Università degli Studi di Roma Tor Vergata

Tesi di Dottorato in Matematica

Mathematical Modelling of HIV-1 dynamics in vivo

Candidato

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c. XXI

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Introduction

The increasing attention in the scientific world towards a quantitative approach to the biological sciences has produced useful and interesting work, giving rise to Biomathematics as a well defined discipline. For applications in some fields of medicine and biology, we also have a theory and a specific formalism [1, 2]. However, in some areas, given the complexity of the studied systems and the difficulty of finding detailed informations, there is no accepted basis for modeling. This is the case for the HIV infection that we are considering in this thesis.

Since we model a complex biological system, in Chapter 1 a brief, but somewhat detailed description is given. We chose to put it at the beginning because we believe that modeling in Biomathematics should always be biologically correct and the extent of approximations should be clear. From this point of view, the models we present in Chapters 3 and 4 represent an effort of synthesis of these two guidelines. An important part of the job has been to get out of the description a bio-mathematical model. In this context, the researcher in Biomathematics needs to master concepts and language of biology and to get continuous exchange with biological researchers.

Moreover, we are well convinced that the bio-mathematical model must be constructed starting from a well defined biological question.

There are some examples in Chapter 2. The main objectives of this thesis are to investigate the basic and most important cause-effect relations of the phenomenon, as we do in Chapter 3, and to estimate biological parameters difficult to measure, starting from the features of the model and its solutions, as is presented in Chapter 4.

Chapter 1

HIV biology, therapy, drug resistance

This chapter presents an introduction to the biology of the HIV/AIDS dynamics in host, focusing on important things for mathematical modelling. Specifically, the chapter deals with: the pathological and epidemiological understanding of the virus; the emergence and evolution of mutations, modelling of HIV viral infection, immune system responses, AIDS progression and drug resistance phenomena.

1.1 Biology of HIV

1.1.1 HIV as a retrovirus

HIV is a member of the genus lentivirus, a family of retroviruses. Retroviruses transfer their genomic sequence information via the process of reverse transcription (i.e., RNA → DNA); this is different from other living cells, in which the genomic sequence information flows as a result of replication (i.e., DNA → DNA), transcription (i.e., DNA → mRNA) or translation (i.e., RNA → protein). HIV primarily infects varieties of immune cells such as macrophages, microglial cells (type of brain glial cell that acts as the immune cells), and lymphocyte T- cells which make up a quarter of the white blood cell count. T-cells can be further divided into CD8 and CD4 T-cells. CD4 T-cells are the main target of HIV infection (by T- trophic strains). HIV infection has three main pathogenic mech-

anisms: direct viral killing of CD4 T-cells, increasing of the rates of apoptosis in the infected cells, and killing of the infected CD4 T-cells by CD8 cytotoxic lymphocytes (the cells that recognize the infected cells). In addition to attacking CD4 T-cells, HIV may directly attack organs such as the kidney, heart and brain, leading to acute renal failure, cardiomyopathy, dementia and encephalopathy. The reverse transcription process of viral RNA changes the functions and genomic structure of CD4 T-cells. These changes damage the immune system and lead to low CD4 T-cell counts. When the CD4 T-cell count declines below a critical level (i.e., with the loss of cell-mediated immunity), the overall immune system fails to hinder the growth of HIV and the body becomes progressively more susceptible to opportunistic illnesses. AntiRetroVs reduce viral replication and are used to contain the damage on the immune system and enable reconstitution and ultimately an improved capacity to fight the virus. However, these agents are highly problematic in terms of efficacy and side-effects [3].

1.1.2   Life cycle

HIV efficiently replicates only in living cells; the virus has no independent existence without infecting and replicating within human cells. The life cycle can be divided as:

A **Entry to the cell** The virus binds itself to the target monocyte/macrophages and CD4 T-cells by adsorbing its surface proteins (the envelop gp120 protein) to two host-cell receptors (proteins): the CD4 molecule receptor and CCR5 or CXCR4 co-receptors. Once HIV has attached to the host cell, the HIV-RNA and enzymes such as reverse transcriptase (RT), integrase (IN), protease (PR), are able to enter into the host cell (cytoplasmic compartment).

B **Replication and transcription** The RT converts the single-stranded RNA genome of the virus into double-stranded DNA, which is used to make doubled-stranded viral DNA intermediate (vDNA) in a process known as reverse transcription; this is prone to errors (mutations). The new vDNA is transported into the cell nucleus to be integrated into the host chromosome.

At this stage, the virus is known as a provirus. The integration process requires the IN enzyme. Thereafter, the virus can enter a latent stage of HIV infection, because the proviral DNA remains permanently within the target cell in either a productive or latent state. The factors that may affect this stage are the HIV variant, the cell type, and the expression capacity of the host cell [4]. Eventually, the transcription of the HIV genomic materials and viral proteins forms the HIV messenger (mRNA) and proteins required for the assembly of the virus. This production is exported from the cell nucleus into the cell cytoplasm. This process remains poorly understood because it involves many viral proteins.

C **Assembly and release** The new mRNA codes for the new viral proteins that will contribute to the reconstruction of the HIV-RNA. The viral proteins help the mRNA and the reconstruction proteins to transport into the cell membrane side. The structural components of the virus accumulate at the membrane of the infected cell to construct the HIV virion. Leftover proteins (cleaved by the protease) associated with the inner surface of the host-cell membrane, along with the HIV RNA, are released to form a bud from the host cell, and can proceed to infect other healthy cells.

HIV has very high genetic variability because of its high rate of reverse transcriptase mediated errors, estimated to be up to five mutations per genome, and because of the high rates of the virion production (estimated to be one billion virions a day)[5]. The ability of the virus to recombine during the replication cycle in vivo increases the complexity of HIV genetic diversity. Therefore, an individual infected with genetically different HIV strains may have viruses with genomic recombinations. The diversity of HIV has allowed the division of the viral strains into groups, subtypes and sub-subtypes. Thus, HIV-1 is subdivided into three groups (*M*, *O* and *N*, respectively, *Major*, *Outlier* and *Not-M*, *Not-O*), and HIV-2 is subdivided into two subtypes (*A* and *B*). The *M* group is the major group in the worldwide pandemic; the other two groups are limited to Cameroon and neighbouring regions. The *M* group involves nine subtypes (*A-D*, *F-H*, *J* and *K*) (*K* is a replacement for subtype *F3*)[6]. The most prevalent, in order, are *B* (North America and Europe), *A* and *D* (Africa), and *C* (Africa and Asia).

Although co-infections with different clades of HIV-1, or with a mixture clades of HIV-1 and HIV-2 subtypes have been reported, it is unknown whether the infection resulted from exposure to different viruses at the same time, or serially. HIV-1 superinfection is documented and is a mechanism whereby drug resistant and multi-drug resistant strains of HIV-1 may become more prevalent in time.

1.2   Clinical aspects of HIV infection and AIDS

AIDS is a late consequence of HIV infection. Progression to AIDS is associated with damage to CD4-T cells, the central cell type of the adaptive specific immune system. Because the immune response to HIV infection varies among individuals, the timing of progression to AIDS is highly variable and may range from one to more than 20 years (known as the clinical latency period), with a small percentage of infected people being extremely slow to progress and are called long-term non progressors. During this period, HIV and the immune system interact dramatically. Without antiretroviral therapy (ARV), the battle usually terminates with progressive irreversible damage to the immune system (i.e., the final stage of the disease) and profound immunodeficiency. This stage (i.e., when the CD4 T-cell counts decline below a critical level) leaves individuals prone to opportunistic infections (OIs).

1.2.1   Diagnosis and stages

Progression to AIDS and the fine pathogenesis of HIV infection vary among individuals. The extent of damage to the immune system and the viral load circulating and whole body levels differ among individuals. Difference in age, viral strains and co-infection with OIs are also contributory factors to the rate of progression of the immunodeficiency. There are four distinct stages of progression of HIV infection to AIDS: primary infection, chronic asymptomatic infection, symptomatic chronic infection and progression to AIDS.

A In the primary infection stage, a large number of HIV virions are produced and the immune system starts developing the antibodies and cytotoxic lym-

phocytes against the virus. This stage lasts for only a few weeks, during which the individual is highly infectious.

B In the chronic asymptomatic stage, the immune system responds by expanding anti-HIV specificity in the immune cells to keep the infection under control. Despite this, HIV replication continues and produces large numbers of HIV virions that impede the production of competent CD4 T-cells. The counts of these cells then gradually decline. This stage lasts a median of about 10 years.

C When the CD4 T-cell counts fall below a critical level ($< 200 cell/mm^3$) and serious OIs appear, HIV infection enters the symptomatic stage. During this stage, microbes that cause no illness in healthy individuals can lead to fatal infections and further impair the immune system. The immune system then fails to reproduce sufficient CD4 T-cells to defend the individual. As more OIs emerge and the immune system is further damaged, the infection leads to AIDS. According to the USA National Institutes of Health (NIH) classification, HIV infection has three patterns of progression to AIDS: rapid, intermediate and late.

1.2.2 Epidemiology

HIV/AIDS is a disastrous health problem. An estimated 39.5 million people worldwide were living with HIV in 2007, of whom 4.3 million were newly infected and an estimated 2.9 million lost their lives to AIDS that year. Worldwide, new HIV infections are heavily concentrated among people aged 15 to 24 years; those people accounted for about 40% of the new infections in 2007. Sub-Saharan Africa remains the worst-affected region with 21.8-27.7 million people living with HIV at the end of 2006.

1.2.3 Clinical laboratory tests

The clinical laboratory assessment of HIV infection and its impact on the individual involve five main tests: HIV antibody, HIV viral antigen (known as the p24 test), nucleic acid-based HIV viral load (known as the VL test), the host immune

system T-cell subset analysis (known as the CD4 T-cell test), and ARV resistance assays (known as drug resistance tests). The HIV antibody test enables the diagnosis of the infection even when asymptomatic; it is the most reachable test for identifying the infection. The viral antigen is used to detect very early infection and to screen for HIV-infected blood (i.e., as an alternative test for identifying HIV infection). Both of these tests contribute to an accurate diagnosis of the infection, and other tests are then used to monitor the response to therapeutic regimens and predict and diagnose disease progression.

CD4 T-Cell count test

The CD4 T-cell count serves as a surrogate for T-cell mediated immune response assays in monitoring the progression of HIV's response to therapy. The test determines the counts of CD4 T-cell per cubic millimetre of a blood sample. An average normal CD4 T- cell count is $1000mm^3$, with a range of $400 - 1200mm^3$ in the RPAH laboratory. This count falls during primary infection and then usually returns to near normal levels. Then, untreated, the CD4 T-cell count falls gradually to about $200mm^3$, or even less, and at this level the incidence of OIs arises; this phase is known as the AIDS incident stage. This reduction is associated with the hyper-activation of CD8 T-cells, which may kill HIV- infected cells. The CD8 T-cell response is thought to be important in controlling the infection; however, the CD8 T-cell counts decline similarly to those of CD4 T-cells over prolonged periods.

