = 0$ is given by

$$\frac{\alpha\delta\beta^2\kappa^2(\beta\lambda\kappa - \alpha\delta\omega) - \alpha\beta\kappa\delta(-\alpha\delta\omega\beta\kappa + \beta\beta\lambda\kappa\kappa + \beta\delta\kappa\delta\omega)}{\beta\delta\kappa\beta\kappa\delta} = 0 \tag{4.22}$$

Although an analytical solution to this equation is not feasible, one can use the criteria in section 3.4.3

$$m_0 = 1 \tag{4.23}$$

$$m_1 = \frac{\alpha\theta(\alpha\theta\omega+\beta\kappa\sigma)+(\alpha\theta\omega+\beta\kappa\sigma)(\beta\kappa\sigma+\theta\omega)+\alpha\lambda\theta\beta\kappa\theta}{\theta(\alpha\theta\omega+\beta\kappa\sigma)} \tag{4.24}$$

$$m_2 = \frac{\alpha\theta(\alpha\theta\omega+\beta\kappa\sigma)+(\alpha\theta\omega+\beta\kappa\sigma)(\beta\kappa\sigma+\theta\omega)+\alpha\lambda\theta\beta\kappa\theta}{\theta(\alpha\theta\omega+\beta\kappa\sigma)} \tag{4.25}$$

$$m_3 = \frac{\alpha\theta(\alpha\theta\omega+\beta\kappa\sigma)+(\alpha\theta\omega+\beta\kappa\sigma)(\beta\kappa\sigma+\theta\omega)+\alpha\lambda\theta\beta\kappa\theta}{\theta(\alpha\theta\omega+\beta\kappa\sigma)} \tag{4.26}$$

$$m_4 = \frac{\alpha\theta(\alpha\theta\omega+\beta\kappa\sigma)+(\alpha\theta\omega+\beta\kappa\sigma)(\beta\kappa\sigma+\theta\omega)+\alpha\lambda\theta\beta\kappa\theta}{\theta(\alpha\theta\omega+\beta\kappa\sigma)} \tag{4.27}$$

This is a stable equilibrium by Routh Hurwitz stability criterion since all the coefficients are positive.

### 4.1.4 Case Two: Strong Antibody Only Immune Response

The antibody response mount strongly, dominate and derive CTL responses to extinction. The strong dominant antibody responses neutralizes the virus particle and hence reducing the virus to levels that cannot stimulate CTL.

The equilibrium of this state are as given below.

$$S_{e3} = \frac{\lambda\phi}{\phi\omega+\beta(\tau-\varepsilon)}, Y_{e3} = \frac{\beta\lambda(\tau-\varepsilon)}{\delta(\phi\omega+\beta(\tau-\varepsilon))}, V_{e3} = \frac{\tau-\varepsilon}{\phi}, A_{e3} =$$

$$\frac{\beta\phi\kappa\lambda}{\delta\rho(\phi\omega+\beta(\tau-\varepsilon))} - \frac{\delta}{\rho}, T_{e3} = 0 \tag{4.28}$$

Equation in

$$A_{e3} = \frac{\kappa Y_{e3} - \alpha V_{e3}}{\rho V_{e3}}$$

from which the alternative expression for

$$V_{e3} = \frac{\kappa Y_{e3}}{\rho A_{e3} - \alpha}$$

For primary infection $\varepsilon \approx \upsilon \approx 0$ hence

$$S_{e3} = \frac{\lambda\phi}{\phi\omega+\beta\tau}, Y_{e3} = \frac{\beta\lambda\tau}{\delta(\phi\omega+\beta\tau)}, V_{e3} = \frac{\tau}{\phi}, A_{e3} = \frac{\beta\phi\kappa\lambda}{\delta\rho(\phi\omega+\beta\tau)} - \frac{\delta}{\rho}, T_{e3} = 0$$

The Jacobian matrix is

$$J = \begin{pmatrix} -\omega-\beta V_{e3} & 0 & -\beta S_{e3} & 0 \\ \beta V_{e3} & -\delta & \beta S_{e3} & 0 \\ 0 & \kappa & -\alpha-\rho A_{e3} & -\rho V_{e3} \\ 0 & 0 & \phi A_{e3} & V_{e3}-\tau \end{pmatrix} \tag{4.29}$$

The characteristic equation is

$$(-\beta\tau\delta\rho(\delta\phi+\Lambda\rho+\rho\tau)+\beta\phi\kappa\lambda\rho\phi-\delta\rho\phi\omega(\delta\phi+\Lambda\rho+\rho\tau))(\beta\delta\rho\kappa\lambda\phi\phi(\Lambda+\omega)$$

$$-(\delta+\Lambda)(\beta\tau+\phi(\Lambda+\omega))(\alpha\delta\rho(\beta\tau+\phi\omega)+\beta\tau\delta\rho(\Lambda-\delta)+\beta\phi\kappa\lambda\rho-$$

$$\delta\delta\rho\phi\omega+\delta\rho\Lambda\phi\omega))$$

$$\delta\rho\rho\tau(\beta\tau+\phi\omega)(\delta+\Lambda)(\beta\tau+\phi(\Lambda+\omega))(\beta\tau\delta\delta\rho-\beta\phi\kappa\lambda\rho+\delta\delta\rho\phi\omega)=0 \quad (4.30)$$

Again the analytical solution of $\det(J-\Lambda I)=0$ is not feasible but as in the previous case , the stability can be determined using Routh's criteria.

$$m_0 = 1 \quad (4.31)$$

all the other coefficients are calculated in likewise manner and are positive hence represents a stable equilibrium.

### 4.1.5 Case Three: Strong CTL and Strong Antibody Immune Responses

Both CTL and antibody responses mount strongly and simultaneously coexist. This equilibrium is described by the following;

$$S_{e4} = \frac{\lambda\phi}{\omega\phi + \beta(\tau - \varepsilon)}, Y_{e4} = \frac{\sigma - \upsilon}{\theta}, V_{e4} = \frac{\tau - \varepsilon}{\phi}, A_{e4} =$$

$$\frac{\kappa\phi(\sigma - \upsilon)}{\theta\rho(\tau - \varepsilon)} - \frac{\alpha}{\rho}, T_{e4} = \frac{\beta\lambda\theta(\tau - \varepsilon)}{\mu(\sigma - \nu)(\omega\phi + \beta(\tau - \varepsilon))} - \frac{\delta}{\mu} \tag{4.32}$$

For primary infection $\varepsilon \approx \upsilon \approx 0$ hence,

$$S_{e4} = \frac{\lambda\phi}{\omega\phi + \beta\tau}, Y_{e4} = \frac{\sigma}{\theta}, V_{e4} = \frac{\tau}{\phi}, A_{e4} = \frac{\kappa\phi\sigma}{\theta\rho\tau} - \frac{\alpha}{\rho},$$

$$T_{e4} = \frac{\beta\lambda\theta\tau}{\mu\sigma(\omega\varphi + \beta\tau)} - \frac{\delta}{\mu} \tag{4.33}$$

The Jacobian is

$$J = \begin{pmatrix} -\omega - \beta V_{e4} & 0 & -\beta S_{e4} & 0 & 0 \\ \beta V_{e4} & -\delta - \mu T_{e4} & \beta S_{e4} & 0 & -\mu Y_{e4} \\ 0 & \kappa & -\alpha - \rho - \rho A_{e4} & -\rho V_{e4} & 0 \\ 0 & 0 & \phi A_{e4} & \phi V_{e4} - \tau & 0 \\ 0 & \theta T_{e4} & 0 & 0 & \theta Y_{e4} - \sigma \end{pmatrix} \tag{4.34}$$

And $\det(J-\Lambda I)=0$ is

$$(\Lambda\mu\mu\sigma\phi(\beta\tau+\omega\phi))(\alpha\beta\tau\mu\mu\sigma+\alpha\mu\mu\sigma\omega\phi+\beta\lambda\theta\tau\mu\rho-\beta\tau\delta\mu\sigma\rho+$$

$$+\beta\tau\Lambda\mu\mu\sigma-\delta\mu\sigma\rho\omega\phi$$

$$+\Lambda\mu\mu\sigma\omega\phi)+\mu\mu\sigma\rho\tau\phi(\beta\tau+\omega\phi)(\beta\lambda\theta\tau\mu-\beta\tau\delta\mu\sigma-\delta\mu\sigma\omega\phi))$$

$$-\Lambda\mu\mu\delta(\beta\tau+\omega\phi)(\theta\mu\mu\sigma\rho\tau\phi^2(\beta\tau+\omega\phi)^2(\beta\tau$$

$$+\Lambda\phi+\omega\phi)(\beta\lambda\theta\tau\mu-\beta\tau\delta\mu\sigma-\delta\mu\sigma\omega\phi)$$

$$(\beta\lambda\theta\tau\mu+\beta\tau\Lambda\mu\delta)$$

$$+(\Lambda\mu\delta\omega\phi)-\Lambda\mu\mu\sigma(\beta\tau+\omega\phi)(-\theta\phi^2)(\beta\tau+\omega\phi)(\beta\tau+$$

$$\Lambda\phi+\omega\phi)(\beta\lambda\theta\tau\mu+\beta\tau\Lambda\mu\delta+\Lambda\mu\delta\omega\phi)$$

$$(\alpha\beta\tau\mu\mu\sigma)+$$

$$+\alpha\mu\mu\sigma\omega\phi+\beta\lambda\theta\tau\mu\rho-\beta\tau\delta\mu\sigma\rho+\beta\tau\Lambda\mu\mu\sigma-\delta\mu\sigma\rho\omega\phi+\Lambda\mu\mu\sigma\omega\phi$$

$$-\kappa\mu\mu\sigma\phi(\beta\tau+\omega\phi)-$$

$$\beta\beta\tau\theta\Lambda\lambda\phi\mu\delta\phi^2-\beta\beta\tau\theta\lambda\phi\mu\delta\omega\phi^2-\beta\theta\Lambda\lambda\phi\mu\delta\omega\phi\phi^2-\beta\theta\lambda\phi\mu\delta$$

$$(\omega\omega\phi\phi^2)\theta\mu^3\mu\delta^2\mu\sigma^2\phi^3(\beta\tau+\omega\phi)^5=0 \tag{4.35}$$

The first coefficients is:

$$m_0 = -1 \tag{4.36}$$

Hence the equilibrium is unstable by Routh stability criteria.

### 4.1.6 Limiting Parameter combination for dominance of either CTL, Antibody responses or both

Here the focus is to analytically examine the conditions that must be satisfied to guarantee any of the three possible outcomes described above.

Using the method described in section 3.4.5 , separating immunological events from non- immunological events in the model equations, where an immunological event is any event involving either CTL or Antibody, the next generation matrix is constructed to determine the limiting requirement for mounting either strong and dominant CTL responses, strong and dominant antibody responses or simultaneously strong CTL and antibody responses For CTL dominance let ,

$$f = \begin{bmatrix} \mu Y_{e2} T_{e2} \\ \rho V_{e2} A_{e2} \\ \phi V_{e2} A_{e2} \\ \theta Y_{e2} T_{e2} \end{bmatrix} \tag{4.37}$$

and

$$h = \begin{bmatrix} \beta S_{e2} V_{e2} - \delta Y_{e2} \\ \kappa Y_{e2} - \alpha V_{e2} \\ \tau A_{e2} \\ \sigma T_{e2} \end{bmatrix} \tag{4.38}$$

$$F = \begin{bmatrix} \mu T_{e2} & 0 & 0 & \mu Y_{e2} \\ 0 & \rho A_{e2} & \rho V_{e2} & 0 \\ 0 & \phi A_{e2} & \phi V_{e2} & 0 \\ \theta T_{e2} & 0 & 0 & \theta Y_{e2} \end{bmatrix} \tag{4.39}$$

and

$$H = \begin{bmatrix} -\delta & \beta S_{e2} & 0 & 0 \\ \kappa & -\alpha & 0 & 0 \\ 0 & 0 & \tau & 0 \\ 0 & 0 & 0 & \sigma \end{bmatrix} \tag{4.40}$$

$$H^{-1} = \begin{bmatrix} \frac{-\alpha}{\alpha\delta - \beta S_{e2}\kappa} & \frac{\beta S}{-\alpha\delta + \beta S_{e2}\kappa} & 0 & 0 \\ \frac{\kappa}{-\alpha\delta + \beta S_{e2}\kappa} & \frac{-\delta}{\alpha\delta - \beta S_{e2}\kappa} & 0 & 0 \\ 0 & 0 & \frac{1}{\tau} & 0 \\ 0 & 0 & 0 & \frac{1}{\sigma} \end{bmatrix} \tag{4.41}$$

Substituting the equilibrium points and evaluating $F.H^{-1}$ it is found

$$F.H^{-1} = \begin{bmatrix} \frac{\sigma^2}{\theta^2} & \alpha\beta\lambda\theta\sigma^2 & 0 & \frac{\mu}{\theta} \\ 0 & 0 & \frac{\kappa\rho\sigma}{\alpha\theta\tau} & 0 \\ 0 & 0 & \frac{\kappa\rho\phi}{\alpha\theta\tau} & 0 \\ \frac{\sigma^2}{\theta\mu} & \frac{\alpha\lambda\theta\beta\sigma^2}{\lambda^2\theta^2\omega\mu+\alpha\beta\kappa\sigma\theta\mu} & 0 & 0 \end{bmatrix} \tag{4.42}$$

The Eigenvalues of the system are

$$E_1 = \begin{bmatrix} 0 \\ 0 \\ \frac{\theta^2+\sigma^2}{\theta^2} \\ \frac{\kappa\rho\phi}{\alpha\theta\tau} \end{bmatrix} \tag{4.43}$$

For CTL to dominate $\frac{\kappa\rho\phi}{\alpha\theta\tau} > 1$ and hence

$$\frac{\kappa\rho\phi}{\alpha\theta} > \tau \tag{4.44}$$

Similarly for Antibody responses to dominate the matrix $F.H^{-1}$ is evaluated at $S_{e3}$ , $Y_{e3}$, $V_{e3}$ ,$A_{e3}$ , $T_{e3}$ and Eigenvalues evaluated.

This is found to be

$$E_2 = \begin{bmatrix} 0 \\ 0 \\ \frac{\beta\lambda\tau\theta}{\delta\sigma(\beta\tau+\omega\varphi)} \\ \frac{\beta\tau\delta^3\rho-\beta\delta\rho\kappa\lambda\theta-\beta\phi\kappa\lambda\delta\rho+\delta^3\rho\phi\omega+\alpha\delta^2\rho(\beta\tau+\phi\omega)}{\delta\rho(-\beta\kappa\lambda\theta+\alpha\delta(\beta\iota+\phi\omega))} \end{bmatrix} \tag{4.45}$$

Its is required that

$$\frac{\beta\lambda\tau\theta}{\delta\sigma(\beta\tau+\omega\phi)} > 1 \tag{4.46}$$

and hence

$$\frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} > \sigma \tag{4.47}$$

Therefore whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} < \tau \tag{4.48}$$

and

$$\frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} > \sigma \tag{4.49}$$

the CTL responses will be strong ,dominant and Antibody responses will be extinct , whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} > \tau \& \frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} < \sigma \tag{4.50}$$

the CTL will fail and Antibody response will dominate, and whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} > \tau \& \frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} > \sigma \tag{4.51}$$

both CTL and antibodies will simultaneously mount strongly.

### 4.1.7 VIRAL EVOLUTION

When the viral pathogen is not resolved by the host immune system, the virus may undergo some genetic changes during its life time. These changes arise from the adaptations, aimed at the survival of the virus within the host, in response to the immune system or the environment. These changes leads to development of new viral variants that can escape detection by the already activated antibody response. This is an important aspect in disease progression especially in HCV. This relationship between the immune system and viral pathogens can result in viral persistence and chronicity. Most viral infections with non-cytopathic viruses are resolved by the lytic activity of the CTL.

The lytic activity of CTL contributes to viral resolution by eliminating the source of virus production, the cell. This is important for the clearance and resolution of infection, however, also can have harmful effects on the host. This will happen when significantly large number of cells are virus infected and CTL lytic activity is stimulated. This lead to a substantial loss of cells resulting in tissue malfunctioning, which may affect the ability of the tissue to regenerate the same cell types or long time chronic conditions . This may lead pathology, and eventual death of the host.The

physical harm, resulting from CTL lytic activity against infected cells the is known as *CTL-induced pathology*. This happens when the CTL activity is un able to clear the infection and therefore unsuccessful in keeping the high rate of virus replication at low levels. Consequently the number of infected cells with different viral mutants will keep growing. This diversification of the virus will gradually stimulate the initially weak CTL and immune responses will shift from non lytic to lytic response. The presence of ongoing CTL lytic activity that kill the infected cells result in many cells dying, and the infected organ gradually get thinner and weaker in a way that is unhealthy which can lead to its failure of its critical functions in the host. The model constructed here now will capture the aspect of viral mutants that escape the antibody responses.This is to further explore the course of disease progression when it is not resolved by CTL.

The model make the following further assumptions together with those in section 3.7.1

1. $i = 1, ...n$ virus are produced that are different from each other in their epitopes only but otherwise identical in replication rate.

2. $V_i$ and $Y_i (i = 1...n)$ are virus of strain $i$ and cells infected by strain $i$ respectively

3. The virus $i$ of strain can prompt antibody response $A_i$ that is specific for the strain.

4. The CTL responses are considered cross reactive to recognize and respond to all the variants equally.

### 4.1.8 Model Flow Chart for Viral Evolution

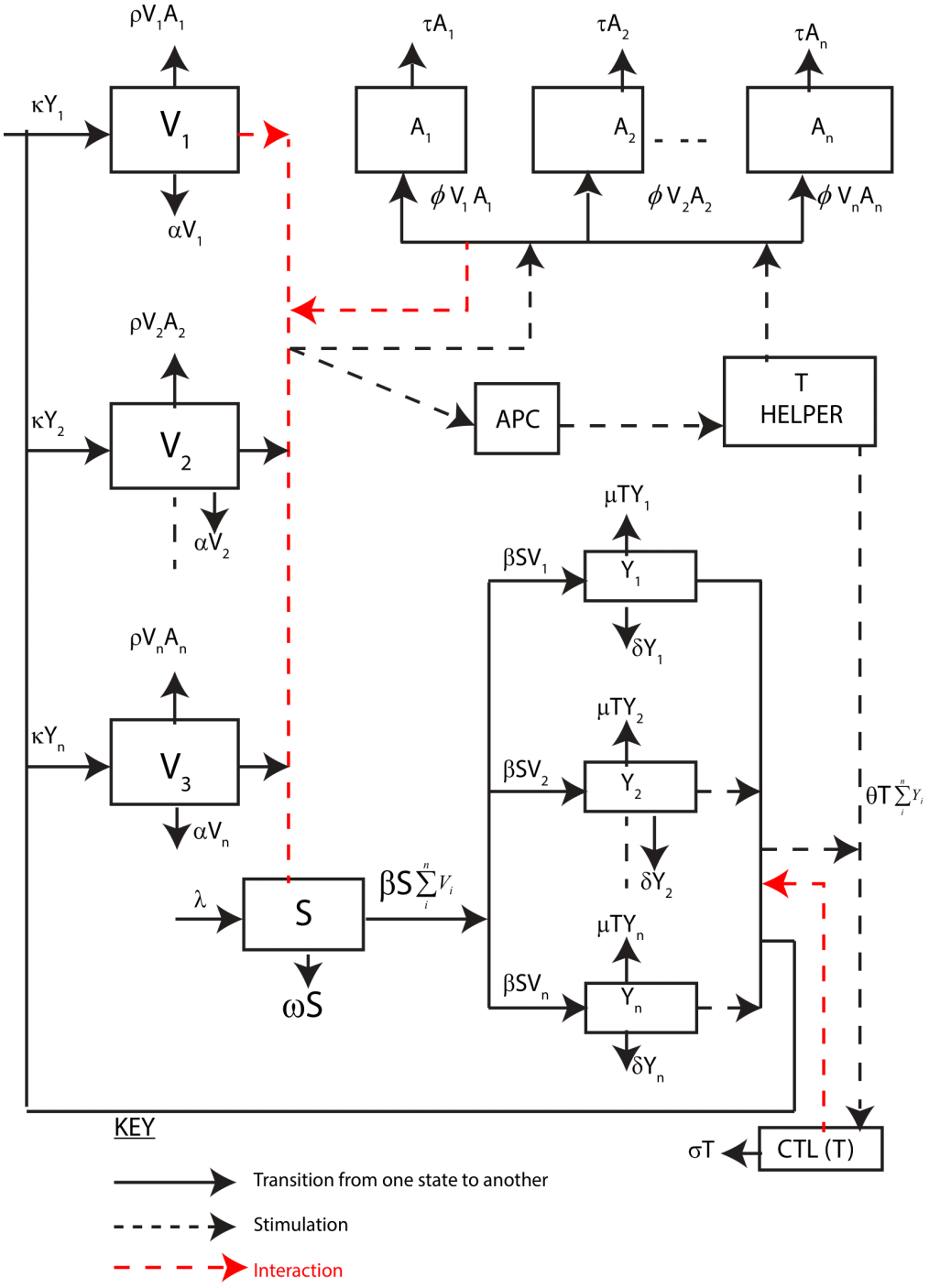


Figure 4.1: *Schematic representation of viral evolution. Source Author*

The following model is developed:

$$\dot{S(t)} = \lambda - \omega S - \beta S \sum_{i=1}^{n} V_i \tag{4.52}$$

$$\dot{Y_i(t)} = \beta S V_i - \delta Y_i - \mu Y_i T \tag{4.53}$$

$$\dot{V_i(t)} = \kappa Y_i - \alpha V_i - \rho V_i A_i \tag{4.54}$$

$$\dot{A_i(t)} = \varepsilon A + \phi A_i V_i - \tau A_i \tag{4.55}$$

$$\dot{T(t)} = \nu T + \theta T \sum_{i=1}^{n} Y_i - \sigma T \tag{4.56}$$

The following equilibrium describe the outcome of the infection with respect to the number of mutants.

$\varepsilon \approx \upsilon \approx 0$

$$S_{e5} = \frac{\lambda \phi}{\phi \omega + n\beta \tau}, {Y_{e5}}^{i} = \frac{n\beta \lambda \tau}{\delta (\phi \omega + n\beta \tau)}, {V_{e5}}^{i} = \frac{\tau}{\phi}, A^{i}_{e5} =$$

$$\frac{\beta \phi \kappa \lambda}{\delta \rho (\phi \omega + n\beta \tau)} - \frac{\alpha}{\rho}, T_{e5} = 0 \tag{4.57}$$

$$J = \begin{pmatrix} -\omega - \beta\frac{\sigma}{\theta} & 0 & -\beta S_{e5} & 0 \\ \beta V_{e5} & -\delta & \beta S & 0 \\ 0 & \kappa & -\alpha - \rho A_{e5} & -\rho V_{e5} \\ 0 & 0 & \phi A_{e5} & \phi V_{e5} - \tau \end{pmatrix} \tag{4.58}$$

and $\det(J - \Lambda I) = 0$

$$\Lambda - \beta\delta\kappa\lambda\phi\phi(\phi(\beta\sigma + \theta(\Lambda + \omega)) - \beta\theta\tau) - (\beta \mathrm{n}\tau - \omega\phi)(\delta + \Lambda)(\beta\sigma + \theta(\Lambda + \omega))$$

$$\delta\phi^2(\alpha + \Lambda) - \mu\tau^2 + \beta\lambda\tau\kappa\tau\phi(\mathrm{n}\beta\tau + \omega\phi) +$$

$$\tau^2(\beta \mathrm{n}\tau - \omega\phi)(\delta + \Lambda)(\beta\sigma + \theta(\Lambda + \omega))(\delta\mu\tau - \beta\lambda\tau\kappa\phi(\mathrm{n}\beta\tau + \omega\phi)) = 0 \tag{4.59}$$

Using Routh-Harwitz stability criteria

$$a_0 = 1 \tag{4.60}$$

$$a_1 = \alpha + \frac{\beta\sigma}{\theta} + \delta - \frac{\mu\tau^2}{\phi^2} + \frac{\beta\lambda\tau\kappa\tau(\mathrm{n}\beta\tau + \omega\phi)}{\delta\phi} + \omega \tag{4.61}$$

$$a_4 = -\frac{\tau^2(\beta\sigma+\theta\omega)(\delta\mu\tau-\beta\lambda\tau\kappa\phi(\mathrm{n}\beta\tau+\omega\phi))}{\theta\phi^2} \tag{4.62}$$

This equilibrium is stable if

$$\delta\mu\tau^3(\beta\sigma+\theta\omega) > \beta\lambda\tau\kappa\tau^2\phi(\mathrm{n}\beta\tau+\omega\phi)(\beta\sigma+\theta\omega) \tag{4.63}$$

When the CTL responses are weak the increase in antigenic mutants escalates the antigenic drive promoting the expansion of the initially weak CTL. below expressions describing this equilibrium .

$$S_{e6} = \frac{\lambda\phi}{\phi\omega+n\beta\tau}, {Y_{e6}}^i = \frac{\sigma}{n\theta}, {V_{e6}}^i = \frac{\tau}{\phi}, {A_{e6}}^i = \frac{\kappa\phi\sigma-n\mu\tau\theta}{n\theta\rho\tau}, T_{e6} =$$

$$\frac{n\beta\tau(\lambda\theta+\delta\sigma)-\delta\theta\sigma\omega}{\mu\sigma(\omega\phi-n\beta\tau)} \tag{4.64}$$

$$J = \begin{pmatrix} -\omega-n\beta\frac{\tau}{\phi} & 0 & -\beta S_{e6} & 0 & 0 \\ \beta V_{e6} & -\delta-\mu T_{e6} & \beta S_{e6} & 0 & -\mu T_{e6} \\ 0 & \kappa & -\alpha-\rho A_{e6} & -\rho V_{e6} & 0 \\ 0 & 0 & \phi A_{e6} & \phi V_{e6}-\tau & 0 \\ 0 & \theta n T_{e6} & 0 & 0 & 0 \end{pmatrix} \tag{4.65}$$

then $\det(J-\Lambda I)=0$ is

$$\Lambda\mu\sigma(\mathrm{n}\beta\tau-\omega\phi)(\rho\tau(\mathrm{n}\beta\tau+\phi\omega)(-(\mathrm{n}\mu\tau\theta-\kappa\phi\sigma))(\phi(\Lambda+\omega)+\mathrm{n}\beta\tau)(-\mu\sigma\omega\phi(\delta))+$$

$$+\Lambda+\delta\theta\sigma\omega\mu+\mathrm{n}\beta\tau(\mu\sigma(\delta+\Lambda)-\delta\sigma\mu-\lambda\theta\mu)-\Lambda\beta\kappa\lambda\phi\mu\sigma\mathrm{n}\theta\rho\tau(\mathrm{n}\beta\tau)-$$

$$-\omega\phi(\beta\tau+\phi(\Lambda+\omega)+\mathrm{n}\beta\tau)-(\mathrm{n}\beta\tau+\phi\omega)(\phi(\Lambda+\omega)+\mathrm{n}\beta\tau)\mathrm{n}\theta\rho\tau(\alpha+\Lambda)+$$

$$(\rho(\kappa\phi\sigma-\mathrm{n}\mu\tau\theta))(-\mu\sigma\omega\phi(\delta))$$

$$+\Lambda+\delta\theta\sigma\omega\mu+\mathrm{n}\beta\tau(\mu\sigma(\delta+\Lambda)-\delta\sigma\mu-\lambda\theta\mu)+\theta\mu(\mathrm{n}\beta\tau+\phi\omega)(\delta\theta\sigma\omega)$$

$$-\mathrm{n}\beta\tau((\delta\sigma+\lambda\theta))^2(\phi(\Lambda+\omega)+\mathrm{n}\beta\tau)(\Lambda\mathrm{n}\theta\rho\tau(\alpha+$$

$$\Lambda)-\rho(\Lambda+\tau)(\mathrm{n}\mu\tau\theta-\kappa\phi\sigma))=0 \tag{4.66}$$

$$a_0=-1 \tag{4.67}$$

$$a_1=\frac{-\mu\sigma\mathrm{n}\beta\tau^2\mathrm{n}\theta\rho\tau+\mathrm{n}\beta\tau(\mathrm{n}\theta\rho\tau(-\mu\sigma(\alpha\phi+\delta\phi-\omega\phi+\omega\phi)+\delta\sigma\mu\phi+}{\mu\sigma\mathrm{n}\theta\rho\tau\phi(\mathrm{n}\beta\tau-\omega\phi)}$$

$$\frac{\lambda\theta\mu\phi)+\mu\sigma\rho\phi(\mathrm{n}\mu\tau\theta-\kappa\phi\sigma))+\phi(\mathrm{n}\theta\rho\tau(\mu\sigma\omega\phi(\alpha+\delta+\omega)-}{\mu\sigma\mathrm{n}\theta\rho\tau\phi(\mathrm{n}\beta\tau-\omega\phi)}$$

$$\frac{\delta\theta\sigma\omega\mu)+\mu\sigma\rho\omega\phi(\kappa\phi\sigma-\mathrm{n}\mu\tau\theta))}{\mu\sigma\mathrm{n}\theta\rho\tau\phi(\mathrm{n}\beta\tau-\omega\phi)} \tag{4.68}$$

The coefficient of the polynomial are negative.

The characteristic equation fails to meet the necessary condition of stability by Routh

and it is therefore an unstable equilibrium.

### 4.1.9 Sensitivity Analysis

Sensitivity analysis studies the effect of different parameters in the output of a system. It has been used in many scientific fields to highlight important data, optimize the design of a system, and rank the influence of various parameters on the system. The primary goal of sensitivity analysis is to find out which modeling parameter has the biggest impact on the system and what causes the effect.

Using the equation in section 3.4.6

$$S_{\lambda}{}^{R_0} = \frac{\partial R_0}{\partial \lambda}\frac{\lambda}{R_0} = 1, S_{\omega}{}^{R_0} = \frac{\partial R_0}{\partial \omega}\frac{\omega}{R_0} = -1 \tag{4.69}$$

$$S_{\beta}{}^{R_0} = \frac{\partial R_0}{\partial \beta}\frac{\beta}{R_0} = 1, S_{\delta}{}^{R_0} = \frac{\partial R_0}{\partial \delta}\frac{\delta}{R_0} = -1, S_{\mu}{}^{R_0} = \frac{\partial R_0}{\partial \mu}\frac{\mu}{R_0} = 0 \tag{4.70}$$

$$S_{\kappa}{}^{R_0} = \frac{\partial R_0}{\partial \kappa}\frac{\kappa}{R_0} = 1, S_{\alpha}{}^{R_0} = \frac{\partial R_0}{\partial \alpha}\frac{\alpha}{R_0} = -1, S_{\rho}{}^{R_0} = \frac{\partial R_0}{\partial \rho}\frac{\rho}{R_0} = 0 \tag{4.71}$$

$$S_{\varphi}{}^{R_0} = \frac{\partial R_0}{\partial \phi}\frac{\phi}{R_0} = 0, S_{\tau}{}^{R_0} = \frac{\partial R_0}{\partial \tau}\frac{\tau}{R_0} = 0, \tag{4.72}$$

$$S_{\theta}{}^{R_0} = \frac{\partial R_0}{\partial \theta}\frac{\theta}{R_0} = 0, S_{\sigma}{}^{R_0} = \frac{\partial R_0}{\partial \sigma}\frac{\sigma}{R_0} = 0 \tag{4.73}$$

### 4.1.10 Limiting Parameter combination for CTL induced pathology

As the virus population continues evolves away from the antibodies, virus load grow and diversification of the antigenic drive increases. The number of cell initially remain the same until a limiting threshold number is attained. This will correspond to absence of pathology The dynamics will however when the limiting threshold is reached which will indicate the setting in of pathology .This threshold is calculated as given below

Using $F$ and $H$ as defined and equilibrium points $S_{e5}$ , $T_{e5}$ , $V_{e5}$ , $A_{e5}$ and $T_{e5}$ it is found

$$H = \begin{bmatrix} -\delta & \frac{\beta\lambda\phi}{\mathrm{n}\beta\tau+\phi\omega} & 0 & 0 \\ \kappa & -\alpha & 0 & 0 \\ 0 & 0 & \tau & 0 \\ 0 & 0 & 0 & \sigma \end{bmatrix} \tag{4.74}$$

$$F = \begin{bmatrix} 0 & 0 & 0 & \frac{\mathrm{n}\beta\lambda\tau\mu}{\delta(\beta\tau+\phi\omega)} \\ 0 & \rho\left(\frac{\beta\phi\kappa\lambda}{\delta\rho(\mathrm{n}\beta\tau+\phi\omega)} - \frac{\alpha}{\rho}\right) & \frac{\rho\tau}{\phi} & 0 \\ 0 & \phi\left(\frac{\beta\phi\kappa\lambda}{\delta\rho(\mathrm{n}\beta\tau+\phi\omega)} - \frac{\alpha}{\rho}\right) & \tau & 0 \\ 0 & 0 & 0 & \frac{\mathrm{n}\beta\lambda\tau\theta}{\delta(\beta\tau+\phi\omega)} \end{bmatrix} \tag{4.75}$$

$$F.H^{-1} = \begin{bmatrix} 0 & 0 & 0 & \frac{n\beta\lambda\tau\mu}{n\beta\tau\sigma+\delta\sigma\varphi\omega} \\ \frac{\kappa(n\beta\tau\alpha\delta\rho-\beta\varphi\kappa\lambda\rho+\delta\alpha\rho\varphi\omega)}{\delta\rho(n\beta\tau\alpha\delta-\beta\kappa\lambda\varphi+\alpha\delta\varphi\omega)} & \frac{\delta(n\beta\tau\alpha\delta\rho-\beta\varphi\kappa\lambda\rho+\delta\alpha\rho\varphi\omega)}{\delta\rho(n\beta\tau\alpha\delta-\beta\kappa\lambda\varphi+\alpha\delta\varphi\omega)} & \frac{\rho}{\varphi} & 0 \\ \frac{\kappa\varphi(n\beta\tau\alpha\delta\rho-\beta\varphi\kappa\lambda\rho+\delta\alpha\rho\varphi\omega)}{\delta\rho^2(n\beta\tau\alpha\delta-\beta\kappa\lambda\varphi+\alpha\delta\varphi\omega)} & \frac{\delta\varphi(n\beta\tau\alpha\delta\rho-\beta\varphi\kappa\lambda\rho+\delta\alpha\rho\varphi\omega)}{\delta\rho^2(n\beta\tau\alpha\delta-\beta\kappa\lambda\varphi+\alpha\delta\varphi\omega)} & 1 & 0 \\ 0 & 0 & 0 & \frac{n\beta\lambda\tau\theta}{n\beta\tau\delta\sigma+\delta\sigma\varphi\omega} \end{bmatrix} \tag{4.76}$$

The Eigen values of the system are

$$E_3 = \begin{bmatrix} 0 \\ 0 \\ \frac{n\beta\lambda\tau\theta}{n\beta\tau\delta\sigma+\delta\sigma\varphi\omega} \\ \frac{2n\beta\tau\alpha\delta^2\rho-\beta\delta\rho\kappa\lambda\varphi-\beta\varphi\kappa\lambda\delta\rho+2\alpha\delta^2\rho\varphi\omega}{n\beta\tau\alpha\delta-\beta\kappa\lambda\varphi+\alpha\delta\varphi\omega)} \end{bmatrix} \tag{4.77}$$

$$\frac{n\beta\lambda\tau\theta}{n\beta\tau\delta\sigma+\delta\sigma\varphi\omega} > 1 \tag{4.78}$$

from which it follows

$$n > \frac{\delta\phi\sigma\omega}{\beta\tau(\lambda\theta-\delta\sigma)} = \psi \tag{4.79}$$

With weak CTL and strong antibody responses the virus replicates at a higher rate that CTL responsiveness. This means that CTL induced pathology can set in when the number of viral variants reaches a certain threshold. As more variants continue to escape from the antibodies there will be a shift from antibody response dominance to the expanded CTL responses. The antibodies prevent pathology while CTL promotes it the level of pathology will continue to grow with diversification of mutants. When CTL attains maximum dominance relative to antibodies Liver pathology is expected to be at peak also . At this level viral evolution is expected to slow down because most of the target cells will have been infected and any more new variants will have too few cells to infect.