Viral load test

The nucleic acid-based HIV VL test is used to decide when to start an HIV therapy regimen. The test determines the number of HIV copies in a blood sample. VL is a strong predictor of the likelihood of disease progression and provides strong prognostic value, when paired with CD4 T-cell counts. Therefore, the current guidelines of US NIH and WHO for monitoring the HIV infection in developed countries advocate the use of VL assays for determining initiation of treatment regimens, monitoring the responses to these therapies, and switching drug regimens [7]. Many assays methods have been developed and shown to be

robust and their use attests to the diagnostic power of VL.

Drug resistance tests

Genotypic assays for ARV resistance produce nucleotide sequence data that may be used to determine the HIV-RNA strains and structure. Recommendations of the International AIDS Society indicate that, despite limitations, resistance testing should be incorporated into patient management. These tests are recommended to guide the choice of new regimens after treatment failure. The recommendations indicate that resistance testing should be considered in treatment-naive patients with established infection and before initiating therapy in patients with acute HIV infection, although therapy should not be delayed pending the results. Given the complexity of results and genotypic assay limitations, an expert interpretation is also recommended.

1.2.4 Course of HIV and CD4 interaction

Within a few weeks after HIV infection, the multiple viral quasispecies accumulate to reach a high level in the blood of an infected individual. As a result of the response of the immune system and in those cases where there is commencement of ART, this level falls to the point where the infection remains stable in the long terms, up to and beyond 20 years, whereas without ARV progression is almost universal.

Untreated, CD4 T-cell counts fluctuate gradually from about 600 to 800 $cell/mm^3$ and then over many years decline towards an undetectable level. The virus takes advantage of this period to evolve during killing and infecting progeny of the initially infected CD4 cells. The plasma virus increases from high early levels and peaks at very high level, and then increases forward as it reaches the symptomatic AIDS phase [21]. Further complexities occur during this period because of the immunological and virological interdependencies. Thus, the infected CD4 cells are impeded in their containment of HIV and the virus gains an advantage by infecting other healthy CD4 cells. The result of this dynamics is frequently severe immunodeficiency as the immune system becomes *exhausted* and unable to generate a replacement quantity of CD4 cells daily.

In the few very slow (or non-progressor) cases the mechanisms of the infection containment and protection against progressive disease after initial infection are not yet entirely understood, illustrating the complexity of the dynamic interactions between HIV populations and immune system cells, and the genetic variations that evolve at the sites of the PR and RT genes. In progressors these variations enable the evolution of new virions with immune system evasion mechanisms evident. CD4 T-cell counts and ranges of VL continue to fluctuate according to this evolution. Figure 1.1 illustrates a generalised relationship between the HIV copies and CD4 T-cell counts during the typical course of the disease in untreated individuals.
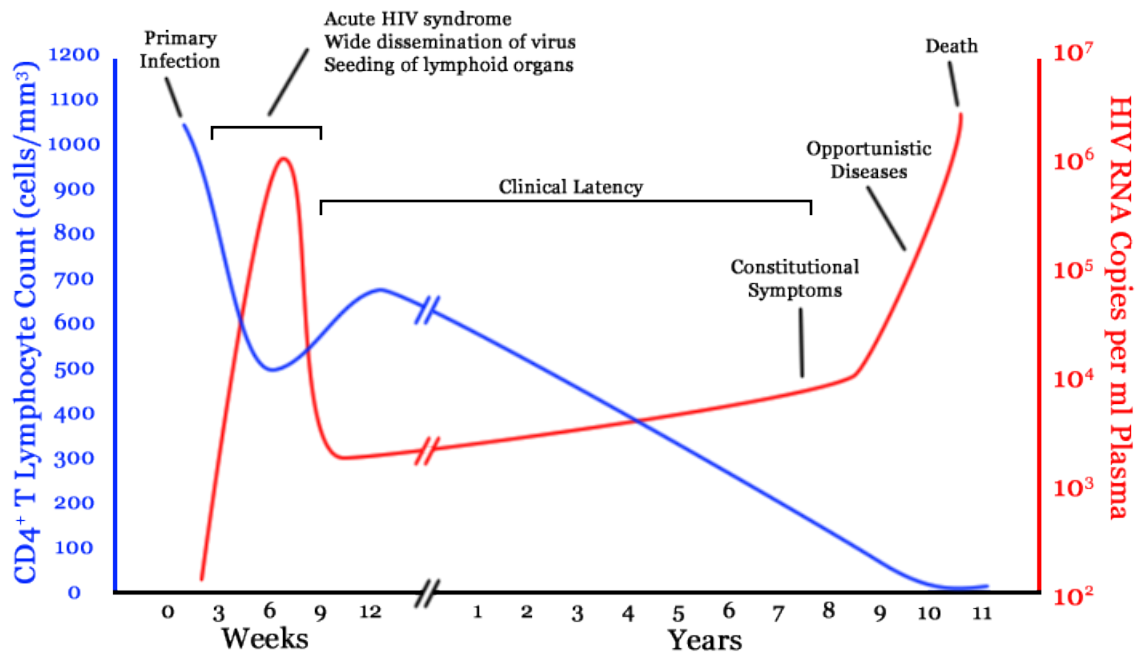
Figure 1.1: HIV viral load and CD4 cells counts in a human over the course of a treatment-naive HIV infection

Chapter 2

Modeling of HIV/AIDS

Here it is presented a brief description of a wide range of mathematical models describing the dynamics and the evolution of HIV and/or its interactions with immune systems, drugs, other infections. Focusing on a complex phenomenon, as described in the previous chapter, mathematical models of HIV need to be enough rich to contain relevant biological aspects, but not so much to prevent mathematical analysis and simulations. So, any mathematical model of a complex biological phenomenon not only need to be an approximation of real world but also has to be addressed to a specific target, to respond a precise question about the phenomenon. Namely, HIV models are used to estimate specific immuno-virological parameters, an optimized therapy or the expected number of newly infected cells (or individuals). Other models are essential in understanding cause-effect relation between different biological processes of the virus. Any different question about HIV gives rise to different models with different approximations regarding the overall phenomenon. This clearly explains why there are many different models that can be classified to be HIV Models, but, in fact, there are different models because they address to different aspects of the complex phenomenon as synthetically named HIV/AIDS.

HIV/AIDS models provide crucial insights into understanding the biological and clinical behavior of HIV infection, during the immune response, asymptomatic phase and during ARV treatment. The two major broad research domains where modeling has been active are AIDS epidemiology and HIV pathogenesis (including treatment). Obviously there are two approaches in both of

these areas: deterministic and stochastic. The dominant parameters in pathogenesis/treatment HIV/AIDS models include immunological and virological responses and their interactions, the dynamics of HIV drug resistance and effects of treatment regimens and practices.

2.1 Deterministic models

Deterministic models, especially any kind of ODE systems, have been successfully used to investigate the interactions of HIV with the immune system [8, 9, 10, 11, 12, 13] and to create better clinical protocols and therapy optimization [14, 15, 16]. The most part is based on the model developed by Nowak and May [17] as shown by Perelson and Callaway [18]. ODE systems using an SIR-like modelization are also successfully used to explain the transmission and the evolution of the virus among hosts [19]

2.1.1 The basic deterministic model of HIV

Many HIV/AIDS models are derived from the first model of viral dynamics by Nowak and May (i.e., the basic model of HIV). Here, we provide a brief description of this model (see also related Equation 3.1.1 and Appendix A) [2]. This model encompasses three variables: the population size of uninfected cells, infected cells, and free virions, termed as x, y and v, respectively. These values can indicate either the total load in a host, or the load in a given volume of blood or tissue.

$$\begin{aligned}
\dot{x} &= \Lambda - x(\mu_x + \alpha v) \\
\dot{y} &= \alpha v x - \mu_y y \\
\dot{v} &= ky - \mu_v v
\end{aligned} \qquad (2.1.1)$$

According to this model, the free virus population v infects the uninfected cells x at a rate proportional to the product of their loads, $-\alpha x v$, where α is the rate of infection (changing healthy cells to infected ones). The infected cells y start producing free virions at a rate proportional to their load, ky, where k is

the production rate of virions by the infected cells, and die at a rate $\mu_y y$, while the free virions v are removed from the system at rate $\mu_v v$. For $R_0 = \frac{k\Lambda\alpha}{\mu_x\mu_y\mu_v} \leq 1$ (R_0 is termed the basic reproductive ratio [17]) there is only one stationary point; the healthy state which is stable ($x_{eq} = \Lambda/\mu_x, y_{eq} = 0, v_{eq} = 0$). For $R_0 > 1$, there are two stationary points: the healthy state becomes unstable and the stable infectious state appears, $(x_{eq}, y_{eq}, v_{eq}) = \left(\frac{\Lambda}{\mu_x R_0}, \frac{\Lambda(R_0-1)}{\mu_y R_0}, \mu_x/\alpha(R_0-1) \right)$.

Starting from equations 2.1.1 there are a lot of models that investigate the coupled dynamics between HIV and Immune System, long-time asymptomatic phase and co-receptor switching from $X4$ to $R5$. They differ, for example, for the form of infection term in x, like $\frac{\alpha vx}{\alpha'+\rho x+\rho'v}$[18], or for the presence of a logistic growth of target cells, where $(\Lambda - \mu_x x)$ is replaced by $rx(1 - x/\bar{x})$.
Depending on which biological problem the models are focused, they include more detailed modelization, for example in modeling drug-resistance there are also populations of resistant virus and resistant infected cells [20]; if the focus is immune system responses one can introduce new population of immune specific response (c) and a killing term in equation for y, like $-\beta yc$, or introduce mutation among viral strains and, consequently, different viral strains, which vary continuously[1] $v(t,s), \quad s \in [0,1]$ or discretely $v_i(t) \quad \forall i = 1, 2, \ldots, N$. Resuming, there are a lot of models developed starting from the basic one, 3.1.1, that focused on different aspects of biology of HIV like quantification of the parameters of the coupled dynamics HIV infection/immune system, or the evolutionary and clinical latency that affect the timing of progression to AIDS.

Others have aimed to examine the role of the immune system with respect to the emergence of resistance mutations [21], viral decay during the intra-cellular phase [22], and long-term HIV infection dynamics.

2.1.2 Modeling HIV dynamics with antiretrovirals therapy

In clinical studies, when three or more drugs are given to HIV-infected patients, to analyze the effects of giving an antiretroviral drug, equations have to be modified. Reverse transcriptase (RT) inhibitors block the ability of HIV to successfully

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[1]In this case the virus genotype is an index s wich vary continuosly in $[0; 1]$

infect a cell. Protease inhibitors (PI) cause the production of non-infectious viral particles.

In the presence of these drugs there are two different virus populations v_i and v_{ni}, infectious and non-infectious viral particles, respectively. So, the model equations become:

$$
\begin{aligned}
\dot{x} &= \Lambda - x\left(\mu_x + (1 - U_{RT})\alpha v_i\right) \\
\dot{y} &= (1 - U_{RT})\alpha v x - \mu_y y \\
\dot{v}_i &= (1 - U_{PI})ky - \mu_v v_i \\
\dot{v}_{ni} &= U_{PI}ky - \mu_v v_{ni}
\end{aligned}
\qquad (2.1.2)
$$

where $U_{RT}, U_{PI} \in [0,1]$ are the efficacies of RT and PI ($U_\bullet = 1$ being a perfect drug), $v = v_i + v_{ni}$ is the total amount of viruses.