This maximum is calculated using the equilibrium points $S_{e6}, Y_{e6}, V_{e6}, A_{e6}$ and $T_{e6}$ as illustrated below.

$$F = \begin{bmatrix} \frac{\sigma\left(\frac{\alpha\lambda\theta\beta\kappa\sigma}{n\theta\alpha(\alpha\theta\omega+\beta\kappa\sigma)} - \frac{\delta\sigma}{n\theta}\right)}{n\theta} & 0 & 0 & \frac{\mu\sigma}{n\theta} \\ 0 & 0 & \frac{\kappa\rho\sigma}{n\theta\alpha} & 0 \\ 0 & 0 & \frac{\kappa\sigma\phi}{n\theta\alpha} & 0 \\ \frac{\theta\sigma\left(\frac{\alpha\lambda\theta\beta\kappa\sigma}{n\theta\alpha(\alpha\theta\omega+\beta\kappa\sigma)} - \frac{\delta\sigma}{n\theta}\right)}{n\theta\mu} & 0 & 0 & \frac{\theta\sigma}{n\theta} \end{bmatrix} \tag{4.80}$$

$$H = \begin{bmatrix} -\delta & \frac{\alpha\lambda\theta\beta}{\alpha\theta\omega+\beta\kappa\sigma} & 0 & 0 \\ \kappa & -\alpha & 0 & 0 \\ 0 & 0 & \tau & 0 \\ 0 & 0 & 0 & \sigma \end{bmatrix} \tag{4.81}$$

$$F^{-1}H = \begin{bmatrix} \frac{\sigma^2}{n\theta^2} & \frac{\alpha\lambda\theta\beta\sigma^2}{n\theta^2\alpha(\alpha\theta\omega+\beta\kappa\sigma)} & 0 & \frac{\mu}{n\theta} \\ 0 & 0 & \frac{\kappa\rho\sigma}{n\theta\alpha\tau} & 0 \\ 0 & 0 & \frac{\kappa\sigma\phi}{n\theta\alpha\tau} & 0 \\ \frac{\theta\sigma^2}{n\theta^2\mu} & \frac{\alpha\lambda\theta\beta\theta\sigma^2}{\alpha\alpha\theta\omega\mu n\theta^2+\alpha\beta\kappa\sigma\mu n\theta^2} & 0 & \frac{\theta}{n\theta} \end{bmatrix} \tag{4.82}$$

Whose Eigen values are

$$E_4 = \begin{bmatrix} 0 \\ 0 \\ \frac{\theta n\theta+\sigma^2}{n\theta^2} \\ \frac{\kappa\sigma\phi}{\alpha n\theta\tau} \end{bmatrix} \tag{4.83}$$

and found to be

$$n > \frac{\phi\kappa\sigma}{\alpha\theta\tau} = \zeta \tag{4.84}$$

## 4.2 Numerical Results

The model variables describe cell populations and therefore all of them are taken to be non negative, that is

$$S(t) > 0, Y(t) \geq 0, V(t) \geq 0, A(t) \geq 0 \,\&\, T(t) \geq 0$$

To make the analytical results discussed in section 4.1 clear, the model is used to simulate the interactions between the Cell mediated and antibody immunity against a viral pathogen. Data of HCV infection in human beings is used to simulate the results. Complete list of parameters and their estimated values used for numerical

simulations are given in Table 4.1. The majority of the values have been taken from the data found in scholarly articles published in various journals and others have been estimated. Much of these parameters were adopted from Kim et al. (2011) and Ahmed et al. (2011).

These data do not depict a strict situation of the entire patients range but the parameter range is within the plausible and realistic values.

## Table 4.1: Description of Variables

| Item | Parameter description | Symbol | Value | source |
|---|---|---|---|---|
| 1 | Proliferation rate Healthy cells | $\lambda$ | 1-10 | Ahmed et al. (2011) |
| 2 | Rate of Natural Loss of healthy hepatic cells | $\omega$ | 0.1 | Ahmed et al. (2011) |
| 3 | Rate at which Virus Infect Healthy cells | $\beta$ | 0.01-0.03 | Ahmed et al. (2011) |
| 4 | Death rate of infected hepatic cells | $\delta$ | 0.1-0.3 | Wodarz et al. (2006) |
| 5 | Rate at which CTL eliminate Infected cells | $\mu$ | 1 | Wodarz et al. (2006) |
| 6 | Proliferation rate of Virions from Infected cells | $\kappa$ | 1-2.5 | Wodarz et al. (2006) |
| 7 | Rate of Natural Loss of Virus | $\alpha$ | 1-5 | Wodarz et al. (2006) |
| 8 | Rate at which Antibodies Eliminate Virus | $\rho$ | 1-10 | Wodarz et al. (2006) |
| 9 | Rate of activation of Antibodies | $\phi$ | 0.1-2.5 | Kim et al. (2011) |
| 10 | Rate of natural Loss of the Antibodies | $\tau$ | 0.1-0.25 | Kim et al. (2011) |
| 11 | Rate at which Virus Induce CTL Proliferation | $\theta$ | 0.01-4.5 | Kim et al. (2011) |
| 12 | Rate of natural Loss of CTL population | $\sigma$ | 0.1-0.2 | Kim et al. (2011) |
| 13 | Threshold antibody response | $\chi$ | 0.1944-2.5 | Calculated |
| 14 | Threshold CTL response | $\eta$ | 0.2125-11.3 | calculated |
| 15 | Minimum viral variants that induce pathology | $\psi$ | 3.2 | calculated |
| 16 | Maximum CTL dominance | $\xi$ | 10 | calculated |

### 4.2.1 Infection free Dynamics

The healthy target cell population is at the disease-free equilibrium value and the number of infected cells is zero. The initial values are zeros, except for the supply of healthy cell, representing a state at of no infection

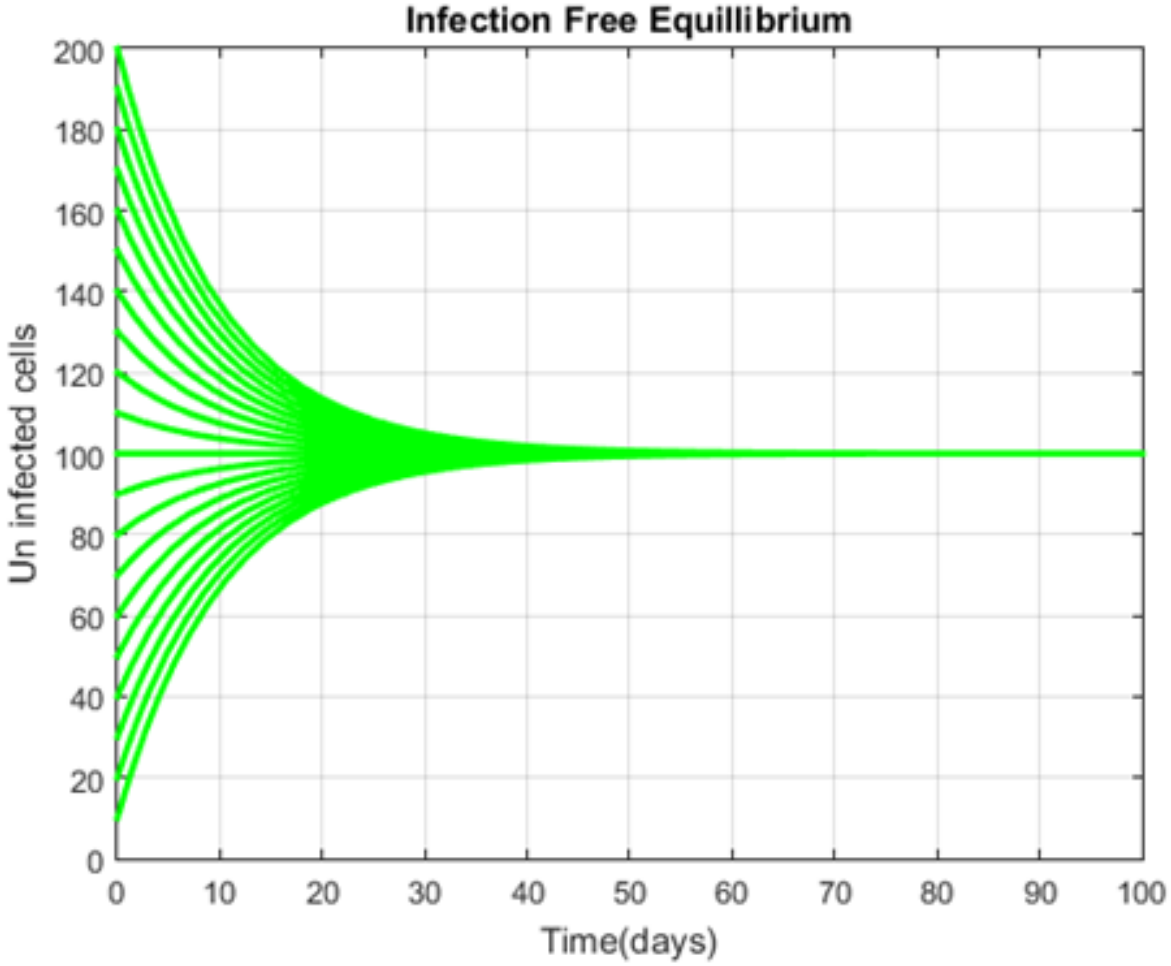


Figure 4.2: *Infection free state of the immune system*

*The following parameters are used:*

$$\lambda = 8,\ \omega = 0.1,\ \beta = 0.0,\ \delta = 0.,\ \mu = 0.0,\ \kappa = 0,\ \alpha = 0.0,\ \rho = 0.0,\ \varepsilon = 0.00,$$

$$\phi = 0,\ \tau = 0.0,\ \upsilon = 0.0,\ \theta = 0,\ \sigma = 0.0$$

*The initial conditions considered are*

$$S(0) = 10,\ Y(0) = 0.0,\ V(0) = 0,\ A(0) = 0.0,\ T(0) = 0.0$$

### 4.2.2 Immunity Free dynamics

The disorders of the immune system lowers is effectiveness in eliminating infective pathogens. These disorders could be due to immune over activity or deficiency.Over activity causes the body to attach its own tissue leading to auto immune diseases while Immune deficiency lowers the ability to defend the body against infectious agents unduly making the body susceptible to infections. A case of immune deficiency is depicted below when the body is attacked by a non-cytopathic virus

*The values of parameters used are:*

$$\lambda = 10,\ \omega = 0.1,\ \beta = 0.01,\ \delta = 0.1,\ \mu = 0.0,\ \kappa = 1.0,\ \alpha = 1.0,\ \rho =$$

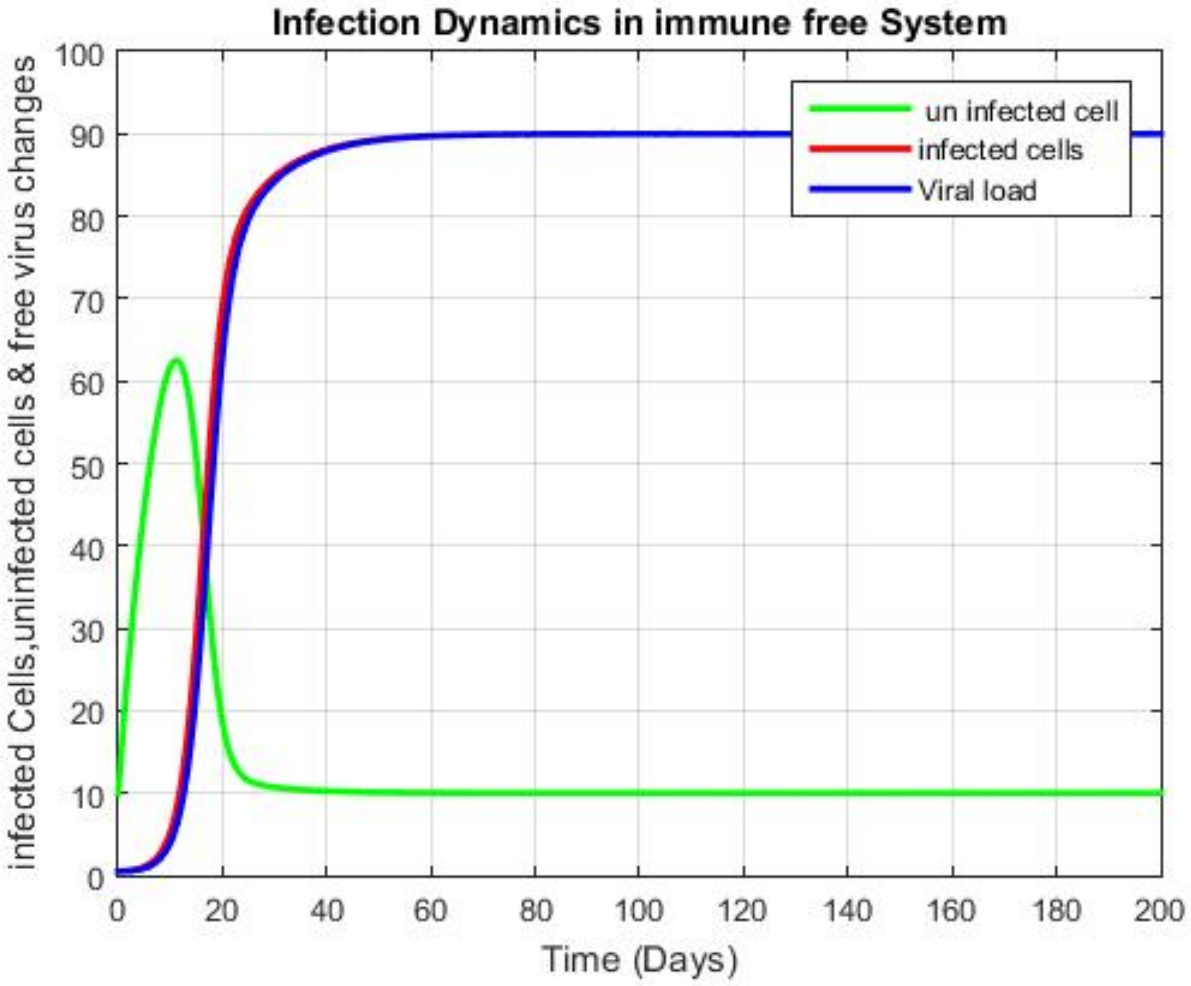


Figure 4.3: *Immunity free state of the immune system*

$$0.0,\ \varepsilon = 0.00,\ \phi = 0,\ \tau = 0.0,$$

$$\upsilon = 0.0,\ \theta = 0,\ \sigma = 0.0$$

*The initial conditions considered are:*

$$S(0) = 10,\ Y(0) = 0.5,\ V(0) = 0.5,\ A(0) = 0.0,\ T(0) = 0.0$$

This represents a high viral load meaning that the body is not able to defend itself against. Since HCV is non-cytopathic and death of infected cells is almost the same

as the death of un infected cells,in the acute infection no pathology is expected.This would however be different for a highly cytopathic virus.

### 4.2.3 Competition dynamics

In the event that immune response will mount, either CMI or humoral, the following conditions must be met; $\phi V(0) > \tau$ and $\theta T(0) > \sigma$.

This guarantees development of immune response but not dominance of any branch of immunity. The initial values are small, representing a state at the initiation of infection, but they are also chosen to be large enough so that there will be an immune responses, either through the CTL or the antibodies, or both.

Three possible outcomes of this are expected and are observed in HCV infection. Each case is discussed below.

#### 4.2.3.1 Case One: Strong CTL only Immune response

Since the CTL performs antiviral activities, strong responses will cause viral load to decrease and resolution of the virus. After the resolution, the stimulation of CTL decay and settle at an elevated level compared to where it had begun and remains at this level for a while in absence of exposure to the same virus.This is known as the contraction phase. This is referred to as the immunological memory and is observed in cellular and humoral immunity. This means that the remaining CTL can respond more efficiently and with grater efficacy in the case of re-challenge by the same virus. Such re-challenge is suppressed and resolved faster than in the first exposure.

The model approximates clearance of the virus between eighth and tenth month. This

is supported by the work Grebely et al. (2012) that spontaneous viral clearance occurs within twelve months , no case of spontaneous clearance is reported after this period.