The infectious steady state exists for $R_0' \leq 1$ and becomes
$(x^{eq}, y^{eq}, v_i^{eq}, v_{ni}^{eq}) = \left(\frac{\Lambda}{\mu_x R_0'}, \frac{\Lambda(R_0'-1)}{\mu_y R_0'}, \frac{\mu_x}{\alpha}(R_0' - 1)\right)$ where $R_0' = R_0(1 - U_{PI})(1 - U_{RT})$ and $R_0 \leq R_0' \leq 1$

If a 100% effective PI is given to an individual at infectious steady state with viral load v_0, and one assumes that over the time period of interest x remains constant, the viral load decay will be explicitly given by:

$$
v(t) = v_0 exp(-\mu_v t) + \tfrac{\mu_v v_0}{\mu_v - \mu_y}\left(\tfrac{\mu_v v_0}{\mu_v - \mu_y}\left(exp(-\mu_y t) - exp(-\mu_v t)\right) - \mu_y t \quad exp(-\mu_v t)\right)
$$

2.1.3   Control Problem of an HIV model

In a clinical point of view, the problem it to assure that the total amount of free virion particles is below a certain level in a period of time τ. As an example, Nowak el al. [23], set deterministic control problem, consider a finite number of virus strains, or quasi-species, and allow mutations from one strain to another. A finite number of therapeutic options are allowed, where each option consists of the simultaneous application of one or more RT inhibitors. The model incorporates uninfected CD4$^+$ T cells, and infected cell and infectious free virus associated with each virus strain; in this context, the RT inhibitors prevent the free virus

from successfully infecting uninfected CD4$^+$ cells. Each drug option has a different efficacy against each virus strain, thereby allowing for complex drug-virus interactions. Because of the high dimensionality of the control problem, they resort to approximation methods: more specifically, the perturbation methods and the policy improvement algorithm are used to derive a closed form dynamic policy. For $i = 1, ..., I$, let $y_i(t)$ be the density of CD4 cells infected by strain i at time t, and let $v_i(t)$ denote the density of infectious free virus of strain i; non-infectious virions are ignored in this model. If $x(t)$ is defined to be the density of uninfected CD4$^+$ cells at time t, then the state of the system at time t is given by $(x(t), y_1(t), ..., y_I(t), v_1(t), ..., v_I(t))$, which is denoted by $(x(t), y_i(t), v_i(t))$.

The controller has J therapeutic options at time t, where each option corresponds to a prespecified combination of RT inhibitors, each used in a prespecified dosing schedule. For example, a typical combination might be $100mg$ of AZT taken three times daily with $200mg$ of ddI taken twice daily. Control variables $d_j(t)$ satisfy:

$$\sum_{j=1}^{J} d_j(t) \leq 1$$
$$d_j(t) \in \{0, 1\}$$

where $d_j(t) = 1$ if option j is applied at time t, and equals zero otherwise. The assumptions are the following:

- at most one drug combination can be used at each point in time;

- each virus strain has its own infectivity rate, denoted by b_i, which is the rate at which it infects uninfected CD4$^+$ cells.

The RT inhibitors reduce virus infectivity in the following manner:

- $\forall i = 1, \ldots, I$ and $j = 1, \ldots, J$ let p_{ji} denote the efficacy of drug combination j in blocking new infections by virus strain i.

- Under a generic drug policy $d_j(t)$, the infectivity of virus i is $\tilde{\beta}_i[1 - \sum_{j=1}^{J} p_{ji} d_j(t)]$, assuming that the values of p_{ji} are chosen so that the infectivity of each strain is non-negative under all feasible therapeutic strategies.

The dynamics of the system is described by the following set of ordinary differential equations:

$$\dot{x}(t) = \lambda - x(t)\left(\mu + \sum_{i=1}^{I}\tilde{\beta}_i v_i(t)\left(1 - \sum_{j=1}^{J} p_{ji}d_j(t)\right)\right)$$

$$\dot{y}_i(t) = x(t)\left(\sum_{k=1}^{I}q_{ki}\tilde{\beta}_k v_k(t)\left(1 - \sum_{j=1}^{J} p_{jk}d_j(t)\right)\right) - \alpha_i y_i(t)$$

$$\dot{v}_i(t) = \pi_i y_i(t) - \left(k_i + \tilde{\beta}_i x(t)\right)$$

The rate at which uninfected cells are invaded by virus strain i at time t is $\tilde{\beta}_i v_i(t)x(t)$, and each of these potential infections leads to a reduction in free virus. The rate of successful infections by strain i is $\tilde{\beta}_i[1 - \sum_{j=1}^{J} p_{ji}d_j(t)]v_i(t)x(t)$, and these infections cause a simultaneous decline in uninfected cells $x(t)$ and rise in infected cells $y_i(t)$. The mutation rate q_{ij} is the fraction of reverse transcriptions of strain i that result in a cell infected by strain j. Hence, $\sum_{j=1}^{J} q_{ij} = 1$ and the diagonal terms of the mutation matrix are close to one in value, while the off-diagonal terms are nearly zero. Strain i replicates at rate π_i after it has infected a CD4 cell, and thus free virus of strain i is produced at rate $\pi_i y_i(t)$.

In [23], the authors explicitly state: *Because the primary focus of this paper is on therapeutic regimens and not on natural disease progression, we purposely do not incorporate the human immune response into the model. Hence, we implicitly assume that the strength of the immune response remains constant over the time horizon under study. The model also ignores latently infected cells; although most plasma virus comes from actively infected cells, this does not imply that latently infected cells are unimportant for the emergence of drug resistance. Finally, although the lymph system is the location of considerable production of plasma virus and many new infections, our model focuses on the blood and essentially assumes that the blood and lymph system are in equilibrium.*

If τ is the time horizon, the mathematical control problem is to choose the binary controls $(d_j(t), t \geq 0)$ to minimize $\int_0^\tau \sum_{i=1}^{I} v_i(t)dt$

The approximation method, which uses perturbation analysis and the policy improvement algorithm, gives rise to a dynamic index policy: each drug combination has an associated dynamic index, and at each point in time the policy uses

the drug combination with the largest index. The dynamic indices succinctly summarize the efficacy of each drug combination on each virus strain and the marginal benefit of blocking a new cell infection by each virus strain; the latter quantity changes over time as a function of an individual's CD4$^+$ count, viral load and viral mix.

Numerical results for a two-virus, two-drug model suggest that dynamic multi-drug therapies outperform their static counterparts: the total viral load is reduced, the uninfected CD4$^+$ count is increased, and the emergence of drug resistant strains is delayed.

2.2 Stochastic models

Stochastic models, as applied to HIV, proceed from the assumption that the effective population size is so small (or that selective forces are so weak) that random drift dominates over selection. This is particularly true in the first step of infection, after the first peak of infection ($\simeq$ 2-3 weeks after the infections, see figure 1.1. In this case, the hypothesis of selectively neutral mutations (small populations, not uniformly distributed, and very low mutation rates) works fine. Rouzine et al [24] developed a general theory that includes the effects of both selection and drift on a population. They use a set of assumptions appropriate to virus populations, focusing on the interplay between deterministic and stochastic behavior in the context of virologically realistic experiments, applying these to the simplest possible model: mutation at a single site with only two alleles, replicating in a steady-state system (constant number of infected cells) under the influence of constant selective pressure in a single isolated population. They do not consider recombination explicitly, neither allelic dominance. They consider the evolution of one nucleotide position at a time, and they assume that each nucleotide has a choice between only two alleles, conventionally denoting the better-fit allele as wild type and the less-fit allele as mutant. A deleterious mutation event (from wild type to mutant) is referred to as forward mutation, and an advantageous mutation event will be referred to as reverse mutation. Each separate nucleotide is characterized by two parameters, which are both assumed to be much less than unity: the mutation cost (or selection coefficient), s, which is the relative

difference in fitness between the two alleles, and the mutation rate per base per replication cycle, μ. They assume that mutations at different nucleotides have a weak additive effect on virus fitness.

They introduce the virus population model that considers an asexual population of N cells infected with two genetic variants of a virus: n cells are infected with *mutant* virus, and $N - n$ cells are infected with *wild-type* virus. The total population size N is fixed, while n changes in time. During a generation step, each mutant-infected cell produces b_1 mutant virions and then dies, and each wild-type-infected cell produces b_2 wild-type virions and dies. The respective numbers of virions per cell, b_1 and b_2, are assumed to be large, $b_1, b_2 \gg 1$, and differ slightly for the two alleles: $b_1 = b_2(1 - s)$, where s, $s \ll 1$, is, by definition, the selection coefficient (mutation cost), reflecting the difference in fitness. From all the virions produced per generation, N virions are sampled randomly to infect new generation of cells. Each virion, on infecting a cell, can mutate into the opposite genetic variant with a probability μ, $\mu \ll 1$. The virus population model described is a particular case of the Wright-Fisher population with discrete time.

Then, they get out the stochastic equation of evolution, in terms of a discrete Markovian equation.

Let $p(n, t)$ be the probability of n mutant cells at time t, where t is an integer that numbers generations and n can change from 0 through N. If consecutive generations do not overlap, $p(n, t)$ is a Markov chain described by a discrete evolution equation

$$p(n, t+1) \quad = \quad \sum_{n'=0}^{N} P(n|n')p(n', t)$$

(2.2.1)

where $P(n|n')$ is the conditional probability of having n mutants, given that their number at the previous step was n'.

Neglecting mutation events, suppose that the number of mutants in some generation is n'. The total numbers of virions produced by all mutant and all wild-type-infected cells are

$$B_1 \quad = \quad b_1 n'$$
$$B_2 \quad = \quad b_2(N - n')$$

respectively. If n is the number of new mutant-infected cells, then the numbers of mutant and wild-type virions which infect must be n and $N - n$, respectively. The probability of n new mutant cells, $P_0(n|n')$, is proportional to the number of possible ways in which one can choose n mutant virions from B_1 possible mutant virions and $N - n$ wild-type virions from B_2 possible wild-type virions

$$P_0(n|n') \;=\; A\frac{(n')^n(N - n')^{N-n}(1 - s)^n}{n!(N - n)!}$$

$$A \;:\; \sum_n P_0(n|n') = 1$$

Taking mutations into consideration, suppose, at the moment of infection of new cells by n mutant and $N - n$ wild-type virions, m_1 forward and m_2 reverse mutations occur. The resulting number of mutant-infected cells, n'', will be $n'' = n + m_1 - m_2$. The probability of m_2 reverse mutations among n infecting virions, if n is large, is given by Poisson statistics with the average μn

$$\pi(m_2|n) \;=\; \frac{(\mu n)^{m_2}}{m_2!}\exp(-\mu n)$$

$$m_2 \;=\; 0, 1, \ldots \tag{2.2.2}$$

Analogously, the probability of m_1 forward mutations is $\pi(m1|(N - n))$. Finally, the conditional probability $P(n''|n)$ is

$$P(n''|n) \;=\;$$
$$\sum_{n=0}^{N}\sum_{m_1=0}^{N-n}\sum_{m_2=0}^{n}\delta_{n'',n+m_1-m_2}\pi(m_1|(N - n))\pi(m_2|n)P_0(n|n')$$

where the Kronecker symbol $\delta_{i,j}$ is 1 if $i = j$ and 0 otherwise.