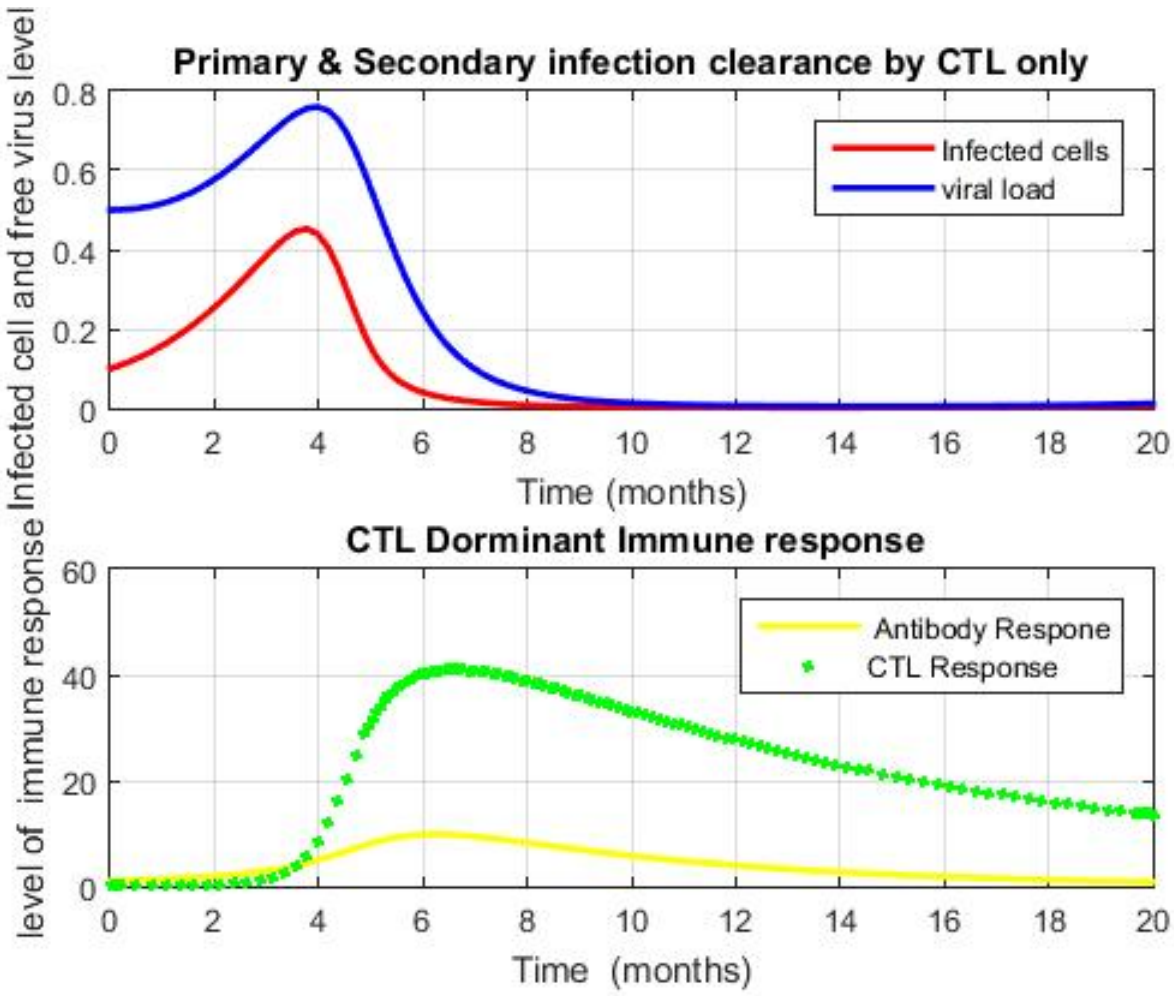


Figure 4.4: *CTL Dominant immune response .The lytic activity of CTL resolve the viral infection. On decay it settles around heightened level in the long-term.*

*Parameters are taken as follows:*

$$\lambda = 10,\ \omega = 0.1,\ \beta = 0.01,\ \delta = 0.1,\ \mu = 0.1,\ \kappa = 1,\ \alpha = 1,$$

$$\rho = 0.1,\ \varepsilon = 0.0,\ \phi = 1,\ \tau = 0.25,$$

$$\upsilon = 0.0,\ \theta = 4.5\ \sigma = 0.1$$

*The initial conditions considered are*

$$S(0) = 10,\ Y(0) = 0.1,\ V(0) = 0.5,\ A(0) = 1,\ T(0) = 0.1,$$

$$\chi = 0.22 < 0.25, \eta = 11.13 > 0.1$$

Before the first exposure to the virus the host *naive.* to the infection.At the naive phase the level of immune effector cells is generally low. After infection the naive cells are stimulated to become effector cells to fight the virus, and subsequently settles in a relatively heightened stable level than in the a host that had not encountered the virus. These cells remains for a longer time after virus resolution.When the host is infected again with the same virus their will be a standing stock of virus specific CTL with greater efficacy ready to fight the virus. As a result the resolution is faster.(fig 4.5).

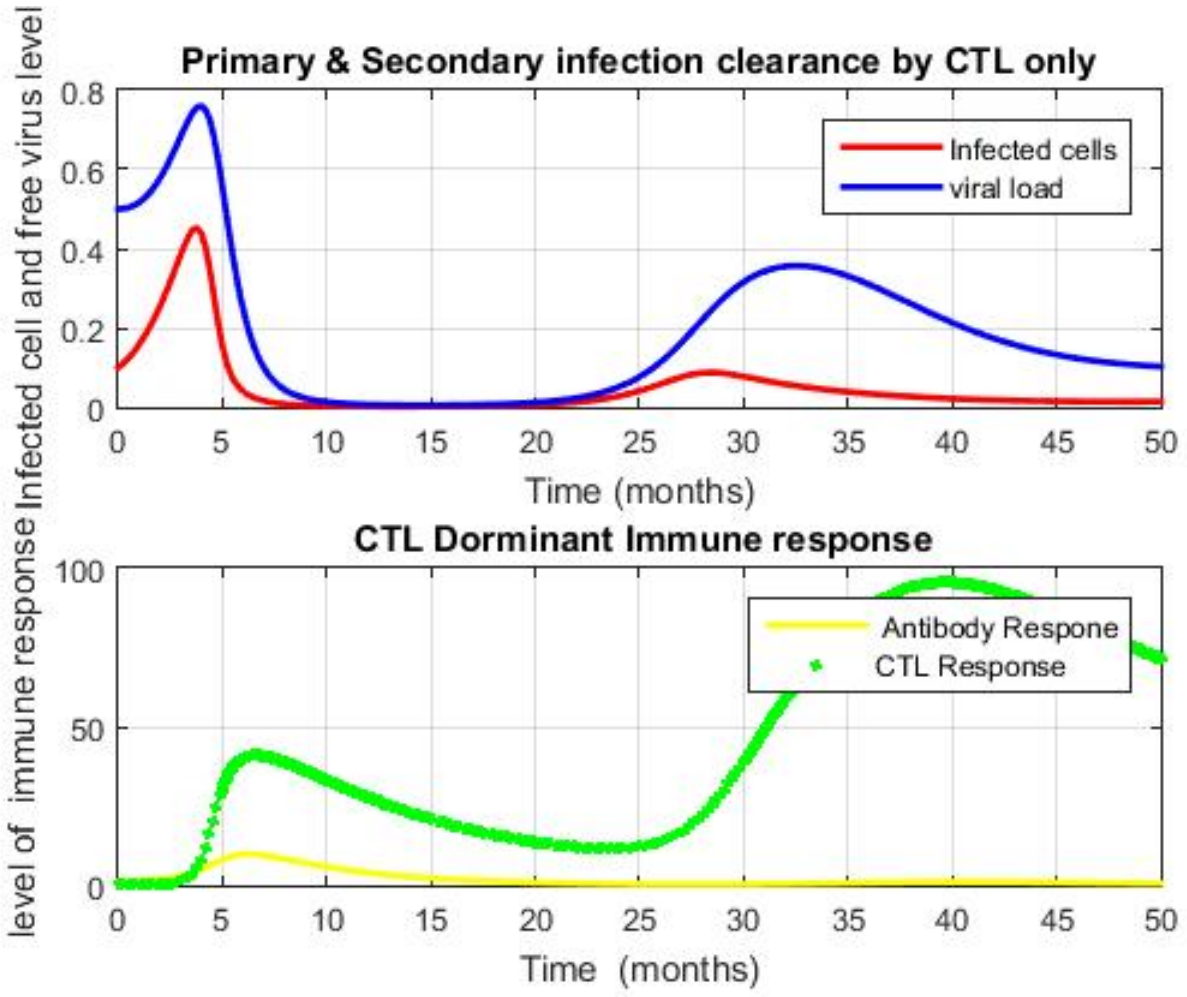


Figure 4.5: *Due to some standing stock OF immunological memory CTL specific to the virus mounts faster and resolves the secondary infection*

#### 4.2.3.2 Case Two: Strong Antibody only Immune Response

Antibodies prevents the rate of virus spread by neutralizing the free virus in the extracellular phase and without killing the infected cells. Antibodies contribute significantly to non lytic activity . As a result the virus continue to proliferate despite the antibody immunity, since the source of the virus (infected cells) still remains active. In the case the CTL response is diminished and un sustained, the host does not clear the virus leading to chronic infection and progression to cirrhosis in 5 - 10 % of

individuals within 20 years Grebely et al. (2012).

Since neutralizing pathogens or changed cells is one of the most important tasks of antibodies they attach directly to the surface of a virus and stop the virus from attaching itself to a normal body cell and subsequently infecting it. These substances can then no longer enter the host cells to damage them. The antibodies however have no ability to control the virus once inside the cell. This is demonstrated by the simulation results below (fig 4.6).

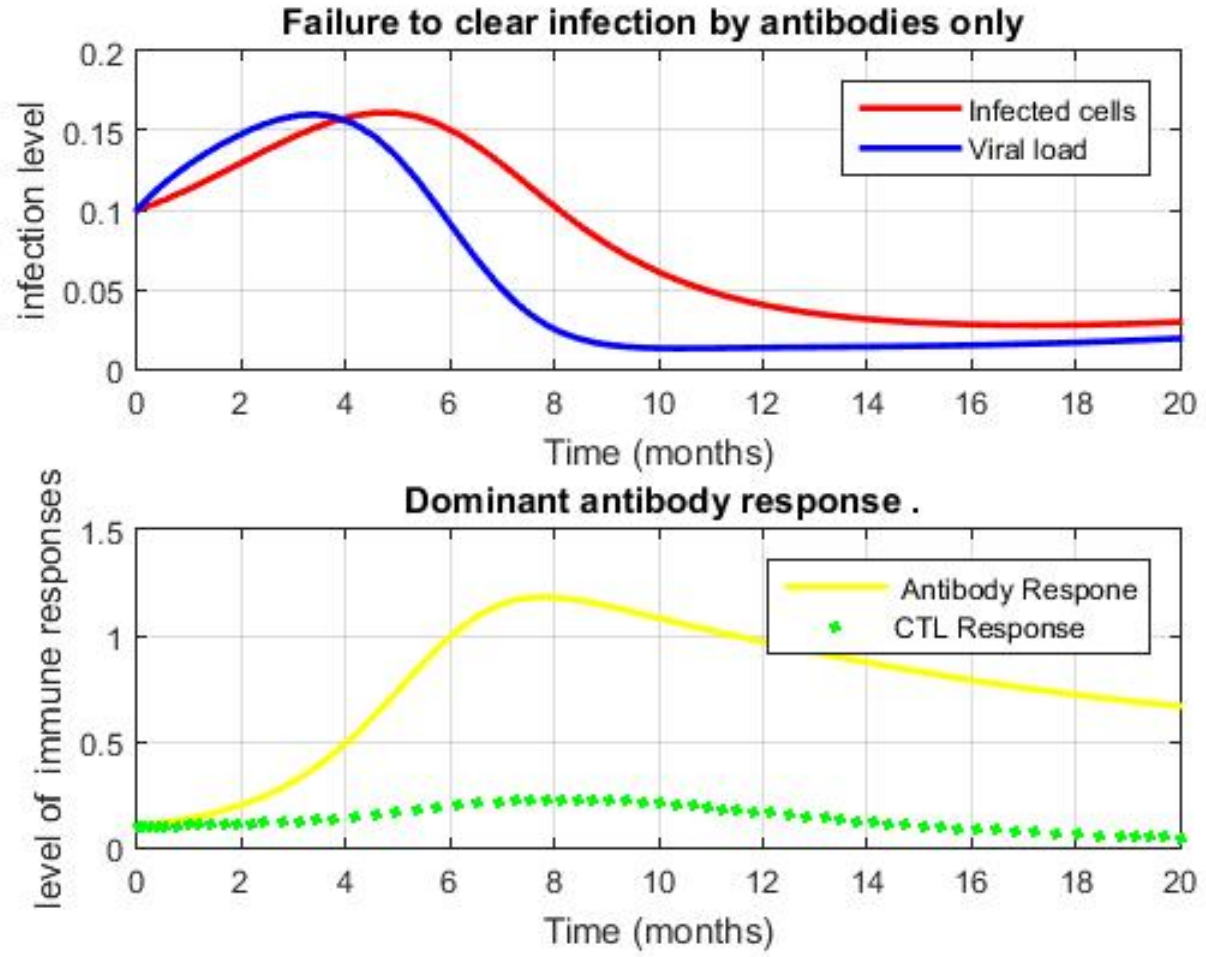


Figure 4.6: *Antibody Dominant immune response*

*The choice of parameters is as given below:*

$$\lambda = 1,\ \omega = 0.1,\ \beta = 0.03,\ \delta = 0.1,\ \mu = 1,\ \kappa = 1.5,\ \alpha = 1.0,$$

$$\rho = 1.2,\ \varepsilon = 0.0,\ \phi = 3.5,\ \tau = 0.1,\ \upsilon = 0.0,\ \theta = 2.5,\ \sigma = 0.24$$

*The initial conditions considered are*

$$S(0) = 10,\ Y(0) = 0.1,\ V(0) = 0.1,\ A(0) = 0.1,\ T(0) = 0.1, \chi = 2.5 > 0.1,$$

$$\eta = 0.2125 < 0.24$$

#### 4.2.3.3 Case Three:Strong CTL and strong Antibody Responses

Both cellular and humoral immunities mount relatively strong leading to clearance of the viral pathogen. It is worthy noting that when the host immune system mount both cellular and humoral responses strongly the virus is cleared much earlier that when the immune system mounts strong cellular immunity only.

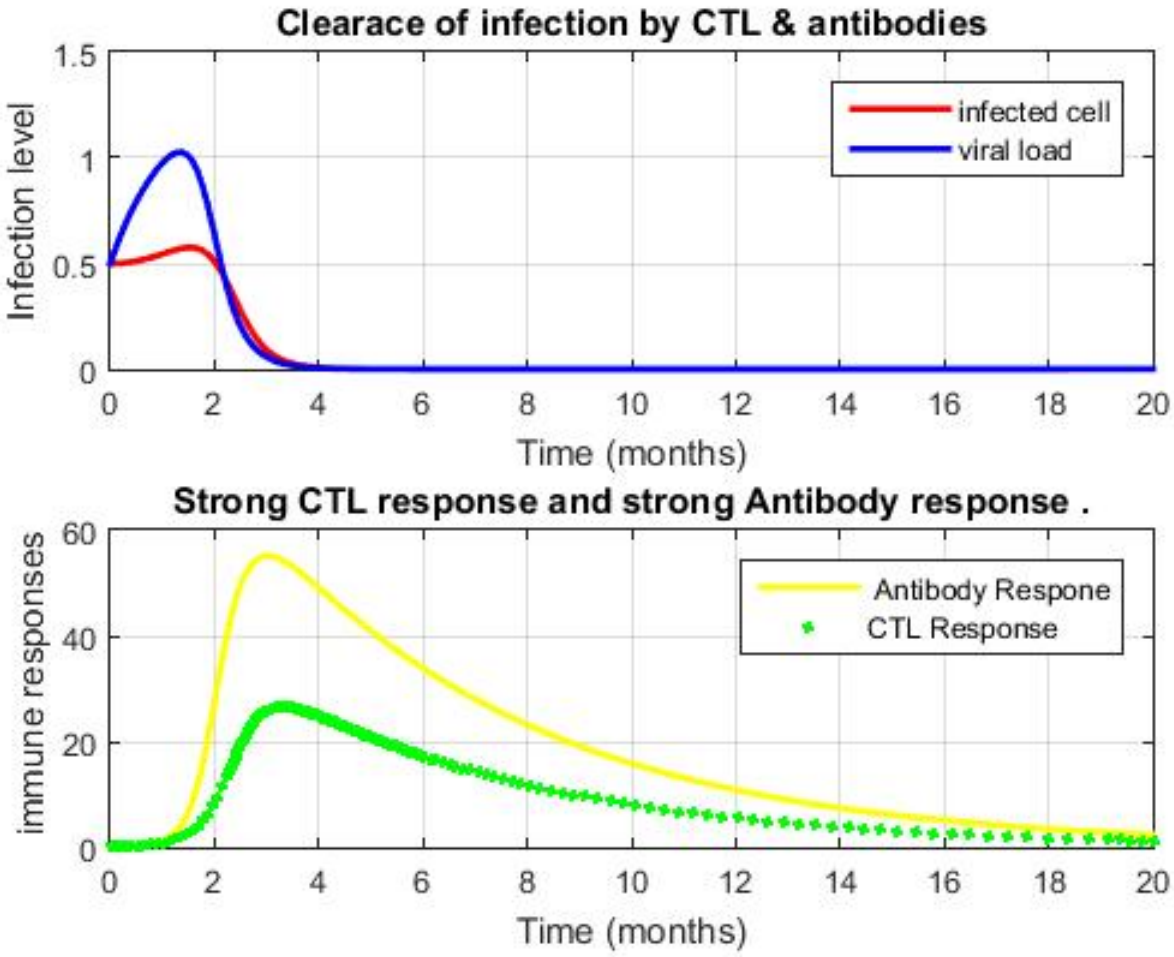


Figure 4.7: *strong CTL and Antibody responses*

*The parameters are chosen as follows:*

$$\lambda = 10,\ \omega = 0.1,\ \beta = 0.01,\ \delta = 0.1,$$

$$\mu = 0.1,\ \kappa = 1,\ \alpha = 1.0,\ \rho = 0.1,\ \varepsilon = 0.0,\ \phi = 3.5,\ \tau = 0.2,$$

$$\upsilon = 0.0,\ \theta = 3.5,\ \sigma = 0.2$$

*The initial conditions considered are*

$$S(0) = 10,\ Y(0) = 0.5,\ V(0) = 1,\ A(0) = 0.13,\ T(0) = 0.13, \chi = 0.1944 > 0.1,$$

$$\eta = 1.2821 > 0.2$$

### 4.2.4 Viral Evolution in Chronic HCV

When the CTL response is weak and unable to clear the virus and the antibody responses are strong, the virus will evolve to evade the antibody response.This evolution causes production of new viral variant. In response to this evolution the antibody responses will also expand to neutralize these new variants leading to a repertoire of immune responses. According to Jirillo (2007) six major genotypes of HCV have been identified.