Then they simplify the expression for the full conditional probability useng the fact that mutations are rare ($\mu \ll 1$), so that the probability values of m_1 and m_2 are much smaller than those of $N - n$ and n.

The corresponding evolution equation and the boundary conditions have the form:

$$\frac{\partial \rho}{\partial t} \;=\; -\frac{\partial q}{\partial f}$$

$$q(f, t) \;=\; -\frac{1}{2N}\frac{\partial}{\partial f}\left[f(1 - f)\rho\right] - sf(1 - f)\rho - \mu(2f - 1)\rho$$

$$q(f, t)_{f\to 0} \;=\; q(f, t)_{f\to 1} = 0$$

where f is the mutant frequency, ρ is the probability density of the mutant frequency, q is the probability density flux, t is the generation number and N is the population size. Equations are valid under the conditions $s, \mu \ll 1$ and $\mu N \gg 1/lnN$ (large population). In this case, t and f can be treated (approximately) as continuous variables.

In the particular case where the probability density, $\rho(f,t)$, it is possible, combining the first two equations, to derive the evolutionary equation:

$$\frac{\partial \rho}{\partial t} = \frac{f_{max}(1 - f_{max})}{2N} \frac{\partial^2 \rho}{\partial f^2} \qquad + s f_{max}(1 - f_{max}) \frac{\partial \rho}{\partial f} \qquad + \mu(2f_{max} - 1)\frac{\partial \rho}{\partial f}$$
$$Drift(\mu = s = 0) \qquad Selection(N \to \infty, \mu = 0) \quad Mutation(N \to \infty, s = 0)$$

In small populations, where $\mu N \ll 1/[\mu ln(1/\mu)]$, the population can be found, with a finite probability, in a purely monomorphic state of f (0 or 1). In this interval, the total probability density can be broken up into a sum of the continuous probability density and of two singular terms, as given by:

$$\rho(f,t) \;\; = \;\; p_0(t)\delta(f) + p_1\delta(1 - f) + g(f,t)$$

where p_0 and p_1 are the probabilities of having pure wild-type and pure mutant, respectively; $\delta(f)$ denotes the Dirac delta function; and $g(f,t)$, where $f(1 - f) \gg 1/N$, is the continuous part of the probability density. The boundary conditions are:

$$\frac{dp_0}{dt} = -q(0,t), \qquad \frac{dp_1}{dt} = q(1,t) \qquad N \ll 1/[\mu ln(1/\mu)]$$
$$2\mu N p_0 = [fg(f)]_{f \to 0}, \quad 2\mu N p_1 = [(1 - f)g(f)]_{f \to 1}$$

The differential equation for the continuous part of the probability density, $g(f,t)$, has a form:

$$\frac{\delta \rho}{\delta t} \;\; = \;\; -\frac{\delta q}{\delta f}$$
$$q(f,t) \;\; = \;\; -\frac{1}{2N}\frac{\delta}{\delta f}[f(1 - f)\rho] - sf(1 - f)\rho$$

With numerical simulations the authors find that (as long as the mutation rate is lower than the selection coefficient) the dynamic properties differ drastically in three wide intervals of the population size, called the drift, selection-drift,

and selection regimes. Transition between stochastic and deterministic behavior of genetic evolution occurs in the intermediate selection-drift regime, which is expected to be very wide in the population size, especially for DNA systems.

Estimates of typical population sizes and of the time in which new advantageous alleles appear and become fixed in the population suggest that higher organisms may evolve during the selection-drift regime. If this is the case, the speed of evolution depends on three parameters: mutation rate, selective advantage, and population size. Hence, selection pressure and random drift are equally important, although they act differently: selection promotes evolution, and random drift slows it down.

For HIV populations in vivo, theory based on the purifying selection alone predicts either a weak diversity or a very low genetic turnover rate. Experimental searches for rapidly varying bases can provide biological evidence for selection for diversity due to different environments, a changing immune response, changes in host cell populations with time, and other important aspects of HIV infection.

Chapter 3

Toward a new basic Model

The modelization by Nowak et al. [17] shew by simulations and some further discussion the consequences of the increase of diversity of HIV virus population, just by analyzing the critical role of the associated index (Simpson's index), which changes the qualitative behavior of the system; the way by which new strains arise in the game is modelized there by a one-step increase of the dimensionality of the viral population space at random times. In this chapter we propose to model mutations by means of a diffusion term in the genomic space. In this way we are able to follow via computer simulations and numerical analysis all the relevant quantities, highlighting the role of several parameters involved in the model. Another attempt of our models is to consider a more biological situation whith respect to Nowak [17] and Perelson [8], in which we consider the uninfected cells as also an immune system response to infection.

3.1   Nowak-May-like models

Here we list some models of viral infection, ordered by complexity, starting from the basic one by Nowak an May [17], see section 2.1.1.

x denotes the population of healthy immune cells, v that of virions, y that of infected immune cells; further c denotes the specific immune cell population. The parameter $\mu_\bullet$ denotes the specific rate of death of the $\bullet$ population (so $\bullet$ may mean x, v, y, c). Other common parameters are:

- Λ, the immune cell production rate,

- α, the specific rate of infection (changing healthy cells to infected ones),

- k, the production rate of virions by the infected cells.

3.1.1 Analysis of Nowak-May "basic" model

After rescaling[1], assuming $\mu_x = \mu_y$, and keeping the same symbols, the system can be rewritten in the following way:

$$
\begin{aligned}
\dot{x} &= 1 - x(1 + v) \\
\dot{y} &= vx - y \\
\dot{v} &= ky - \mu_v v
\end{aligned}
\tag{3.1.1}
$$

Stationary points

For $R_0 = k/\mu_v \leq 1$, (R_0 is termed the basic reproductive ratio [17]), there is only one stationary point $(x_{eq} = 1, y_{eq} = 0, v_{eq} = 0)$, which represents the healthy state and it is stable. For $R_0 > 1$, there are two stationary points: the healthy state becomes unstable and stable infectious state appears, $(x_{eq}, y_{eq}, v_{eq}) = (1/R_0, 1/R_0(R_0 - 1), R_0 - 1)$, see fig. 3.1.

3.1.2 Nowak-May with immune system responses

Starting from model 3.1.1, the next step is to include that x cells are, in HIV case, immune cells. For this reason, we introduced another population c, coming from x's, and loss terms, $-\beta_x xy$ and $-\beta_c cy$, where $\beta_\bullet$ is the killing rate for immune cells $\bullet$, in the evolution equation for the infected cells y. The c's represent more specialized immune cells stemming from the unspecialized x's which kill the infected ones more efficiently ($\beta_c > \beta_x$).
The equations for the system are:

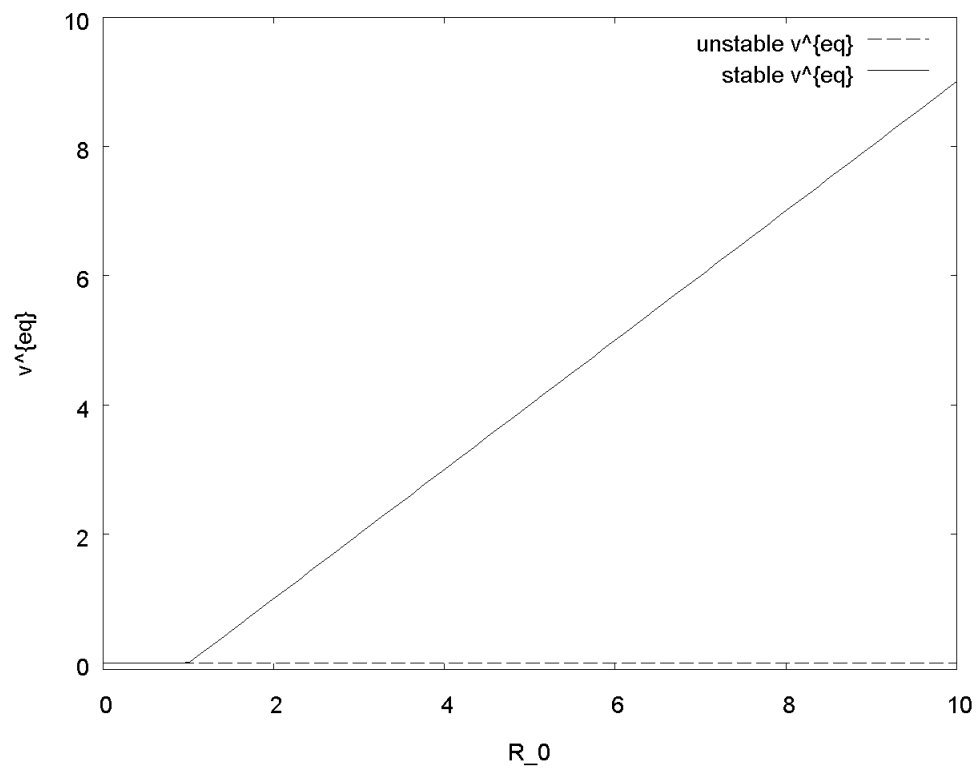[1]see A.1 for detailed calculations of scaling and stationary points

Figure 3.1: Stationary solutions for Nowak-May basic model and their R_0-dependent stability

$$
\begin{aligned}
\dot{x} &= \Lambda - x(\mu_x + \alpha v + Fy) \\
\dot{c} &= Fxy - c(\mu_c + \alpha v) \\
\dot{y} &= \alpha v(x + c) - y(\mu_y + \beta_x x + \beta_c c) \\
\dot{v} &= ky - \mu_v v
\end{aligned}
\qquad (3.1.2)
$$

After the assumption $\mu_x = \mu_c = \mu_y$, several scaling of variables and parameters[2] and keeping the same symbols, the system can be rewritten in the form:

$$
\begin{aligned}
\dot{x} &= 1 - x(1 + v + Fy) \\
\dot{c} &= Fxy - c(1 + v) \\
\dot{y} &= v(x + c) - y(1 + \beta_x x + \beta_c c) \\
\dot{v} &= ky - \mu_v v
\end{aligned}
\qquad (3.1.3)
$$

Stationary points

The healthy state, $(x^{eq}, c^{eq}, y^{eq}, v^{eq}) = (1, 0, 0, 0)$, is a persistent stationary point iff $R_0 < 1 + \beta_x$ (see appendix A). The presence of term $-\beta_x xy$ increases the immunization whit respect to the basic model, where the healthy state is stable iff $R_0 < 1$.

For $F = 0 = \beta_c$, $c^{eq} = 0$ always, and only the aspecific response $(\beta_x \neq 0)$ is active. In this case, iff $R_0 > \beta_x + 1$, just one infectious state solution exists, $(\bar{x}^{eq}, \bar{c}^{eq}, \bar{y}^{eq}, \bar{v}^{eq}) = (1/(R_0 - \beta_x),\ 0,\ 1/R_0(R_0 - \beta_x - 1),\ R_0 - \beta_x - 1)$ and it is stable. When only the aspecific immune reaction is present, the behavior of v^{eq} as a function of R_0 is, except for a shift from 1 to $1 + \beta_x$, exactly the same of the basic model.

So, the presence of an aspecific immune reaction doesn't change the behavior of the system, see figure 3.2.

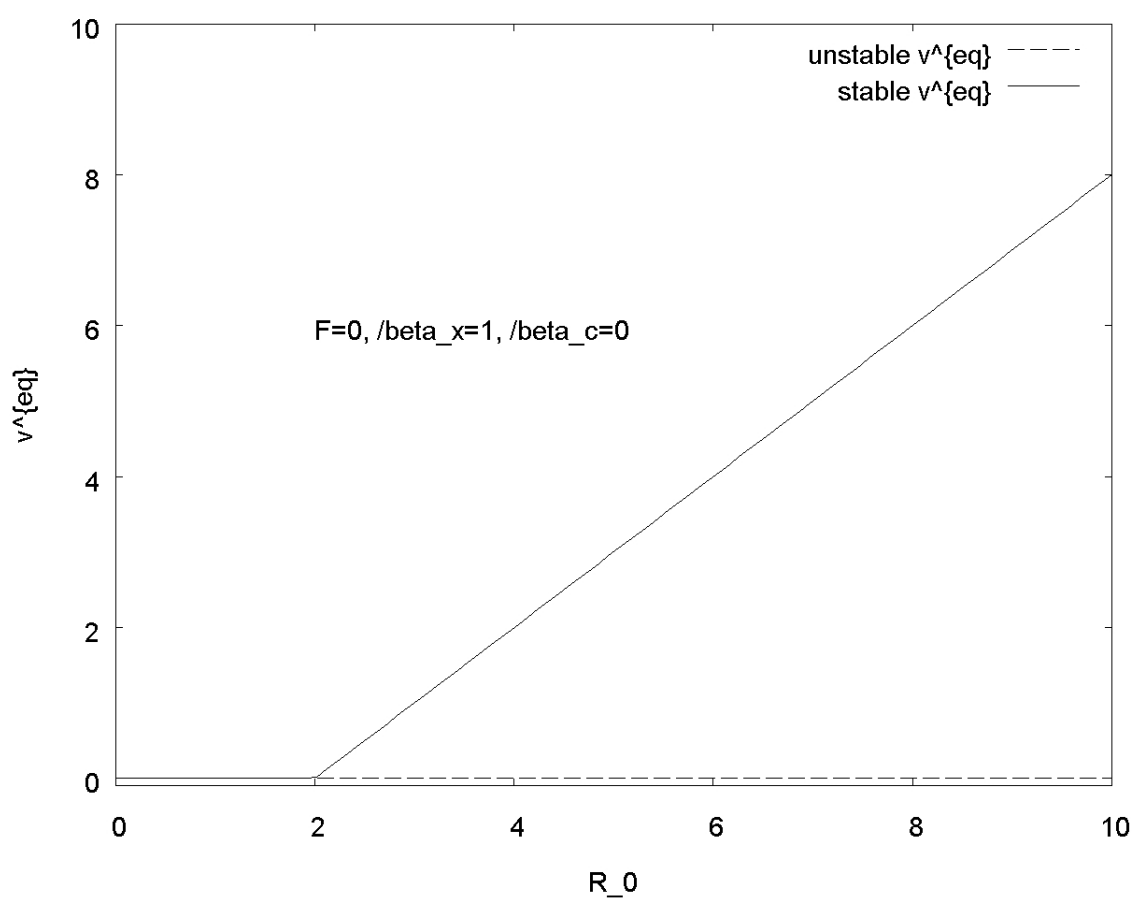[2]See appendix for detailed calculations of scaling and stationary points stability

Figure 3.2: Stationary solutions for Nowak-May with aspecific immune system $(F = \beta_c = 0, \beta_x = 1)$ and their R_0-dependent stability

For the whole system, $(\beta_c, F \neq 0)$, iff $R_0 > 1 + \beta_x$ just one positive solution exist,

$$(\bar{x}^{eq}, \bar{c}^{eq}, \bar{y}^{eq}, \bar{v}^{eq}) = \left((1 + \bar{v}^{eq} + \bar{v}^{eq} F/R_0)^{-1}, \frac{F\bar{v}^{eq}}{R_0(1+\bar{v}^{eq})(1+\bar{v}^{eq}+\bar{v}^{eq}F/R_0)}, \bar{v}^{eq}/R_0, \bar{v}^{eq} \right)$$

where

$$\bar{v}^{eq} = \frac{-[1 + (R_0 - \beta_x - 1) + F(1/R_0 - 1) + \beta_c F/R_0]}{2(1 + F/R_0)} +$$
$$\frac{\sqrt{[1 + (R_0 - \beta_x - 1) + F(1/R_0 - 1) + \beta_c F/R_0]^2 + 4(R_0 - \beta_x - 1)(1 + F/R_0)}}{2(1 + F/R_0)}$$

which is stable, see figure 3.3(a).

In this case, relying on biological facts, we argue that the specific immune responses start only if the aspecific system fails. This means $R_0 > \beta_x + 1$, and in this case we have the above solution for the infectious state[3].

It's interesting to note that $\bar{v}^{eq}(R_0)$, for the whole system, is always less than the corresponding value for the aspecific one, and that $\bar{v}^{eq}(R_0) \simeq R_0 - \beta_x - 2$, as $R_0 \to \infty$, while in the aspecific case, for $R_0 > \beta_x + 1$, $\bar{v}^{eq}(R_0) = R_0 - \beta_x - 1$, see figure 3.3(b).

3.2   First purpose of a Model

As announced before, here we analyze a model that takes into account, differently from Nowak-May models, mutations and effect of immune system. For simplicity, here we state the equations for infected cells, considering N virus strains V_σ, $\sigma = 1, \ldots, N$, and X_σ corresponding immune system responses. We introduce, in the equations for the X's, a term $-\alpha_1 X_\sigma V_\sigma$, $\alpha_1 \in \Re$ that is the sum of two effect. The first one is the stimulated production of immune system cells by the presence of the virus and it is proportional to $X_\sigma V_\sigma$. The second one is due to the fact that infection of cell X_σ by the corresponding V_σ is slightly more probable because, due to immune response, virus V_σ can encounter slightly more likely X's with same σ. The latter term is proportional to $-X_\sigma V_\sigma$. As a consequence, we

---

[3]See appendix A for detailed calculations
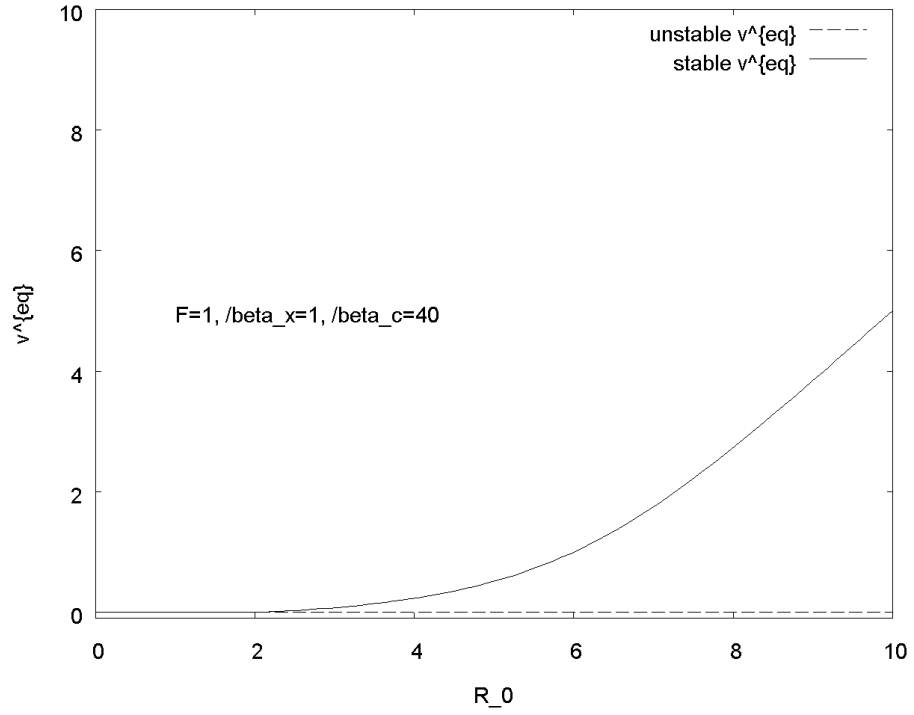
(a)
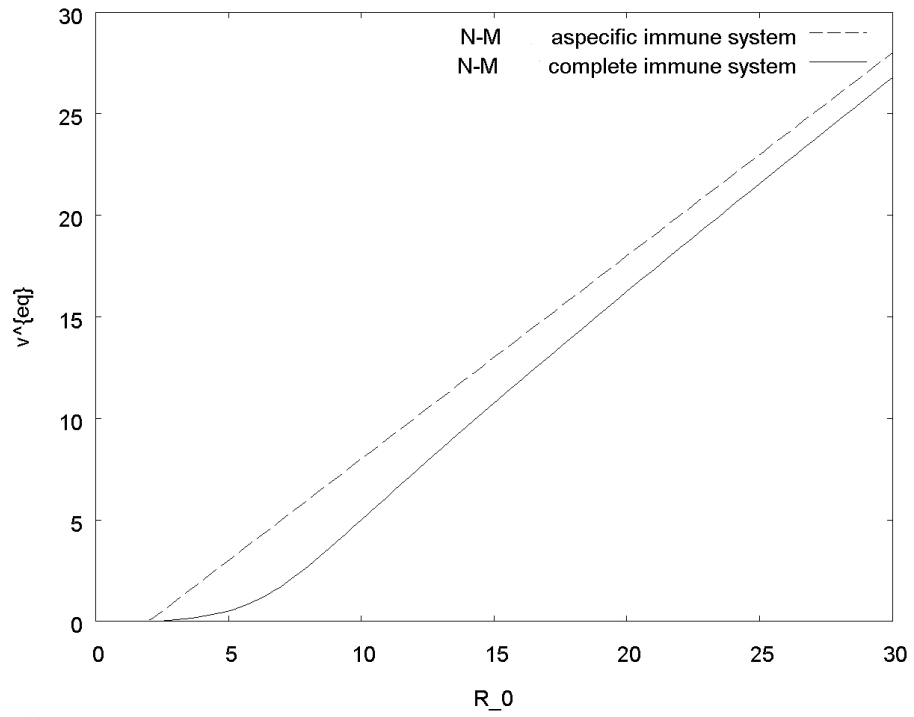
(b)

Figure 3.3: (a): Stationary solutions for Nowak-May model with complete immune system $(F = 1, \beta_c = 10, \beta_x = 1)$ and their R_0-dependent stability(b): Comparison of stationary values of v^{eq} as functions of R_0 between aspecific immune system$(---)$ and complete immune system $(—)$.

introduce a term $\alpha_2 X_\sigma V_\sigma$, $\alpha_2 \in \Re$, that is the sum of a term for immune system killing, proportional to $-X_\sigma V_\sigma$, and a term of virus production, proportional to $X_\sigma V_\sigma$, due to the term of specific infection in equation for the X's.