When the condition,

$$n > \frac{\delta\phi\sigma\omega}{\beta\tau(\lambda\theta - \delta\sigma)} = \psi \tag{4.85}$$

is satisfied the CTL response increase and elevate to dominance driving antibody responses to near extinction.That is for the initially weak CTL responsiveness , the accumulation of variants that have evaded antibody responses creates diversification of antigens which gradually stimulate the CTL. Thompson et al. (1997).

Five variants are considered for this illustration. The outcome of viral evolution with respect to the number of viral mutants that escape the antibodies is plotted below.

CTL-induced pathology is expected,the evolution will stop when substantially many cells of the liver are infected. This is because there will be but just a few cells of the liver that are purely susceptible and a gain the liver will be substantially damaged, Fig 4.9..

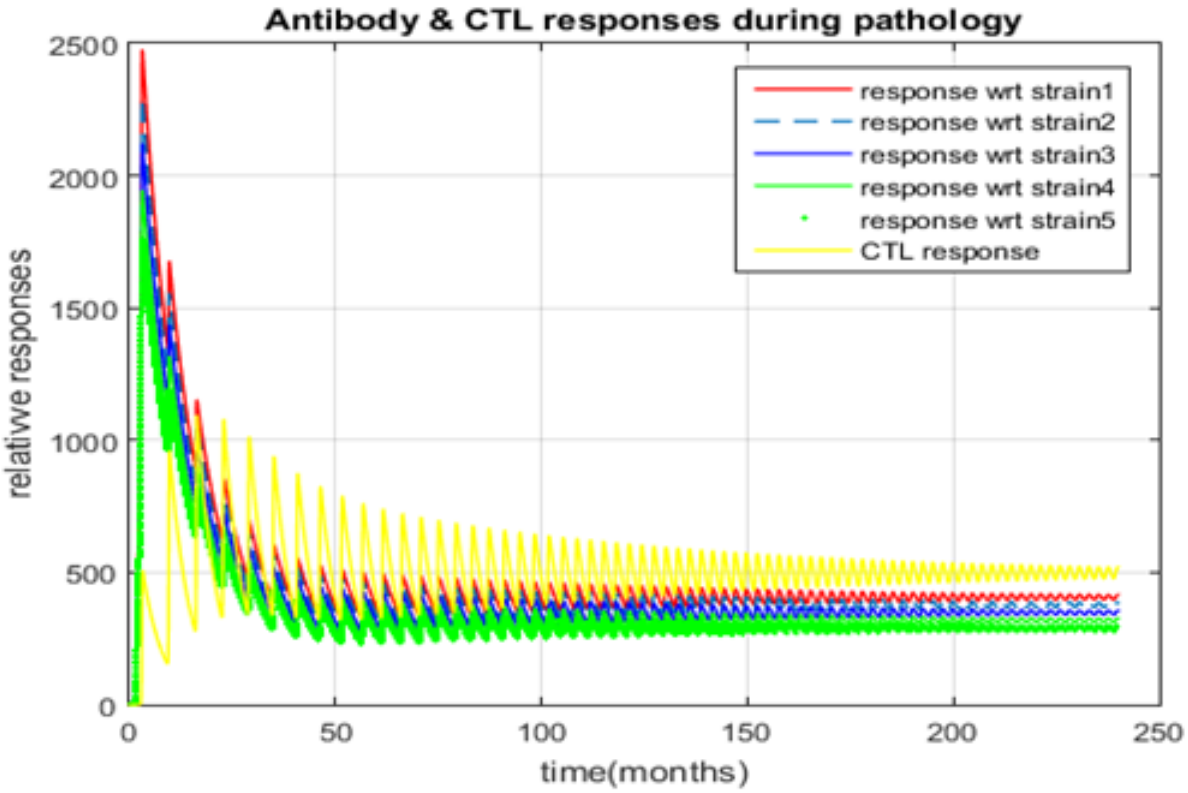


Figure 4.8: *With increased antigenic diversity the initially weak CTL gradually begin to grow.*

*The initial conditions considered are*

$$S(0) = 10,\ Y_1(0) = 0.1, Y_2(0) = 0.2, Y_3(0) = 0.3, Y_4(0) = 0.4, Y_5(0) = 0.5,\ V_1(0) = 0.1,$$

$$V_2(0) = 0.1, V_3(0) = 0.1, V_4(0) = 0.1, V_5(0) = 0.1,\ A_1(0) = 0.1, A_2(0) = 0.1,$$

$$A_3(0) = 0.1, A_4(0) = 0.1, A_5(0) = 0.1,\ T(0) = 0.2\ ,\ \psi = 3.2 < 5$$

*The parameters used are :*

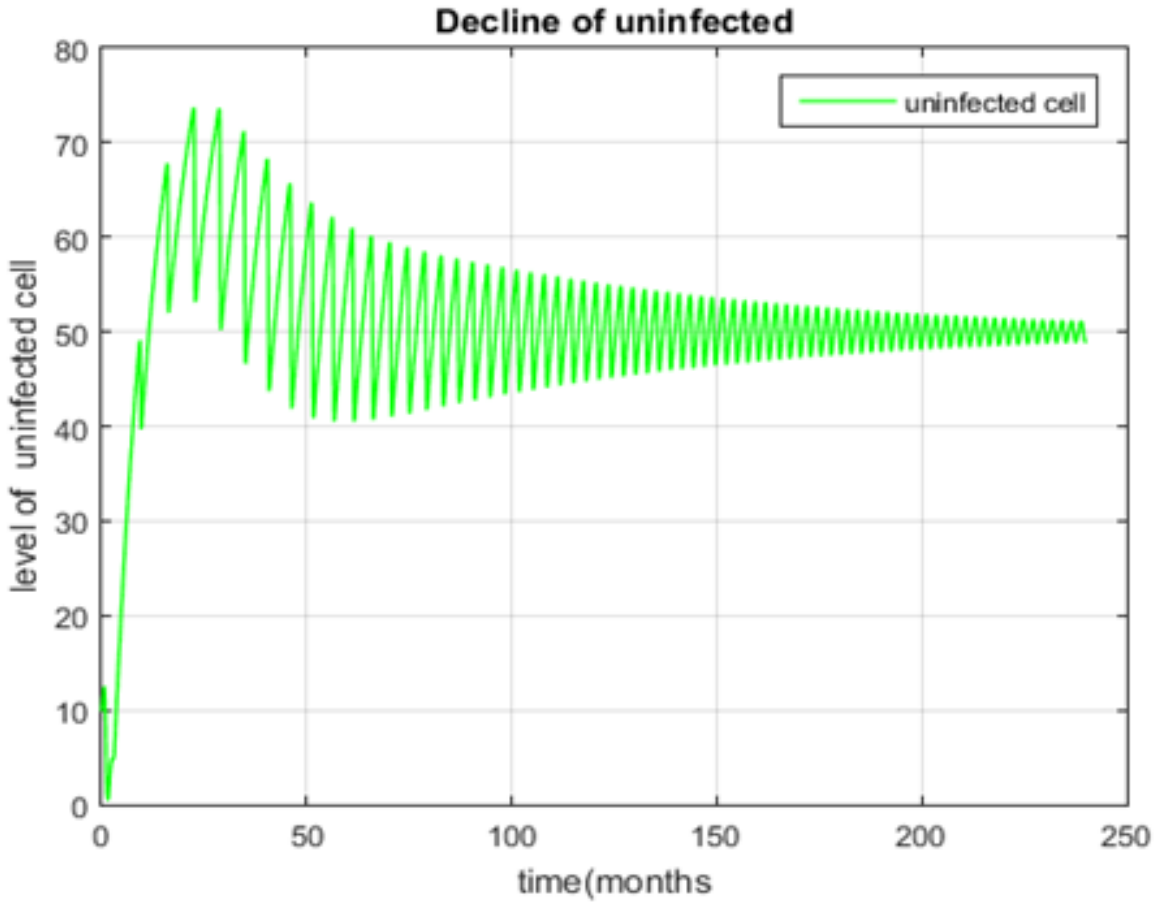


Figure 4.9: *With the setting in of severe pathology the number of uninfected cells will oscillate towards equilibrium and then start to decrease*

$$\lambda = 1,\ \omega = 0.1,\ \beta = 0.03,\ \delta = 0.3\ \mu = 2,\ \kappa = 2.5,\ \alpha = 1.0,\ \rho = 1,$$

$$\varepsilon = 0.0,\ \phi = 2,\ \tau = 0.1,\ \upsilon = 0.0,\ \theta = 0.1,\ \sigma = 0.2$$

The level of pathology is determined by the number of viral mutants.This gradual escaped will eventually stimulate the CTL shifting the balance from humoral to CIM. Since humoral responses hinder pathology and CTL promote it, the level of liver

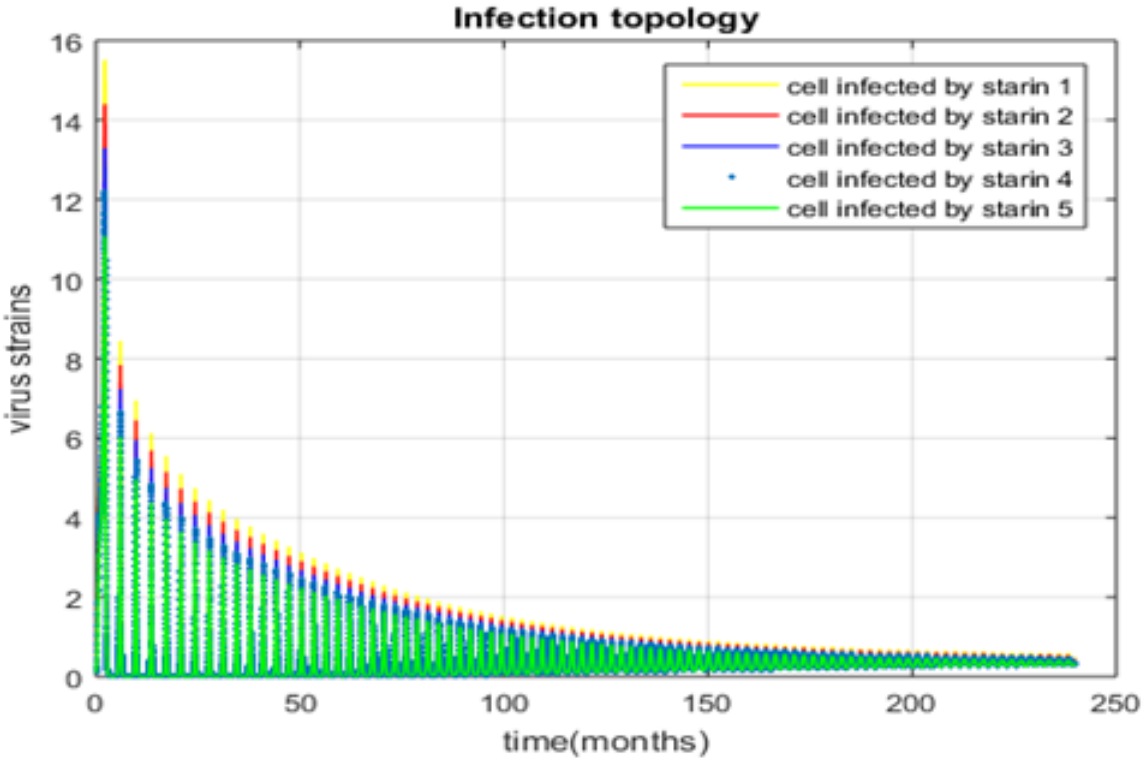


Figure 4.10: *The overall number of infected cells decline due to the Cytotoxic activity of CTL* .

physical harm escalate with the increase of viral variants. When the number of the variants reaches ascertain number, the CMI response will dominate antibody responses when $n > \frac{\varphi\kappa\sigma}{\alpha\theta\tau} = \xi$.

The dynamics of CMI can be explained as competition of interaction of CTL and Antibody responses. This competition has been proved experimentally Wodarz (2003) ) Coexistence of CTL and antibody responses is possible when their respective relative response rates are close.

## 4.3 Results and Discussion

### 4.3.1 Introduction

With the help of the mathematical model,a study of different developmental pathways of the immune response to HCV and conditions in which each of them can explain the biological data is carried out. Such a scheme is particularly appropriate for use in human studies, where data is sparse and several sources of uncertainty and noise have an influence on parameter values. In order to keep the model tractable, several simplifying assumptions have been made as outlined in section 3.7.1

In an effort to keep the model simple, a five-phase deterministic model (Eq. (3.19) to Eq. (3.23)) that describes the way immune system responds to viral pathogen stimulus was developed. The model describes the immune system where the size of immune responses is proportional to the number of pre-existing immune cells and viral load.

### 4.3.2 Results And Discussion

The mathematical model developed in section 3.7 is based on simplification of the complex dynamics of the immune response to viral pathogens as diagrammed in the model flow chart. The model is a system of five differential equations that describe the dynamics of viral pathogen and its interaction with a target cell population as well as the subsequent immune response to the pathogen. The Law of Mass Action or the usual type of qualitative behavior observed in population interactions and chemical reactions has been used to provide a basis for constructing the model equations.The

model examines the ability of the immune cell and viral pathogens populations growing from low numbers.

In the absence of any pathogen the immune system is always at equilibrium $S = \frac{\lambda}{\omega}$ fig 4.2, there is neither the stimulation of CTL nor antibodies. This is the scenario where the pathogen has not invaded or has suffered extinction and every cell in the population is susceptible. The pathogen-free equilibrium is stable whenever $R_0 < 1$. This makes good sense because the pathogen cannot invade if each infected host cell passes on the infection to fewer than one other host cell. In the immune free scenario, the CTL response is extinct, and the virus replicates at high levels this could be an indication of CTL exhaustion. CTL exhaustion implies that the lytic activity of CTL is diminished and the virus can now replicate persistently unchecked and at elevated rate. Exhaustion or functional impairment of the T cell compartment hampers T cell immunity. Loss of functional capacity, like cytotoxicity, cytokine production and proliferative capacity, is thought to reflect prolonged excessive immune inactivation and to correlate with pathogen progression.

Immunodeficiency diseases and syndromes could cause inactivation of antibody response caused by either by pathological conditions that affect the immune system or by the administration of therapeutic compounds with immuno suppressive effects. Because HCV is non-cytotoxic,the aggregate tissue cells is assumed to be unchanged for a while, and no pathology will be observed, fig 4.3.

The CMI and antibody mediated responses require stimulation by antigen to mount.They can therefore be considered to be in competition where the antigen is the common resource. The outcome of this competition therefore depends on the

interplay between these two branches. When CTL drive antibodies responses to extinction , then the interplay is shifted towards lytic responses and the outcome is possible resolution of the virus. The model estimates that the HCV will be cleared in six to eight months, fig 4.4. the host is also likely to clear the infection in case of a secondary re-challenge fig 4.5.

When antibodies drive CTL responses to extinction , then the interplay is shifted towards non-lytic immunity, the result is persistent, chronic and possible viral evolution despite the presence of an ongoing antibody response. Fig 4.6. The dominance of antibody mediated branch of immunity in fighting a viral pathogen lead to viral evolution that result in emergence of new viral mutants.

When CTL and antibody responses are both strong and become sufficiently established,the two branches coexist and jointly fight the viral pathogen, the outcome is virus clearance. The model suggests that it would take a shorter period to clear the infection compared to when CTL respond to the virus only. It is estimated to take less than four months ,fig 4.7.

Viral evolution is also considered and a discussion on its influence in changing the immune and disease dynamics from disease free to liver pathology has been given. Weak and unestablished CTL responses give way for the dominance of antibody responses. As a result, there is persistent replication and new variants evolve that escape the antibodies. Each new variant leads to generation of a specific antibody response. The number of viral mutants increase with time. fig 4.8. The antibody response equally diversify with respect to these new viral mutants by producing new antibody responses specific to them . As the diversity of the viral mutants expand, the

initially weak CTL response begin to expand. However, this late CTL expansion does not significantly contribute to viral clearance .This will mark the genesis of liver pathology.The CTL lytic activity against infected cell at this stage will now contribute to tissue damage and the total number of liver cells is expected to decline which will correspond to the wasting away leading to the eventual death of hosts Fig 4.9.

Among the many infections that exemplify competition dynamics between CMI and humoral branches of immunity is HCV. Each branch is important in its own right in determining the short term and long term outcome of the infection.

A mathematical model on immune responses with respect to HCV is critical in understanding the experimental data of the infection. CTL-mediated clearance is associated with the relative absence of strong antibody responses. Persistence and chronicity are however associated with strong and dominant antibody responses. The argument presented here is that CMI is responsible for viral clearance . On the other hand, antibody responses are crucial for deciding the long term outcome of infection. Antigenic escape from antibody responses allows the virus to persist. Continued infection in the presence of an immune response creates a conducive survival tactic for the virus to mutate and escape the antibody responses.

With the establishment of the infection, the model suggests that viral mutants are responsible of shifting the balance from humoral to CMI.

The relative balance of humoral and CMI determines whether the HCV will be cleared and whether persistence,chronicity and liver pathology will be observed.It is suggested here that for chronic and persistent HCV patients the therapy should be directed more to controlling of antigenic mutants and suppression of CTL rather than

clearance of the virus. The central concept is that viral evolution is the major drive to chronic infection.