The mutation term contributes to the evolution of V_σ's like a discrete diffusion term, $\varepsilon(\Delta V)_\sigma$, where $(\Delta V)_\sigma = V_{\sigma+1} + V_{\sigma-1} - 2V_\sigma$. The single population X_σ can be infected by the overall virus populations V_T, defined by $V_T \sum_\sigma V_\sigma$; as consequence the growth of a single population V_σ as a positive term proportional to the overall immune cells population X_T, defined as $X_T = \sum_\sigma X_\sigma$ The resulting equations for the system are:

$$
\begin{aligned}
\forall \sigma &= 1, \ldots, N \\
\dot{X}_\sigma &= \Lambda - X_\sigma \left(\alpha V_T + \alpha_1 V_\sigma + \mu_X \right) \\
\dot{V}_\sigma &= V_\sigma \left(\alpha X_T + \alpha_2 X_\sigma - \mu_V \right) + \varepsilon \left(\Delta V \right)_\sigma
\end{aligned}
\tag{3.2.1}
$$

where:

- Λ is the immune cell production rate,

- α is the rate of infection,

- μ_X, μ_V denote the rate of death of the X's and the V's, respectively.

- $\Lambda, \alpha, \mu_X, \mu_V, \varepsilon \in \Re^+$

Applying the following scaling of variables and parameters to the model 3.2.1

- $t' = \mu_X t$

- $\varepsilon' = \varepsilon/\mu_X$, $\tilde{\alpha} = \alpha\Lambda/\mu_X^2$, $A = \alpha_1/\alpha$, $B = \alpha_2/\alpha$, $C = \mu_V \mu_X/(\alpha\Lambda)$

- $X'_\sigma = X_\sigma \mu_X/\Lambda$, $V'_\sigma = (\alpha/\mu_X)V_\sigma$

we obtain

$$
\begin{aligned}
\forall \sigma &= 1, \ldots, N \\
\dot{X}_\sigma{}' &= 1 - X'_\sigma \left(V'_T + AV'_\sigma + 1 \right) \\
\dot{V}_\sigma{}' &= \tilde{\alpha} V'_\sigma \left(X'_T + BX'_\sigma - C \right) + \varepsilon' \left(\Delta V' \right)_\sigma
\end{aligned}
\tag{3.2.2}
$$

3.2.1 Study of the model with $\varepsilon' = 0$

Under the hypothesis $\varepsilon' = 0$ and that only for $\sigma = 1, \ldots, K$ V'_σ are $\neq 0$ and equal to Z so $V'_T = KZ$, there are K equations of the form $\dot{X}'_\sigma = 1 - X'_\sigma \left((K+A)Z + 1\right)$ and $N - K$ of the form $\dot{X}'_\sigma = 1 - X'_\sigma \left(KZ + 1\right)$ for the X's.

Under these hypotheses, we can reduce the system 3.2.2 to a 3-dimensional system $(X_a, X_{na}, Z)^4$. The X_a's are representative for the K activated X_σ's while the X_{na}'s are for the $N - K$ not activeted X_σ's. The equations are of the form:

$$\begin{aligned}
\dot{X}_a &= 1 - X_a \left((K+A)Z + 1\right) \\
\dot{X}_{na} &= 1 - X_{na} \left(KZ + 1\right) \\
\dot{Z} &= \tilde{\alpha} Z \left((K+B)X_a + (N-K)X_{na} - C\right)
\end{aligned} \qquad (3.2.3)$$

Stationary points

The stationary points are solutions of the following system:

$$\begin{aligned}
0 &= 1 - X_a^* \left((K+A)Z^* + 1\right) \\
0 &= 1 - X_{na}^* \left(KZ^* + 1\right) \\
0 &= \tilde{\alpha} Z^* \left((K+B)X_a^* + (N-K)X_{na}^* - C\right)
\end{aligned}$$

So, neglecting the suffix *, the stationary points are $X_a = \left((K+A)Z + 1\right)^{-1}$ and $X_{na} = \left(KZ + 1\right)^{-1}$. We assume $A > -K$ because we don't want divergence of X_a at stationary point for $Z \geq 0$.

We find an equation for Z:

$$\tilde{\alpha} Z \left(\frac{K+B}{(K+A)Z + 1} + \frac{N-K}{KZ + 1} - C\right) = 0$$

$Z = 0$ gives the solution of non-infectious state $(X_a, X_{na}, Z) = (1, 1, 0)$. For $Z \neq 0$ we find this second order equation in Z:

$$Z^2 + Z\frac{C(2K+A) - K(B-A) - N(K+A)}{CK(K+A)} + \frac{C - N - B}{CK(K+A)} = 0 \qquad (3.2.4)$$

---

[4]a stand for activated and na for not activated

that can be written in the form $Z^2 - (Z_1 + Z_2)Z + Z_1 Z_2 = 0$, where $Z_{1,2}$ are the solutions of the equation.

If $Z_1 Z_2 < 0$, there exists only one positive solution. For $C > 0$, in the region $A > -K$, $CK(K + A) > 0$ the condition $Z_1 Z_2 < 0$ is true iff $C < N + B$. We call this region *Nowak-May* (NM region) because it reproduces the behavior of Nowak-May model.

Otherwise, if $C > N + B$, $Z_1 Z_2 > 0$, that implies only $sign(Re(Z_1)) = sign(Re(Z_2))$ and we need to study the sign of $Z_1 + Z_2$. $Z_1 + Z_2 > 0$ iff $CK(2K + A) - K(B - A) - N(K + A) < 0$. This gives another condition for C: $C < \frac{K(B+N)+A(N-K)}{2K+A}$.

Resuming, the condition for C is $N + B < C < \frac{K(B+N)+A(N-K)}{2K+A}$. This is true iff $\frac{K(B+N)+A(N-K)}{2K+A} > N + B$ and $\frac{K(B+N)+A(N-K)}{2K+A} > 0$.

This gives a condition for B, $-N\frac{K+A}{K} + A < B < -NK\frac{1+A/N}{K+A}$ and, in this case, $B \in \Re^-$. This is possible iff $A > 0$.

If the two above conditions, for B and C, aren't satisfied, $Re(Z_1), Re(Z_2) < 0$ and the system has only one stationary point at $(1, 1, 0)$.

We need to verify when $Z_1, Z_2 \in \Re$. If exist, the solutions are:

$$Z_{1,2} = \frac{(-C(2K + A) + K(B - A) + N(K + A))}{2CK(K + A)} \quad (3.2.5)$$

$$\mp \frac{\sqrt{(-C(2K + A) + K(B - A) + N(K + A))^2 - 4CK(K + A)(C - N - B)}}{2CK(K + A)}$$

$Z_1, Z_2 \in \Re$ is verified iff:

$$\Delta = (-C(2K + A) + K(B - A) + N(K + A))^2 - 4CK(K + A)(C - N - B) > 0$$

So this give to an inequality equation for C:

$$A^2 C^2 - 2AC\left(-K(K + B) + (N - K)(K + A)\right) + (K(K + B) + (K + A)(N - K))^2 > 0$$

which the solution is $C < C_-$ or $C > C_+$,
where $C_\mp = 1/A \left(\sqrt{(K + A)(N - K)} \mp \sqrt{-K(K + B)}\right)^2$,
iff $N + B < C_\mp < \frac{K(B+N)+A(N-K)}{2K+A}$.

But $C_+ > \frac{K(B+N)+A(N-K)}{2K+A}$ and $C_- < \frac{K(B+N)+A(N-K)}{2K+A}$ always if $-N\frac{K+A}{K} + A < B < -NK\frac{1+A/N}{K+A}$.

So C can't be greater than C_+ and there are 2 positive solutions Z_1, Z_2 iff

$$
\begin{aligned}
A &> 0 \\
-N\frac{K+A}{K} + A < B &< -NK\frac{1+A/N}{K+A} \\
N+B < C &< C_-
\end{aligned}
\tag{3.2.6}
$$

we call this parameter region GST[5] zone. So, for $A > 0$, we can represent in the plane (B, C) the different regions for stationary points, graph 3.4.

Stability of Stationary Points

Now we study the conditions 3.2.6 for A, B, C, that give 2 positive solution for Z^{eq}.

The associated characteristic equation for the eigenvalues T, $p(T) = 0$, of the linearized matrix is:

$$
\begin{vmatrix}
-(K+A)Z - 1 - T & 0 & -X_a(K+A) \\
0 & -KZ - 1 - T & -KX_{na} \\
\tilde{\alpha}Z(K+B) & \tilde{\alpha}Z(N-K) & \tilde{\alpha}\left(X_a(K+B) + (N-K)X_{na} - C\right) - T
\end{vmatrix} = 0
$$

For (1,1,0) the matrix becomes:

$$
\begin{vmatrix}
-1-T & 0 & -(K+A) \\
0 & -1-T & -K \\
0 & 0 & \tilde{\alpha}(B+N-C) - T
\end{vmatrix} = 0 = (-1-T)(-1-T)(-(C-N-B)-T)
$$

So $T_1 = T_2 = -1$ and $T_3 = -(C - N - B) < 0$ iff $C > N + B$. $Z^{eq} = 0$ is stable iff $C > N + B$. In the Nowak-May region, $C < N + B$, $Z^{eq} = 0$ is unstable.