If immune responses were to be suppressed,the competition between the various mutants determine the outcome of evolution. Moreover, as the number of susceptible cells will become factor in this scenario limiting, for example when the liver vastly infected the rate of viral evolution may slow down or stop because of the reduced number of susceptible target cell.Therefore, if viral evolution lead to liver pathology, patients at advanced stages may exhibit a lower rate of viral diversification as compared with those in less advanced stages.

The concept presented in this study of viral evolution is supported by Wodarz (2003).This has also been observed in patients who have undergone liver transplants.

It is suggested in this model that new viral variants can always stimulate new antibody responses that are specific to them. With this the virus will continue to mutate in order to evade these specific antibody responses. With the infection progression, there will come a time when the antibody responses are unable to cope with the viral population. This lead to reduced rate of viral evolution , an observation common with chronic HCV patients.

Elimination of virus infected cells by lysis in the main way CMI eradicates the viral infection. A role of CMI in controlling of HCV replication is suggested by the temporal correlation among the detection of CTLs, a rise in transaminase levels, and a fall in viremia Grakoui et al. (2003).

## 4.4 Model limitations

Despite the robustness of the model, its effectiveness in describing CMI immune responses and how well it is able to predict HCV infection progression , there are a few aspects of the immune system that can not be deduced from the model. They include

1. The spatial distribution of the viral infection in the host's organ can not be determined.This distribution of infection is important in determining the portion of the organ infected or not infected.

2. The delay aspects observed in most biological systems can not be deduced from the model. There the effect of these delays therefore cannot be understood from the model

## 4.5 Experimental Data on HCV Infection

Models are vital tools in helping us understand and interpret experimental data. Several experiments on the role of the antibody and CTL responses in HCV infection have been conducted using chimpanzee as a model animal as well as humans. However most of the experimental work has remained a challenge mainly because of ethical issues, lack of another suitable small animal model and partly because HCV infection can be asymptomatic and those infected may be unaware until the late stages of infection. This makes early monitoring of the infection difficult Grakoui et al. (2003). However experiments have provided useful insights on how the immune

system interact with HCV either to eliminate it from the body or to coexist together. Experimental results have confirmed that CMI is critical in the resolution of HCV while antibodies determine the long term outcome of the infection, if it is not resolved, in humans and chimpanzees Experimentally both humans and chimpanzees that cleared the infection were seen to have strong and sustained CTL responses that lasted beyond the acute phase. The chimpanzees that cleared the infection were observed to have undetectable antibody responses. This observation suggests to mean that the two branches of immunity are in competition and CTL responses will have driven the antibody responses to extinction Oniankitan et al. (2004).The results are in agreement with the simulated results for strong CTL and weak antibody responses HCV-specific antibodies have been found to be effective in blocking *invitro* infection of target cells by HCV. However, humans and chimpanzees, naturally acquired anti-HCV antibodies generated during this infection were found to be ineffective in protecting secondary challenge and spread of HCV Scarselli et al. (2002).

Humans and chimpanzees that did not resolve the infection were observed to develop strong and sustained antibody responses and diminished and sustained CTL responses. This led to persistent and chronic infection. It has been shown that those who resolved the infection did show viral evolution status in the virus population. However, in both humans and chimpanzees those who did not resolve the infection and developed persistent and chronic infection were observed to have antigenic diversity in the virus population, which is and indicator of presence of viral mutant that have escaped the antibody responses. Bartosch and Cosset (2006).Failure to clear the infection lead to persistence and chronicity, a phenomena associated with weak

CTL responses. Persistence lead to viral evolution. Experimentally it has been shown that viral evolution is a consequence of viral persistence and it is not the viral evolution that lead to persistence. This is what is assume in the model and results confirmed by the simulations. Though strong antibody and weak CTL responses have been identified as the suitable scenario for viral persistence, it does not mean that the antibody responses are not important in HCV infection Bowen and Walker (2005). According to ,CTL responses against HCV can be divided into three phases:

1. This is in the first few weeks after exposure and infection. The virus titers escalate to high level CTL, HCV specific immune responses are activated. However, among the most remarkable observations that came out of studies investigating the kinetics of HCV infection in both chimpanzees and humans is that these responses are not detected in the blood before 1-3 months after initial infection Bowen and Walker (2005). The median time for the development of a IF-$\gamma$ response is 33 days. The reasons for this delay are not yet understood

2. The second phase is where there is transient acute infection lasting a few weeks. Infected individuals may develop acute hepatitis irrespective of the outcome of infection, whether the infection will be cleared or will be chronic. These responses have been shown to peak between 180-360 days after initial infection.

3. The last phase depends on the outcome of the disease.In about 30% of infected individuals who resolve the infection their is retention a stock of HCV specific CTL cells.In approximately 70% of HCV infections, infection becomes

chronic. This phase is usually characterized by an absent or almost undetectable HCV-specific CTL response.

Experimental studies have shown that antibody responses are responsible for keeping the patient asymptomatic and escape from the antibodies is observed to contribute to the shifting of the immune responses from antibody to CTL. Therefore, the balance between these immune responses determine the ultimate outcome of the infection Neumann-Haefelin and Thimme (2013). Other experiments have quantified the rate of viral evolution in humans and chimpanzees with severe liver disease. The rate of viral evolution and antigenic diversity have shown that if the viral load is suppressed sufficiently by the immune responses this would lower the rate of evolution. But this suppression also allows the coexistence of various antigenic variants. If the immune responses are sufficiently suppressed competition between the various viral mutants determine the rate of viral evolution. For patients with mild liver disease there was constant accumulation of amino acid changing substitutes and a higher rate of viral evolution. Patients with severe liver disease were observed to have lower viral replication rate. This is consistent with the theory presented in this study that at maximum pathology the number of susceptible cells will be few hampering further viral evolution. This was supported by the studies of Neumann-Haefelin et al. (2005)who observed that virus diversity as well as amino acids changing to silent substitutions was higher in humans and chimpanzees with severe liver disease as well as patients who had liver transplant as a result of severe liver disease .

CMI has been investigated extensively in HCV infections. This has been facilitated by the availability of MHC class I tetramers. Studies in infected chimpanzees and humans using both functional methods of CTL identification and MHC class I tetramers have demonstrated that increases in serum transaminase levels and clearance of the virus during the acute phase are generally associated with the emergence of a strong CTL response in the blood and the liver 1 to 3 months after infection Neumann-Haefelin and Thimme (2013). According to Appay et al. (2002) Up to 8% of the blood CD8+ T cells can be specific for a single HCV epitope at the peak of the acute response.

In addition, recent studies using HCV pseudotyped particles indicate that neutralizing anti-HCV antibodies occur far more commonly in persistently infected individuals than in those who clear the virus,Meunier et al. (2005).

Experiments based on IFN-$\gamma$ production have indicated that CTL activities were not detected in the blood of chronically infected patients although CTL clones specific for HCV can be derived from the liver of humans and chimpanzees in which the virus persists and chronic Lanford et al. (2001). The presence of anti-HCV-specific CTLs have been confirmed using MHC class I tetramers. This powerful immunological tool has demonstrated that the liver contains a higher frequency of HCV-specific T cells than the blood Neumann-Haefelin et al. (2005), with HCV-specific T cells enriched within the liver up to 10- to 30-fold for CD8+ T cells, and 2-fold for CD4+ T cells Neumann-Haefelin et al. (2005). Experiment by Bowen and Walker (2005) to investigate the immune responses during HCV infection give similar results as the results obtained by simulation.

## 4.6 Model Validation

Using the experiment parameters giveN in table 4.1 ,the experimental results were compared with the simulation results for a period of twelve months and the following observations were made:

1. The two results show that the virus will initially grow exponentially before reaching the peak in the fifth month,see fig 4.11.However the peak level of the experimental data is slightly higher that the one obtained by simulation

2. There will be an exponential decay, which will not go to zero in twelve months, after the peak.

3. The CTL/CD8+ levels will continue to rise for the twelve months, see fig 4.12.

4. The simulated results show that CTL level is slightly higher that the experiment level

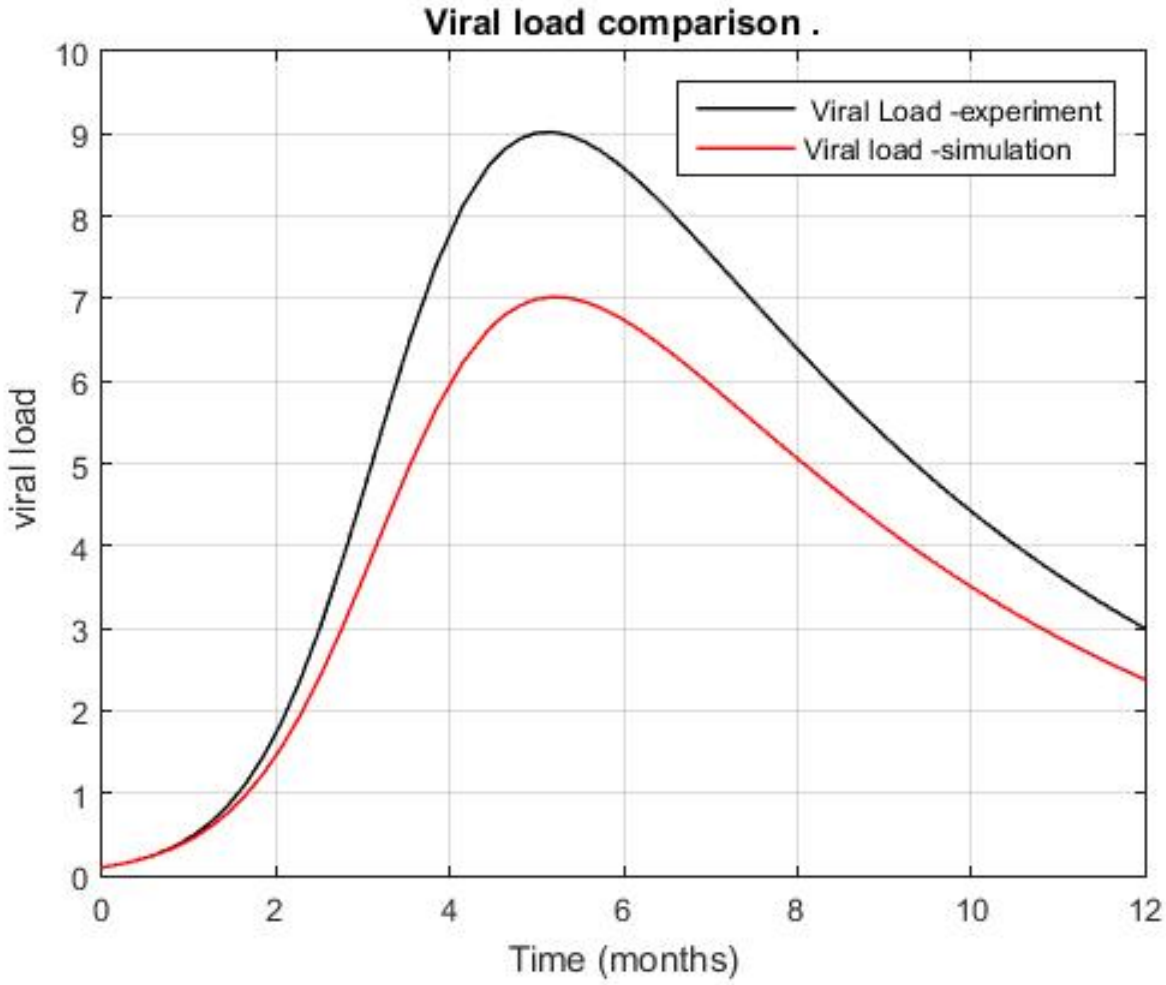


Figure 4.11: *The viral load grow exponentially before reaching the peak in the fifth month.*

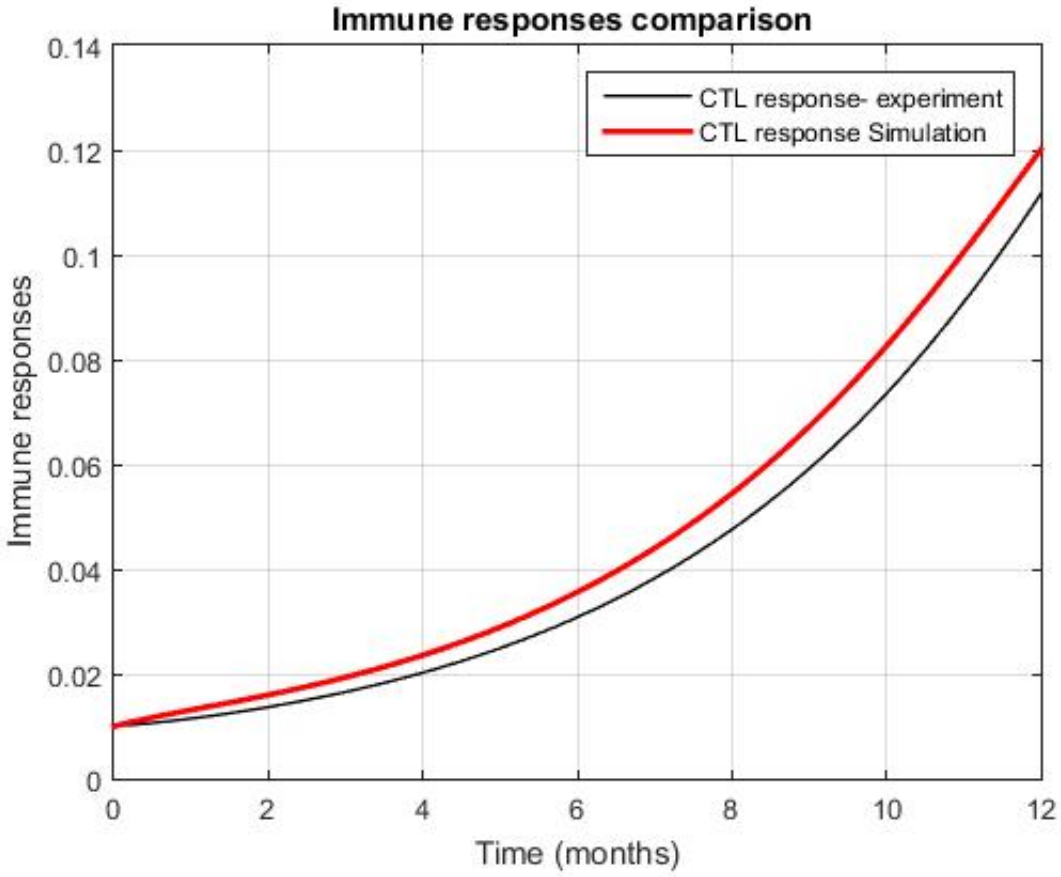


Figure 4.12: *The comparison of immune responses between experimental results and simulations.*

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Introduction

An introduction and discussion at length of the deterministic model, including the case of viral evolution, description of the mechanisms involved in HCV infections in humans has been presented. In addition to finding the equilibrium of the systems, numerical simulations of the models are provided , where it was found that strong CTL response and weak antibody response was likely to clear HCV between six to eight months after infection. Strong antibody and weak CTL responses are however not able to clear the HCV, which might lead to persistent and chronic infection. This may later lead to liver pathology and any intervention by a strong CTL may be of little help. Strong CTL at this period lead to massive killing of infected cells, which are considered to be more than the healthy cells. Eventually the size and functions of the organ are compromised and may lead to the death of the host.

In the normal and natural circumstances both the CTL and antibody responses are likely to mount with sufficient strength, since a viral antigen has both extracellular and intracellular phases. In such a scenario the model predict that HCV will be cleared much earlier (before the fourth month of infection) as compared to when CTL only response clears the infection This response better represents real-life natural processes.

However, an important behavior of viral evolution is the emergence of new viral mutants able to evade the antibodies and thought to highly contribute to drug

resistance during treatments.While the antibody responses neutralize the free virus,and hence contribute to low virus population, the virus is able to escape this neutralization by mutation.The antibody respond to this by generating new specific response to the mutant. However, as new antibody specificities are generated other new viral mutants will continue to emerge. Consequently, there will be diversity of the viral mutants. Therefore, while the antibody are able to adapt and respond to new viral mutants, the virus population will still grow since there will be continuous production of new variants not encountered before.

While the models are a good starting point, they still fall short of providing a comprehensive explanation of why some individuals respond to viral infection by CTL,Antibodies or both responses.

## 5.2 Conclusion

It is important for CTL and antibody responses mount at the right time and strength to reduce the chances of antigenic escape In chapter four the deterministic mathematical model that was used to simulate immune responses to viral pathogen was analyzed. The model has been used to simulate different possible scenarios in HCV infection. It is argued that the two branches of immune system are in completion and either of the branch can derive the other one to extinction or may coexist together.

1. It has been shown that strong (dominant) CTL and weak antibody response is able to clear the infection albeit not as fast as when both branches of immune system mount strongly and become established.These results are confirmed in

studies by Chang et al. 2001 that showed that in acute phase of the infection both humans or chimpanzees who cleared the virus developed high and sustained CTL responses.

Failure to clear the infection in the model is associated with strong (dominant) antibody and weak CTL response. This is supported by Wodarz (2003), it was observed that both humans and chimpanzees that were not able to clear the infection had a characteristic low initial CTL response that was un sustained and decayed during the acute phase of infection. Farci et al. (2000).