For $([(K+A)Z+1]^{-1}, (KZ+1)^{-1}, Z)$, where $Z = Z_{1,2}$,

$$
\begin{vmatrix}
-(K+A)Z - 1 - T & 0 & ((K+A)Z_{1,2}+1)^{-1} - (K+A) \\
0 & -KZ - 1 - T & (KZ+1)^{-1} - KX_{na} \\
\tilde{\alpha}Z(K+B) & \tilde{\alpha}Z(N-K) & -T
\end{vmatrix} = 0
$$

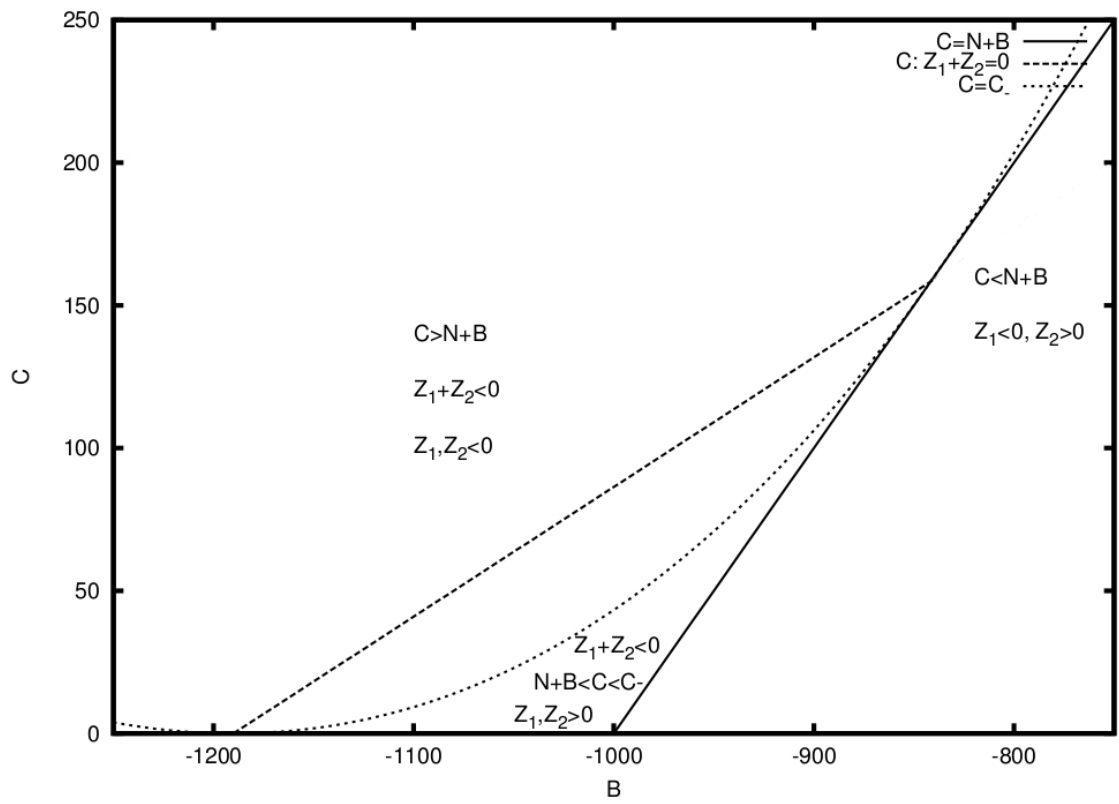
[5]Gobron-Santoro-Triolo

Figure 3.4: Condition for existence of stability points Z_1, Z_2 in the plane (B, C).
Here $N = 1000$, $K = 50$, $A = 10$

we use, for the last term in the diagonal $(-T)$, that these points are solutions for the stationary equation $X_a(K + B) + (N - K)X_{na} - C = 0$.

This gives a third degree equation for T:

$$T^3 +$$

$$T^2(1 + KZ + 1 + K(Z + A)) +$$

$$T\left[[1 + Z(K + A)](1 + KZ) + \frac{\tilde{\alpha}Z\left[(K(N - K))(1 + Z(K + A)) + (K + B)(K + A)(1 + KZ)\right]}{[1 + Z(K + A)](1 + KZ)}\right] +$$

$$\tilde{\alpha}Z\left[\frac{[1 + Z(K + A)]K(N - K)}{1 + KZ} + \frac{(K + B)(K + A)(1 + ZK)}{1 + Z(K + A)}\right] = 0$$

Its solutions satisfy the following relations[6]:

$$S = T_1 + T_2 + T_3 = -(1 + KZ + 1 + K(Z + A)) < 0$$

$$S_2 = T_1T_2 + T_2T_3 + T_3T_1 =$$

$$[1 + Z(K + A)](1 + KZ) + \frac{\tilde{\alpha}Z\left[(K(N - K))(1 + Z(K + A)) + (K + B)(K + A)(1 + KZ)\right]}{[1 + Z(K + A)](1 + KZ)}$$

$$P = T_1T_2T_3 = \tilde{\alpha}Z[1 + Z(K + A)](1 + ZK)\left[-\frac{K(N - K)}{(1 + KZ)^2} - \frac{(K + B)(K + A)}{[1 + Z(K + A)]^2}\right]$$

The condition $S < 0$, gives us the information that almost one solution need to be neagtive. So $\forall\ Z \in \Re^+ \quad \exists\ T_3 < 0$. For the study of the sign of P we use the fact that, at the stationary points Z_1, Z_2, $C = \frac{N - K}{1 + KZ} + \frac{K + B}{1 + Z(K + A)}$. Thinking C as a function of Z, let us derive it, giving $\frac{\partial C}{\partial Z} = -\frac{K(N - K)}{(1 + KZ)^2} - \frac{(K + B)(K + A)}{[1 + Z(K + A)]^2}$, and $sign(P) = sign(\frac{\partial C}{\partial Z})$. Taking into account that $C(Z = 0) = N + B$ and $\frac{\partial C}{\partial Z}|_{Z=0} > 0$, and supposing $C > N + B$ fixed, we find two values of Z: the first, Z_1, in the zone $\frac{\partial C}{\partial Z} > 0$ and the second one, Z_2, in the zone $\frac{\partial C}{\partial Z} < 0$. So, for $Z = Z_1$, $P > 0$, which implies that there exist $T_1 > 0$ ($T_2 < 0$) and Z_1 is always unstable, while for $Z = Z_2$ $P < 0$, which means that $sign(Re(T_1)) = sign(Re(T_2))$ and it is necessary to study the sign of S_2. For $\tilde{\alpha} = 0$ $S < 0$, $P = 0$ and $S_2 > 0$, which

---

[6]We can rewrite $p(T)$ as a combination of its roots:

$$(T - T_1)(T - T_2)(T - T_3) = 0$$
$$T^3 - T^2(T_1 + T_2 + T_3) + T(T_1T_2 + T_2T_3 + T_3T_1) - T_1T_2T_3 = 0$$

means that $Re(T_1), Re(T_2) < 0$ and $T_3 = 0$. For $\tilde{\alpha} \ll 1$ $P < 0$, which implies, for continuity, $T_3 < 0$.

Since $P < 0$ $\forall \tilde{\alpha}$, and S_2 is monotone in $\tilde{\alpha}$, the only way to have either $Re(T_1) > 0$ or $Re(T_2) > 0$ is that they pass trough 0 as opposite purely imaginary, $T_{1,2} = \pm i\theta$. In this case, $P = T_3\theta^2$, $S = T_3$, $S_2 = \theta^2$ which gives the necessary condition $P - SS_2 = 0$. Now we show that is also sufficient to have $T_{1,2} = \pm i\theta$.

i) If T_1, T_2 are complex conjugates, $T_{1,2} = w \pm i\theta$, then:

$$
\begin{aligned}
P &= T_3(w^2 + \theta^2) \\
S_2 &= 2wT_3 + w^2 + \theta^2 \\
S &= T_3 + 2w \\
P - SS_2 &= T_3(w^2 + \theta^2) - (T_3 + 2w)(2wT_3 + w^2 + \theta^2) \\
&= 2w(T_3^2 + 2wT_3 + w^2 + \theta^2)
\end{aligned}
$$

Setting $P - SS_2 = 0$ gives either $w = 0$ or $(w + T_3)^2 + \theta^2 = 0$, but the latter one is absurd. So, only $w = 0$ is the solution for the c.c. case.

ii) Let T_1, T_2 be real and set $P - SS_2 = (T_1 + T_2)(T_1 + T_3)(T_3 + T_2) = 0$.

Because $sign(T_1) = sign(T_2)$, then $(T_1 + T_2) \neq 0$ and if $T_1, T_2 < 0$ there is no solution. On the other hand, if $T_1, T_2 > 0$, let be, without loosing generality, $T_1 = -T_3$ a possible solution; this gives $P = -T_3^2 T_2 < 0$ and $S = T_1 + T_2 + T_3 = T_2 < 0$, that is absurd due to the hypothesis $T_1, T_2 > 0$.

Finally we can find the critical value of $\tilde{\alpha}$, $\tilde{\alpha}^*$, in which $Re(T_1) = Re(T_2) = 0$, applying the condition $P - SS_2 = 0$. Introducing $q_1 = 1 + KZ_2$ and $q_2 =$

$1 + K(Z_2 + A)$ we have:

$$S = -(1 + KZ_2 + 1 + K(Z_2 + A)) = -q_1 - q_2$$

$$S_2 =$$
$$[1 + Z_2(K + A)](1 + KZ_2) + \frac{\tilde{\alpha}Z_2(K(N - K))(1 + Z_2(K + A))}{[1 + Z_2(K + A)](1 + KZ_2)}$$
$$+ \frac{\tilde{\alpha}Z_2(K + B)(K + A)(1 + KZ_2)}{[1 + Z_2(K + A)](1 + KZ_2)}$$

$$= q_1 q_2 + \tilde{\alpha}\left[\frac{(N - K)(q_1 - 1)}{q_1} + \frac{(K + B)(q_2 - 1)}{q_2}\right]$$

$$P = \tilde{\alpha}^* Z_2[1 + Z_2(K + A)](1 + Z_2 K)\left[-\frac{K(N - K)}{(1 + KZ_2)^2} - \frac{(K + B)(K + A)}{[1 + Z_2(K + A)]^2}\right]$$

$$= -\tilde{\alpha}^*\left[\frac{q_2(q_1 - 1)(N - K)}{q_1} + \frac{q_1(q_2 - 1)(K + B)}{q_2}\right]$$

$$P - SS_2 =$$
$$= \tilde{\alpha}^*\left[\frac{(q_1 - 1)(N - K)}{q_1}(q_1 + q_2 - q_2) + \frac{(q_2 - 1)(K + B)}{q_2}(q_1 + q_2 - q_1)\right] + q_1 q_2(q_1 + q_2) = 0$$

$$\tilde{\alpha}^* = \frac{-q_1 q_2(q1 + q_2)}{(N - K)(q_1 - 1) + (K + B)(q_2 - 1)} =$$

$$= \frac{-(1 + KZ_2)[1 + Z_2(K + A)][1 + KZ_2 + 1 + Z_2(K + A)]}{Z_2[(N - K)K + (K + B)(K + A)]}$$

$$(3.2.7)$$

Under the condition $-N\frac{K+A}{K} + A < B < -NK\frac{1+A/N}{K+A}$, $\tilde{\alpha}^* > 0$. Resuming, for stationary points we find that:

- $(X_a, X_{na}, Z) = (1, 1, 0)$ is locally stable if $C < N + B$, locally unstable if $C > N + B$;

- $(X_a, X_{na}, Z) = ([1 + Z_1(K + A)]^{-1}, (1 + KZ_1)^{-1}, Z_1)$, when exists (see conditions 3.2.6) is locally unstable;

- $(X_a, X_{na}, Z) = ([1 + Z_2(K + A)]^{-1}, (1 + KZ_2)^{-1}, Z_2)$, when exist $(C > N + B$ or conditions 3.2.6) is locally stable iff $\tilde{\alpha} < \tilde{\alpha}^*$.

Numerical simulations

In this section we show some numerical simulations of the system 3.2.3, obtained
fixing some parameters and varying others, as follows:

- $N = 1000$, $K = 50$, $C = 20$, $A = 10$ fixed

- $B = -950$ (Nowak-May zone) or $B = -1050$ (GST zone)

- $\tilde{\alpha} < \tilde{\alpha}^*$ or $\tilde{\alpha} > \tilde{\alpha}^*$

We show the simulations by drawing two graphs: the first one represents the time
evolution of $V_T/K = Z$ and of the average number of total X cells, X_T/N. The
second one is the plot of $(V_T/K, X_T/N)$ that give an idea of the trajectories in
the phase space; it will be very useful to compare these results whit simulations
in the case $\varepsilon \neq 0$. A very useful index to understand the evolution of the virus
distribution, in other words its variability (see Nowak and May [17]), is the Simp-
son Index (SI), defined as $SI = \frac{\sum_\sigma V_\sigma^2(t)}{(\sum_\sigma V_\sigma(t))^2}$. Note that $1/N < SI < 1$ and in our
cases $1/N = 10^{-3}$. We show it on the same graph for V_T/K and X_T/N.