2. The steady state analysis that followed reveal qualitatively the nature of the equilibrium points. It was observed that the system yielded computationally infeasible characteristic polynomials in all possible immune system response scenarios. The Eigen value method is therefore not recommended for this analysis. Routh stability criterion is therefore used and found that ;

   a) In the cases where no immune response is mounted the equilibrium is always unstable

   b) For dominant CTL response the equilibrium is always stable. c) For antibody dominant response the equilibrium is always stable.

   d) When both branches of immune system coexist and are both strong the equilibrium is always unstable

   e) For viral evolution, when the threshold for CTL induced pathology is not achieved the stability condition is

$$\delta\mu\tau^3(\beta\sigma+\theta\omega) > \beta\lambda\tau\kappa\tau^2\phi(\mathrm{n}\beta\tau+\omega\phi)(\beta\sigma+\theta\omega) \qquad (5.1)$$

f) When the threshold of CTL induced pathology is reached, the equilibrium is always unstable.

3. Minimum parameter combinations for mounting immune responses is established using the method of next generation matrix. It was seen that whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} < \tau \tag{5.2}$$

and

$$\frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} > \sigma \tag{5.3}$$

CTL dominate and derive to extinction antibody responses, whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} > \tau \tag{5.4}$$

and

$$\frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} < \sigma \tag{5.5}$$

the antibody dominate and CTL will fail and, and whenever

$$\frac{\kappa\rho\phi}{\alpha\theta} > \tau \tag{5.6}$$

and

$$\frac{\beta\lambda\tau\theta}{\delta(\beta\tau+\omega\phi)} > \sigma \tag{5.7}$$

both branches of the immune system will co-exist and mount strongly.

This is confirmed by the simulations and whenever the established criterion is violated the obtained simulations do not reflect the accepted knowledge of immune system.

## 5.3 Recommendations

Nutrition contributes immensely to the immunity status of an organism. Immune system, relative to its size, is one of most energy consuming systems in an organism and hence sensitive to nutrients intake and consumption in the body. The T cell being in the heart of an effective and efficient cellular mediated immune responses receives nutrients from its environment and therefore the dynamics of nutrition status affects its functions. The study makes the following recommendations.

1. The general public be made aware of the overall effect of proper diet and nutrition in reducing increased health cost, morbidity, and mortality.

2. The model be adopted as a tool to simulate different treatment protocols before administering the to patients.

3. Due to the unapparent and asymptomatic nature of persistent and chronic of HCV infection more awareness to the general public and regular medical checkups for all is recommended.

4. Continuous, collaborative and multi-disciplinary research in the human disease is essential, for development of new molecular tools for dissecting the intriguing bio-pathogenesis of chronic hepatitis C in man. Just as the progress

on this disease to date has been phenomenal, so too will be the future progress in furthering our understanding of HCV infection, replication, and molecular biology, and in improving the treatment of hepatitis C.

5. For all infected patients, increased vigilance for signs of hepatotoxicity is highly recommended since HCV infected patients are at high risk of hepatocellular carcinoma (cancer of the liver cells).

## 5.4 Future work

1. For successful treatment of HCV immunological data show that boosting of HCV specific immunity is necessary. It is recommended that treatment factor be included in the model with different drug efficacies to determine whether disease progression and chronicity can be prevented or delayed.

2. It is also essential that more flexible and robust infection models be continuously developed that could capture stochastic aspects of the immune system.

3. Since HCV attacks the liver a spatial model can be developed to determine the distribution of the infected cells with time.

4. The aspect of delay exhibited by most biological systems can be included in the model to see the effects of this delays in pathogen progression.

5. The role of CMI in fighting intracellular bacteria and protozoa can be explored.

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# APPENDIX

## A Basic Reproductive ratio

```
\frac{-\mu \sigma  \text{n$\beta \tau $}^2 \text{n$\theta \rho \tau $}+
\text{n$\beta \tau $} (\text{n$\theta \rho \tau $} (-\mu \sigma
(\alpha  \phi +\delta  \phi -\omega \phi +\omega  \phi )+\delta
\sigma  \mu  \phi +\lambda \theta  \mu  \phi )+\mu \sigma
\rho  \phi  (\text{n$\mu \tau \theta $}-\kappa \phi \sigma
)+\phi  (\text{n$\theta \rho \tau $} (\mu \sigma  \omega
\phi  (\alpha +\delta +\omega )-\delta \theta \sigma
\omega  \mu )+\mu \sigma  \rho  \omega \phi  (\kappa \phi
\sigma -\text{n$\mu \tau \theta\\ $}))}{\mu \sigma
\text{n$\theta \rho \tau $} \phi  (\text{n$\beta \tau
$}-\omega \phi )}
```

## B Stability of Immune Free Response

```
\frac{\kappa  (\beta \lambda \kappa -\alpha \delta \omega )}{\beta
\delta \kappa  \delta }\left( \begin{array}{ccc}
-\frac{\beta  (\beta \lambda \kappa -\alpha \delta \omega )
\kappa }{\beta \delta \kappa  \delta }-\omega  & 0 & -\frac{\alpha
\delta  \beta }{\beta \kappa } \\ \frac{\beta  (\beta \lambda
\kappa -\alpha \delta \omega ) \kappa }{\beta \delta \kappa  \delta
```

```
} & -\delta  & 0 \\ 0 & -\kappa  & -\alpha  \\
\end{array}
\right)
\frac{\alpha  \alpha \delta \omega  \beta  \eta  \kappa }{\beta
\delta \kappa  \delta }+\frac{\alpha  \alpha \delta \omega  \beta
\kappa }{\beta \delta \kappa }-\frac{\alpha  \beta  \beta
\lambda \kappa  \eta  \kappa }{\beta \delta \kappa  \delta
}-\frac{\alpha  \beta  \beta \lambda \kappa  \kappa }{\beta
\delta \kappa }-\alpha  \delta  \eta -\alpha  \delta  \omega
-\alpha  \eta ^2-\alpha  \eta  \omega -\frac{\alpha \delta  \alpha
\delta \omega  \beta ^2 \kappa ^2}{\beta \delta \kappa  \beta
\kappa  \delta }+\frac{\alpha \delta  \beta ^2 \beta \lambda
\kappa  \kappa ^2}{\beta \delta \kappa  \beta \kappa  \delta
}+\frac{\alpha \delta \omega  \beta  \eta ^2 \kappa }{\beta \delta
\kappa  \delta }+\frac{\alpha \delta \omega  \beta  \eta  \kappa
}{\beta \delta \kappa }-\frac{\beta  \beta \lambda \kappa  \eta
^2 \kappa }{\beta \delta \kappa  \delta }-\frac{\beta  \beta
\lambda \kappa  \eta  \kappa }{\beta \delta \kappa }-\delta
\eta ^2-\delta  \eta  \omega -\eta ^3-\eta ^2 \omega =0
\frac{-\alpha  \beta \kappa  (\delta +\eta ) (\beta  \kappa
(\beta \lambda \kappa -\alpha \delta \omega )+\beta \delta \kappa
\delta  (\eta +\omega ))+\beta  \kappa  (\alpha \delta \omega
-\beta \lambda \kappa ) (\beta \kappa  \eta  (\delta +\eta
```

```
)-\alpha \delta  \beta  \kappa )-\beta \delta \kappa  \beta
\kappa  \delta  \eta  (\delta +\eta ) (\eta +\omega )}{\beta \delta
\kappa  \beta \kappa  \delta }=0

\text{Null}

(-1)
\left(\text{a0}=\text{Simplify}\left[\text{Coefficient}\left[K,\eta
^3\right]\right]\right)

\frac{(-\alpha  \beta \delta \kappa  \delta +\beta  \kappa  (\alpha
\delta \omega -\beta \lambda \kappa )-\beta \delta \kappa  \delta
(\delta +\omega ))
\left(\text{a1}=\text{Simplify}\left[\text{Coefficient}\left[K,\eta
^2\right]\right]\right)}{\beta \delta \kappa  \delta }

\frac{(-(\alpha  (\beta  \kappa  (\beta \lambda \kappa -\alpha
\delta \omega )+\beta \delta \kappa  \delta  (\delta +\omega
))+\delta  (-\alpha \delta \omega  \beta  \kappa +\beta  \beta
\lambda \kappa  \kappa +\beta \delta \kappa  \delta  \omega )))
\left(\text{a2}=\text{Simplify}\left[\text{Coefficient}\left[K,
\eta ^1\right]\right]\right)}{\beta \delta \kappa  \delta }
```

```
\frac{(\text{a3}=\text{Simplify}[\text{Coefficient}[K,\eta ,0]])
\left(\alpha \delta  \beta ^2 \kappa ^2 (\beta \lambda \kappa
-\alpha \delta \omega )-\alpha  \beta \kappa  \delta  (-\alpha
\delta \omega  \beta  \kappa +\beta  \beta \lambda \kappa  \kappa
+\beta \delta \kappa  \delta  \omega )\right)}{\beta \delta
\kappa  \beta \kappa  \delta }

\text{b1}=\text{Simplify}\left[\frac{\text{a1} \text{a2}-\text{a0}
\text{a3}}{\text{a1}}\right]

-\frac{\beta \delta \kappa  \delta  \left(\alpha \delta  \beta ^2
\kappa ^2 (\beta \lambda \kappa -\alpha \delta \omega )-\alpha
\beta \kappa  \delta  (-\alpha \delta \omega  \beta  \kappa +\beta \beta \lambd
)\right)+\beta \kappa  (\alpha  \beta \delta \kappa  \delta +\beta

\kappa  (\beta \lambda \kappa -\alpha \delta \omega )+\beta \delta
\kappa  \delta  (\delta +\omega )) (\alpha  (\beta  \kappa  (\beta
\lambda \kappa -\alpha \delta \omega )+\beta \delta \kappa
\delta  (\delta +\omega ))+\delta  (-\alpha \delta \omega
\beta  \kappa +\beta  \beta \lambda \kappa  \kappa +\beta
\delta \kappa  \delta  \omega ))}{\beta \delta \kappa  \beta
\kappa  \delta  (\alpha  \beta \delta \kappa  \delta +\beta
\kappa  (\beta \lambda \kappa -\alpha \delta \omega )+\beta \delta
```

```
\kappa  \delta  (\delta +\omega ))}


\frac{\left(\text{c1}=\text{Simplify}\left[\frac{\text{a3}
\text{b1}-\text{a1} \text{b2}}{\text{b1}}\right]\right) \left(-\left(\beta \kap
+\beta  \kappa  (\beta \lambda \kappa -\alpha \delta \omega )+\beta
\delta \kappa  \delta  (\delta +\omega )) \left(\text{b2} \beta
\delta \kappa  \delta  (\alpha  \beta \delta \kappa  \delta
+\beta  \kappa  (\beta \lambda \kappa -\alpha \delta \omega
)+\beta \delta \kappa  \delta  (\delta +\omega
))-\frac{\left(\alpha \delta  \beta ^2 \kappa ^2 (\beta \lambda
\kappa -\alpha \delta \omega )-\alpha  \beta \kappa  \delta
(-\alpha \delta \omega  \beta  \kappa +\beta  \beta \lambda
\kappa  \kappa +\beta \delta \kappa  \delta  \omega )\right)
\left(\beta \delta \kappa  \delta  \left(\alpha \delta  \beta ^2
\kappa ^2 (\beta \lambda \kappa -\alpha \delta \omega )-\alpha
\beta \kappa  \delta  (-\alpha \delta \omega  \beta  \kappa
+\beta  \beta \lambda \kappa  \kappa +\beta \delta \kappa
\delta  \omega )\right)+\beta \kappa  (\alpha  \beta \delta \kappa
\delta +\beta  \kappa  (\beta \lambda \kappa -\alpha \delta
\omega )+\beta \delta \kappa  \delta  (\delta +\omega )) (\alpha
(\beta  \kappa  (\beta \lambda \kappa -\alpha \delta \omega
)+\beta \delta \kappa  \delta  (\delta +\omega ))+\delta
(-\alpha \delta
```

```
\omega  \beta  \kappa +\beta  \beta \lambda \kappa  \kappa +\beta
\delta \kappa  \delta  \omega ))\right)}{\beta \kappa ^2 (\alpha
\beta \delta \kappa  \delta +\beta  \kappa  (\beta \lambda \kappa
-\alpha \delta \omega )+\beta \delta \kappa  \delta  (\delta
+\omega ))}\right)\right)\right)}{\beta \delta \kappa  \delta
\left(\beta \delta \kappa  \delta  \left(\alpha \delta  \beta ^2
\kappa ^2 (\beta \lambda \kappa -\alpha \delta \omega )-\alpha
\beta \kappa  \delta  (-\alpha \delta \omega  \beta  \kappa
+\beta  \beta \lambda \kappa  \kappa +\beta \delta \kappa  \delta
\omega )\right)+\beta \kappa  (\alpha  \beta \delta \kappa
\delta +\beta  \kappa  (\beta \lambda \kappa -\alpha \delta \omega
)+\beta \delta \kappa  \delta  (\delta +\omega )) (\alpha  (\beta
\kappa  (\beta \lambda \kappa -\alpha \delta \omega )+\beta
\delta \kappa  \delta  (\delta +\omega ))+\delta  (-\alpha \delta
\omega  \beta  \kappa +\beta  \beta \lambda \kappa  \kappa +\beta
\delta \kappa  \delta  \omega ))\right)}

\text{Null} \text{Null} \text{Null} \text{Null} \text{Null}

\text{Null} \text{Null} \text{Null}
```

# C Stability of CTL only Immune Response

```
\left(
\begin{array}{cccc}
-\Lambda -\omega -\frac{\beta  \kappa  \sigma }{\theta } & 0 &
-\frac{\alpha \lambda \theta  \beta }{\alpha \theta \omega +\beta
\kappa \sigma } & 0 \\
\frac{\beta  \kappa  \sigma }{\theta } & -\frac{\alpha \lambda
\ \theta  \beta \kappa }{\alpha \theta \omega +\beta \kappa \sigma
}-\Lambda  & 0 & -\frac{\mu  \sigma }{\theta } \\
0 & \kappa  & -\alpha -\Lambda  & 0 \\
0 & \frac{\left(\frac{\alpha \lambda \theta  \beta \kappa }{\alpha
\theta \omega +\beta \kappa \sigma }-\delta \right) \theta }{\mu }
& 0 & -\Lambda  \\
\end{array}
\right)


-\frac{\Lambda  \left(-(\alpha +\Lambda ) (\Lambda  (\alpha \theta
\omega +\beta \kappa \sigma )+\alpha \lambda \theta  \beta \kappa )
\ (\beta  \kappa  \sigma +\theta  (\Lambda +\omega ))-\alpha
\lambda \theta  \beta ^2 \kappa ^2 \sigma \right)+\sigma  (\alpha
\ +\Lambda ) (\delta  (\alpha \theta \omega +\beta \kappa \sigma
)-\alpha \lambda \theta  \beta \kappa ) (\beta  \kappa  \sigma
```

```
+\theta  (\Lambda +\omega ))}{\theta  (\alpha \theta \omega
+\beta \kappa \sigma )};

\text{Null}\text{Null}\text{Null}\text{Null}

\left(\text{a0}=\text{Simplify}\left[\text{Coefficient}\left
[H,\Lambda ^4\right]\right]\right)
\frac{\left(\text{a1}=\text{Simplify}\left[\text{Coefficient}
\left[H,\Lambda ^3\right]\right]\right) (\alpha  \theta  (\alpha
\theta \omega +\beta \kappa \sigma )+(\alpha \theta \omega +\beta
\kappa \sigma ) (\beta  \kappa  \sigma +\theta  \omega )+\alpha
\lambda \theta  \beta \kappa  \theta )}{\theta  (\alpha \theta
\omega +\beta \kappa \sigma )}

\frac{\left(\text{a2}=\text{Simplify}\left[\text{Coefficient}
\left[H,\Lambda ^2\right]\right]\right) (\alpha  ((\alpha \theta
\omega +\beta \kappa \sigma ) (\beta  \kappa  \sigma +\theta
\omega )+\alpha \lambda \theta  \beta \kappa  \theta )+\delta
\theta  \sigma  (-(\alpha \theta \omega +\beta \kappa \sigma
))+\alpha \lambda \theta  \beta \kappa  (\beta  \kappa  \sigma
+\theta  (\sigma +\omega )))}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}
```

```
\frac{\left(\text{a3}=\text{Simplify}\left[\text{Coefficient}
\left[H,\Lambda ^1\right]\right]\right) \left(\alpha  (\alpha
\lambda \theta  \beta \kappa  (\beta  \kappa  \sigma +\theta
(\sigma +\omega ))-\delta  \theta  \sigma  (\alpha \theta \omega
+\beta \kappa \sigma ))+\sigma  \left(\alpha \lambda \theta
\left(\beta ^2 \kappa ^2+\beta  \beta \kappa  \kappa  \sigma +
\beta \kappa  \theta  \omega \right)-\delta  (\alpha \theta
\omega +\beta \kappa \sigma ) (\beta  \kappa  \sigma +\theta
\omega )\right)\right)}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}

\frac{(\text{a4}=\text{Cancel}[\text{Coefficient}[H,\Lambda ,0]])
\left(-\alpha  \alpha \theta \omega  \beta  \delta  \kappa
\sigma ^2-\alpha  \alpha \theta \omega  \delta  \theta  \sigma
\omega +\alpha  \alpha \lambda \theta  \beta  \beta \kappa
\kappa  \sigma ^2+\alpha  \alpha \lambda \theta  \beta \kappa
\theta  \sigma  \omega -\alpha  \beta  \beta \kappa \sigma  \delta
\kappa  \sigma ^2-\alpha  \beta \kappa \sigma  \delta  \theta
\sigma  \omega \right)}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}
```

```
\frac{\alpha  \sigma  (\alpha \lambda \theta  \beta \kappa -\delta
(\alpha \theta \omega +\beta \kappa \sigma )) (\beta  \kappa
\sigma +\theta  \omega )}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}

\frac{\alpha  \sigma  (\alpha \lambda \theta  \beta \kappa -\delta
(\alpha \theta \omega +\beta \kappa \sigma )) (\beta  \kappa
\sigma +\theta  \omega )}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}

-\frac{\frac{\alpha \delta  \beta ^2 \kappa ^2 (\beta \lambda \kappa
-\alpha \delta \omega )-\alpha  \beta \kappa  \delta  (-\alpha
\delta \omega  \beta  \kappa +\beta  \beta \lambda \kappa  \kappa
+\beta \delta \kappa  \delta  \omega )}{\beta \kappa
}-\frac{(\alpha  \beta \delta \kappa  \delta +\beta  \kappa
(\beta \lambda \kappa -\alpha \delta \omega )+\beta \delta
\kappa  \delta  (\delta +\omega )) (\alpha  ((\alpha \theta \omega
+\beta \kappa \sigma ) (\beta  \kappa  \sigma +\theta  \omega
)+\alpha \lambda \theta  \beta \kappa  \theta )+\delta  \theta
\sigma  (-(\alpha \theta \omega +\beta \kappa \sigma ))+\alpha
\lambda \theta  \beta \kappa  (\beta  \kappa  \sigma +\theta
(\sigma +\omega )))}{\theta  (\alpha \theta \omega +\beta \kappa
\sigma )}}{\alpha  \beta \delta \kappa  \delta +\beta  \kappa
```

```
(\beta \lambda \kappa -\alpha \delta \omega )+\beta \delta \kappa
\delta  (\delta +\omega )}

\text{Null}

\frac{\left(\text{b2}=\text{Simplify}\left[\frac{\text{a1}
\text{a4}-\text{a0} \text{a5}}{\text{a1}}\right]\right) (\alpha
\sigma  (\alpha \lambda \theta  \beta \kappa -\delta  (\alpha
\theta \omega +\beta \kappa \sigma )) (\beta  \kappa  \sigma
+\theta  \omega ))}{\theta  (\alpha \theta \omega +\beta \kappa
\sigma )}

\text{Null} \left\{\text{TextData}[\text{Parallel$\grave{
}$Preferences$\grave{ }$Private$\grave{
}$b2}],\text{BoxData}\left[\text{FormBox}\left[\text
{InterpretationBox}\left[\text{GridBox}\left[\left(

\begin{array}{c}
\text{GridBox}\left[\left(
\begin{array}{c}
\text{RowBox}[\{\text{b2},=,\text{a4}\}] \\
\end{array}
\right),\text{BaselinePosition}\to
```

```
\{\text{Baseline},\{1,1\}\},\text{GridBoxAlignment}\to
\left\{\text{Columns}\to \left(
\begin{array}{c}
\text{Left} \\
\end{array}
\right),\text{ColumnsIndexed}\to \{\},\text{Rows}\to \left(
\begin{array}{c}
\text{Baseline} \\
\end{array}
\right),\text{RowsIndexed}\to \{\}\right\},\text{GridBoxItemSize}
\to \left\{\text{Columns}\to \left(
\begin{array}{c}
\text{Scaled}[0.999] \\
\end{array}
\right),\text{ColumnsIndexed}\to \{\},\text{Rows}\to \left(
\begin{array}{c}
1. \\
\end{array}
\right),\text{RowsIndexed}\to \{\}\right\}\right] \\
\end{array}
\right),\text{BaselinePosition}\to
\{\text{Baseline},\{1,1\}\},\text{GridBoxAlignment}\to
\left\{\text{Columns}\to \left(
```

```
\begin{array}{c}

\text{Left} \\

\end{array}

\right),\text{ColumnsIndexed}\to \{\},\text{Rows}\to \left(

\begin{array}{c}

\text{Baseline} \\

\end{array}

\right),\text{RowsIndexed}\to

\{\}\right\}\right],\text{Null},\text{Editable}\to

\text{False}\right],\text{StandardForm}\right]\right]\right\}

\frac{\left(\text{c1}=\text{Simplify}\left[\frac{\text{a3}

\text{b1}-\text{a1} \text{b2}}{\text{b1}}\right]\right)

\left(-\left(\alpha  \beta \kappa ^2 \sigma  (\alpha \lambda

\theta  \beta \kappa -\delta  (\alpha \theta \omega +\beta \kappa

\sigma )) (\beta  \kappa  \sigma +\theta  \omega ) (\alpha  \beta

\delta \kappa  \delta +\beta  \kappa  (\beta \lambda \kappa

-\alpha \delta \omega )+\beta \delta \kappa  \delta  (\delta

+\omega ))^2-\left(\alpha \delta  \beta ^2 \kappa ^2 (\beta

\lambda \kappa -\alpha \delta \omega )-\alpha  \beta \kappa

\delta  (-\alpha \delta \omega  \beta  \kappa +\beta  \beta

\lambda \kappa  \kappa +\beta \delta \kappa  \delta  \omega

)\right) \left(\theta  (\alpha \theta \omega +\beta \kappa

\sigma ) \left(\alpha \delta  \beta ^2 \kappa ^2 (\beta
```

```
\lambda \kappa -\alpha \delta \omega )-\alpha  \beta
\kappa  \delta  (-\alpha \delta \omega  \beta  \kappa
+\beta  \beta \lambda \kappa  \kappa +\beta \delta
\kappa  \delta  \omega )\right)+\beta \kappa  (\alpha
\beta \delta \kappa  \delta +\beta  \kappa  (\beta
\lambda \kappa -\alpha \delta \omega )+\beta \delta \kappa
\delta  (\delta +\omega )) (-\alpha  ((\alpha \theta \omega
+\beta \kappa \sigma ) (\beta  \kappa  \sigma +\theta  \omega
)+\alpha \lambda \theta  \beta \kappa  \theta )+\delta
\theta  \sigma  (\alpha \theta \omega +\beta \kappa \sigma
)-\alpha \lambda \theta  \beta \kappa  (\beta  \kappa
\sigma +\theta  (\sigma +\omega
)))\right)\right)\right)}{\beta \delta \kappa  \beta
\kappa ^2 \delta  \theta  (\alpha \theta \omega +\beta
\kappa \sigma ) \left(\frac{\alpha \delta  \beta ^2 \kappa ^2
(\beta \lambda \kappa -\alpha \delta \omega )-\alpha  \beta
\kappa  \delta  (-\alpha \delta \omega  \beta  \kappa +\beta
\beta \lambda \kappa  \kappa +\beta \delta \kappa  \delta
\omega )}{\beta \kappa }-\frac{(\alpha  \beta \delta \kappa
\delta +\beta  \kappa  (\beta \lambda \kappa -\alpha
\delta \omega )+\beta \delta \kappa  \delta  (\delta
+\omega )) (\alpha  ((\alpha \theta \omega +\beta \kappa
\sigma ) (\beta  \kappa  \sigma +\theta  \omega
```

```
)+\alpha \lambda \theta  \beta \kappa  \theta )+\delta  \theta
\sigma  (-(\alpha \theta \omega +\beta \kappa \sigma ))+\alpha
\lambda \theta  \beta \kappa  (\beta  \kappa  \sigma +\theta
(\sigma +\omega )))}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}\right)}
\frac{\alpha  \sigma  (\alpha \lambda \theta  \beta \kappa -\delta
(\alpha \theta \omega +\beta \kappa \sigma )) (\beta  \kappa
\sigma +\theta  \omega )}{\theta  (\alpha \theta \omega +\beta
\kappa \sigma )}
```

# D Weak CTL and Strong Antibody

```
function [Ddv_Div]=cmiwctlsa(I,D)
% IV,I, IVSOLVE-INDIPENDENT VARIABLE
% DV, D, DSOLVE-DEPENDENT VARIABLE
lambda=1; omega=0.1;beta=0.03;delta=0.1;mu=1.0; kappa=1.5;
alpha=1; rho=1.2;eta=0.00; phi=3.5; tau=0.1; nu=0.0;theta=2.5;
sigma=0.240;
x(1)=D(1); x(2)=D(2); x(3)=D(3);x(4)=D(4);x(5)=D(5);
Ddv_Div=[lambda-omega*x(1)-x(3)*beta*x(1);
beta*x(1)*x(3)-delta*x(2)-mu*x(2)*x(5);
kappa*x(2)-alpha*x(3)-rho*x(2)*x(4);
eta*x(4)+phi*x(3)*x(4)-tau*x(4);
```

```
nu*x(5)+theta*x(2)*x(5)-sigma*x(5)]

end

domain=[0 20]

IC1=10;IC2=0.1;IC3=0.1; IC4=0.1;IC5=0.1;

IC=[IC1 IC2 IC3 IC4 IC5]

[IVsol,DVsol]=ode45('cmiwctlsa',domain,IC);

subplot(2,1,1)

plot(IVsol,DVsol(:,1),'--')

grid on

hold on

plot(IVsol,DVsol(:,2),'r','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','y')

grid on

xlabel('Time (months)')

ylabel('infection level')

title('Failure to clear infection by antibodies only ')

legend('infected cells','free virus')

hold on

subplot(2,1,2)

plot(IVsol,DVsol(:,3),'b','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','y' )

legend('Infected cells','Viral load (x 10^4)IU/L')

hold on
```

```
subplot(2,1,2)
plot(IVsol,DVsol(:,4),'y','linewidth',2,'markersize',2,...
'markeredgecolor','g','markerfacecolor','y')
hold on
grid on
subplot(2,1,3)
plot(IVsol,DVsol(:,5),'o','linewidth',2,'markersize',2,...
'markeredgecolor','g','markerfacecolor','r')
ylabel('level of  immune responses')
xlabel('Time (months)')
title( ' Dominant antibody response .')
legend(' Antibody Respone','CTL Response')
```

# E Strong CTL and Strong Antibody

```
function [Ddv_Div]=cmi(I,D)
% IV,I, IVSOLVE-INDIPENDENT VARIABLE
% DV, D, DSOLVE-DEPENDENT VARIABLE
lambda=10;omega=0.1;beta=0.01;delta=0.1;mu=0.1;kappa=2.5;alpha=1;
rho=0.1;eta=0.01; phi=3.5;tau=0.2; nu=0.01;theta=4.5;sigma=0.2;
x(1)=D(1); x(2)=D(2); x(3)=D(3); x(4)=D(4); x(5)=D(5);
Ddv_Div=[lambda-omega*x(1)-beta*x(1)*x(3);
beta*x(1)*x(3)-delta*x(2)-mu*x(2)*x(5);
kappa*x(2)-alpha*x(3)-rho*x(3)*x(4);
```

```
eta*x(4)+phi*x(3)*x(4)-tau*x(4)

nu*x(5)+theta*x(2)*x(5)-sigma*x(5)]

end

domain=[0 20]

IC1=10;IC2=0.5;IC3=0.5;IC4=0.1;IC5=0.1;

IC=[IC1 IC2 IC3 IC4 IC5]

[IVsol,DVsol]=ode45('cmiSCSA',domain,IC);

subplot(5,1,1)

plot(IVsol,DVsol(:,1),'y')

grid on

hold on

subplot(2,1,1)

plot(IVsol,DVsol(:,2),'r','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','y')

grid on

xlabel('Time (months)')

ylabel('Infection level')

title('Infected cells ')

hold on

subplot(5,1,3)

plot(IVsol,DVsol(:,3),'b','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','y')

xlabel('Time (months)')
```

```
ylabel('Infection level')

title('Clearace of infection by CTL & antibodies')

legend('infected cell','viral load (x10^4) IU/L')

subplot(2,1,2)

plot(IVsol,DVsol(:,4),'y','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','y')

hold on

grid on

subplot(5,1,5)

plot(IVsol,DVsol(:,5),'o','linewidth',2,'markersize',2,...

'markeredgecolor','g','markerfacecolor','r')

ylabel(' immune responses')

xlabel('Time (months)')

title( 'Strong CTL response and strong Antibody response .')

legend(' Antibody Respone','CTL Response')
```

# F Liver Pathology with five virus strains

```
function [Ddv_Div]=forloopnopathology(I,D)

% IV,I, IVSOLVE-INDIPENDENT VARIABLE

% DV, D, DSOLVE-DEPENDENT VARIABLE

lambda=10;omega=0.1;beta=0.5;delta=0.1;mu=0.05;kappa=3.5;

alpha=0.2;rho=0.1;eta=0.00;phi=2.5;tau=0.1;nu=0.00;theta=0.1;
```

```
sigma=0.2;
x(1)=D(1);x(2)=D(2);x(3)=D(3);x(4)=D(4);x(5)=D(5);x(6)=D(6);x(7)=D(7);
x(8)=D(8);x(9)=D(9);x(10)=D(10);x(11)=D(11);x(12)=D(12);x(13)=D(13);
x(14)=D(14);x(15)=D(15);x(16)=D(16);x(17)=D(17);
Ddv_Div=[lambda-omega*x(1)-beta*x(1)*(x(7)+x(8)+x(9)+x(10)+x(11));
beta*x(1)*x(2)-delta*x(2)-mu*x(2)*x(17);
beta*x(1)*x(3)-delta*x(3)-mu*x(3)*x(17);
beta*x(1)*x(4)-delta*x(4)-mu*x(4)*x(17);
beta*x(1)*x(5)-delta*x(5)-mu*x(5)*x(17);
beta*x(1)*x(6)-delta*x(6)-mu*x(6)*x(17);
kappa*x(2)-alpha*x(7)-rho*x(7)*x(12);
kappa*x(3)-alpha*x(8)-rho*x(8)*x(13);
kappa*x(4)-alpha*x(9)-rho*x(9)*x(14);
kappa*x(5)-alpha*x(10)-rho*x(10)*x(15);
kappa*x(6)-alpha*x(11)-rho*x(11)*x(16);
eta*x(12)+phi*x(7)*x(12)-tau*x(12);
eta*x(13)+phi*x(8)*x(13)-tau*x(13);
eta*x(14)+phi*x(9)*x(14)-tau*x(14);
eta*x(15)+phi*x(10)*x(15)-tau*x(15);
eta*x(16)+phi*x(11)*x(16)-tau*x(16);
nu*x(17)+theta*x(17)*(x(2)+x(3)+x(4)+x(5)+x(6))-sigma*x(17)]
end
domain=[0 240]
```

```
IC1=10;

IC2=0.014;IC3=0.013;IC4=0.012;IC5=0.011;IC6=0.010;

IC7=0.14;IC8=0.13;IC9=0.12;IC10=0.11;IC11=0.10;

IC12=0.014;IC13=0.013;IC14=0.012;IC15=0.011;IC16=0.010;

IC17=0.002;

IC=[IC1 IC2 IC3 IC4 IC5 IC6 IC7 IC8 IC9 IC10 IC11 IC12 IC13...

IC14 IC15 IC16 IC17]

[IVsol,DVsol]=ode45('forloopnopathology',domain,IC);

subplot(5,1,1)

plot(IVsol,DVsol(:,1),'g', 'linewidth',1,'markersize',2,'markeredgecolor',...

'g', 'markerfacecolor','y')

grid on

title('Decline of uninfected')

hold on

xlabel('time(months')

ylabel('level of  uninfected cell')

legend('uninfected cell')

subplot(3,1,1)

plot(IVsol,DVsol(:,2),'y','linewidth',1,'markersize',1)

grid on

xlabel('Time (arbitrary units)')

ylabel('Infection level')

title('Infected cell dynamics in the face of a strong CTL
```

```
and strong Antibody responses')
hold on
subplot(3,1,1)
plot(IVsol,DVsol(:,3),'r','linewidth',1,'markersize',1)
hold on
grid on
ylabel('level of  immune response')
xlabel('Time (months)')
title('Anibody  response')
subplot(3,1,2)
plot(IVsol,DVsol(:,4),'b','linewidth',1,'markersize',1)
grid on
hold on
ylabel('level of  immune response')
xlabel('Time (months)')
title(  'Anibody  response ')
hold on
plot(IVsol,DVsol(:,5),'*','linewidth',1,'markersize',1)
hold on
plot(IVsol,DVsol(:,6),'g','linewidth',1,'markersize',1)
xlabel('time(months)')
ylabel('virus strains')
legend('cell infected by starin 1','cell infected by starin 2',
```

```
'cell infected by starin 3','cell infected by starin...
4','cell infected by starin 5')
title ('Infection topology')
hold on
plot(IVsol,DVsol(:,7),'r','linewidth',1,'markersize',1)
hold on
plot(IVsol,DVsol(:,8),'--','linewidth',1,'markersize',1)
hold on
subplot(3,1,3)
plot(IVsol,DVsol(:,9),'b','linewidth',1)
ylabel('level of  immune response')
hold on
xlabel('Time (months)')
title(  'CTL response for five strains ')
plot(IVsol,DVsol(:,10),'g','linewidth',1)
hold on
plot(IVsol,DVsol(:,11),'y','linewidth',1)
grid on
xlabel('time(months)')
ylabel('virus load')
legend('strain 1','strain 2','strain 3','strain 4','strain 5')

plot(IVsol,DVsol(:,12),'r','linewidth',1,'markersize',1,...
```

```
'markeredgecolor','r','markerfacecolor','r')
hold on
plot(IVsol,DVsol(:,13),'--','linewidth',1,'markersize',1,...
'markeredgecolor','g','markerfacecolor','g')
hold on
plot(IVsol,DVsol(:,14),'b','linewidth',1,'markersize',1,...
'markeredgecolor','b','markerfacecolor','b')
hold on
plot(IVsol,DVsol(:,15),'g','linewidth',1,'markersize',1,...
'markeredgecolor','g','markerfacecolor','b')
hold on
plot(IVsol,DVsol(:,16),'*','linewidth',1,'markersize',1,...
'markeredgecolor','g','markerfacecolor','r')
plot(IVsol,DVsol(:,17),'y','linewidth',1,'markersize',1,...
'markeredgecolor','y','markerfacecolor','r')
%hold on
title('Antibody & CTL responses during pathology')
ylabel('relativve responses')
xlabel('time(months)')
legend('response wrt strain1','response wrt strain2',...
'response wrt strain3','response wrt strain4',...
'response wrt strain5','CTL response')
grid on
```

```
gtext('x')
```