GST zone, $\tilde{\alpha} = 0.02 < \tilde{\alpha}^*$. For GST zone $\tilde{\alpha}^* = 0.21$. As expected, the
simulation, for $\tilde{\alpha} < \tilde{\alpha}^*$, shows a stable steady state in $Z_2 = 0.061$, graph 3.5. The
initial conditions for V's are $V_T/K(0) = 0.3$, obtained setting $V_\sigma(0) = 0.3 \quad \forall \sigma = 1, \dots, K$ and $V_\sigma(0) = 0 \quad \forall \sigma = K+1, \dots, N$. The initial condition $X_T/N(0) = 1$
was obtained simply setting $X_\sigma(0) = 1 \quad \forall \sigma$

GST zone, $\tilde{\alpha} = 0.23 > \tilde{\alpha}^*$. In this case the simulation shows, as expected, a
stable sready state only in $Z_0 = 0$, graph 3.6. The initial conditions are the same
as in the previous simulation, graph 3.5.

If we set the initial conditions near the stationary point (X_a, X_{na}, Z_2), we
can see, in graph 3.7 that, for $\tilde{\alpha} > \tilde{\alpha}^*$, the trajectory is destabilized due to the
presence of c.c. eigenvalues as predicted in calculations of $\tilde{\alpha}^*$ in equations 3.2.7.
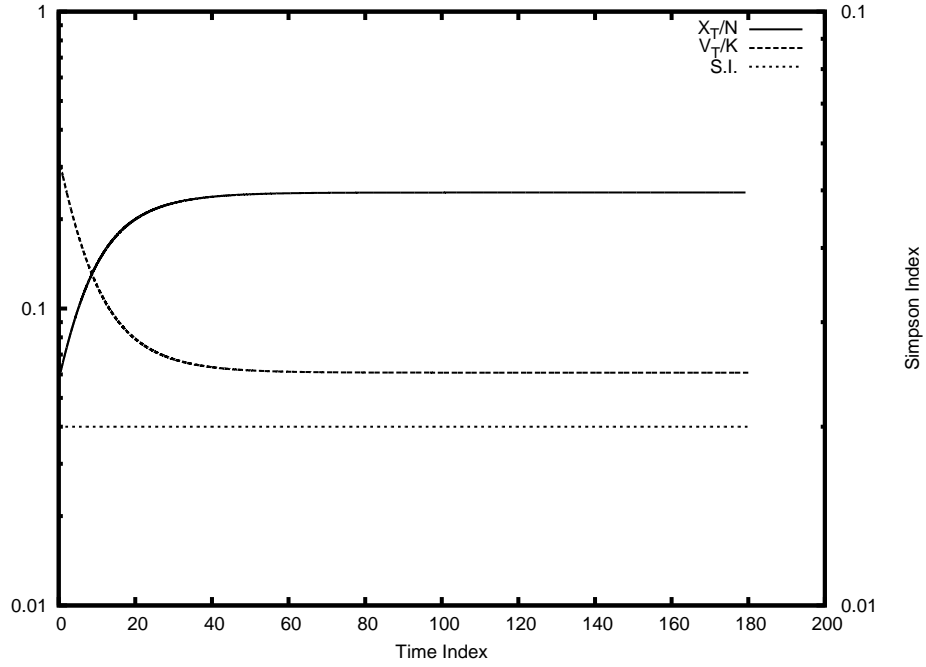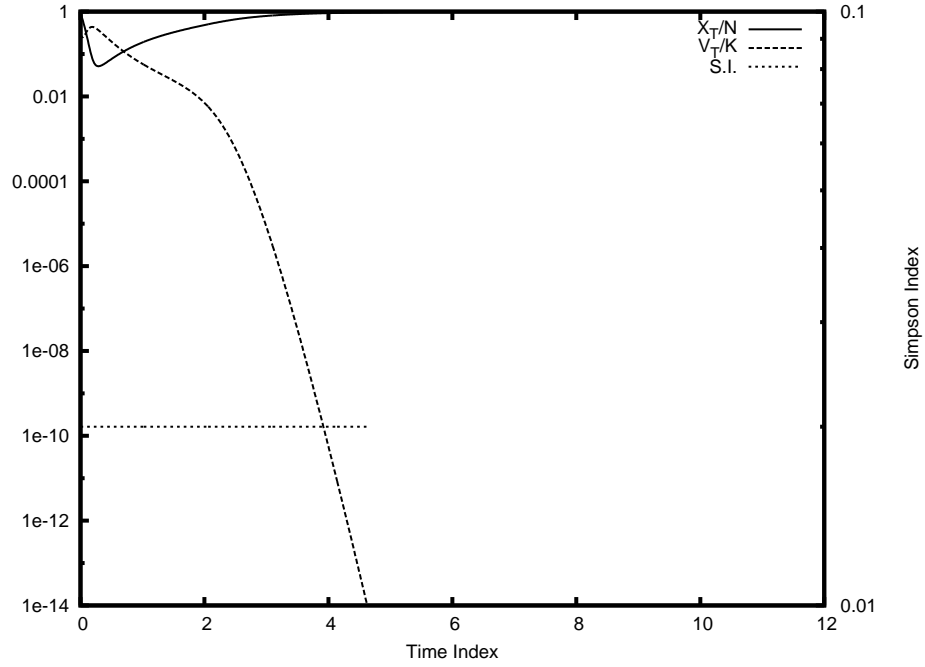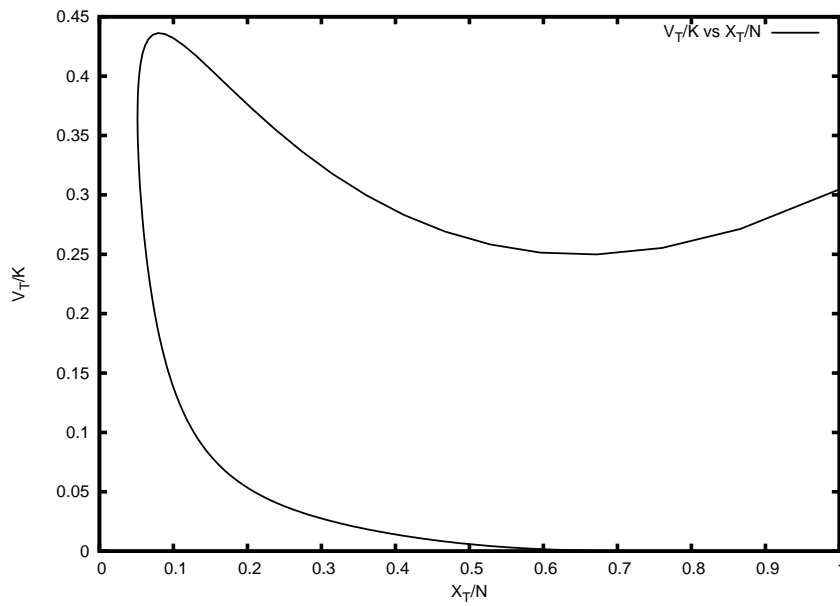
(a)
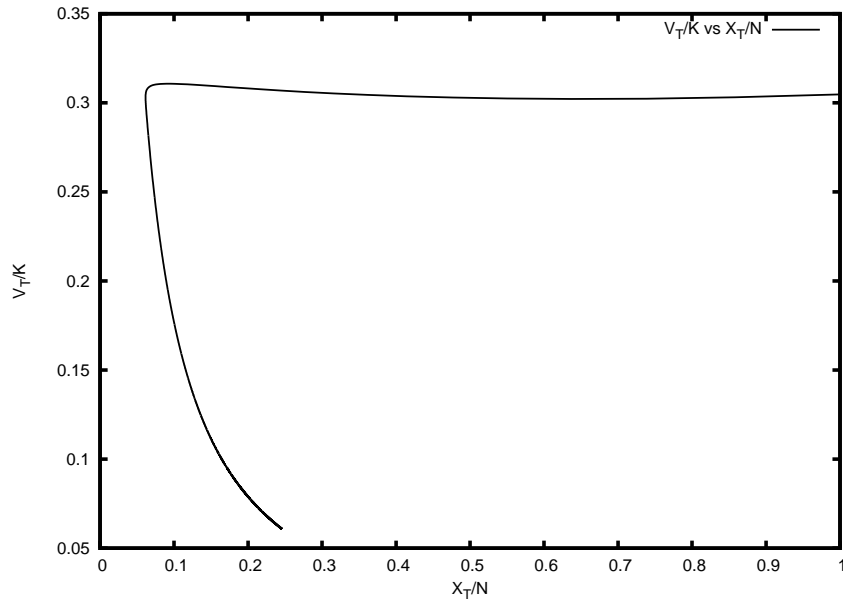
(b)

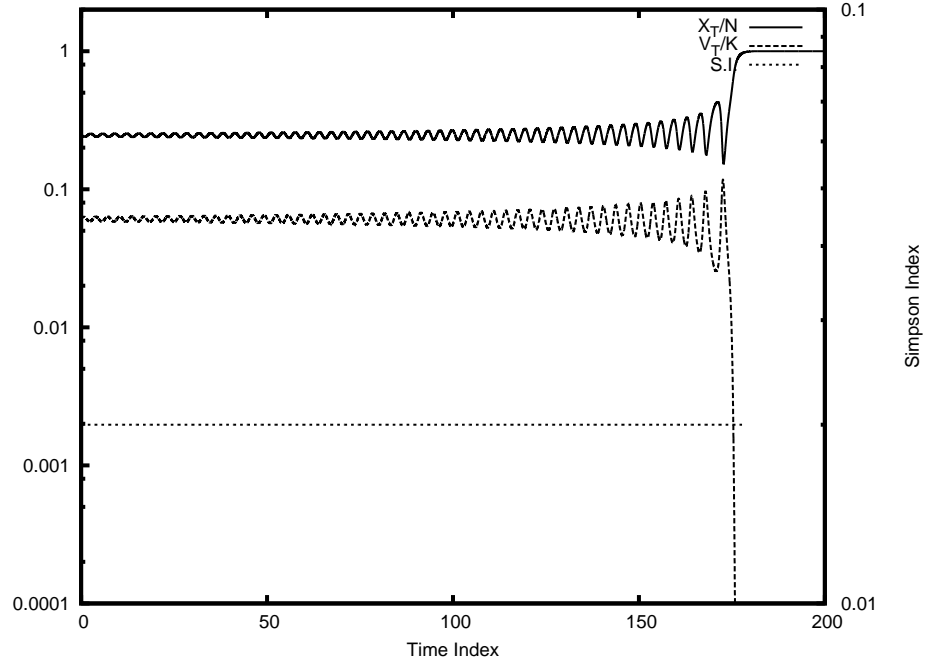Figure 3.5: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$
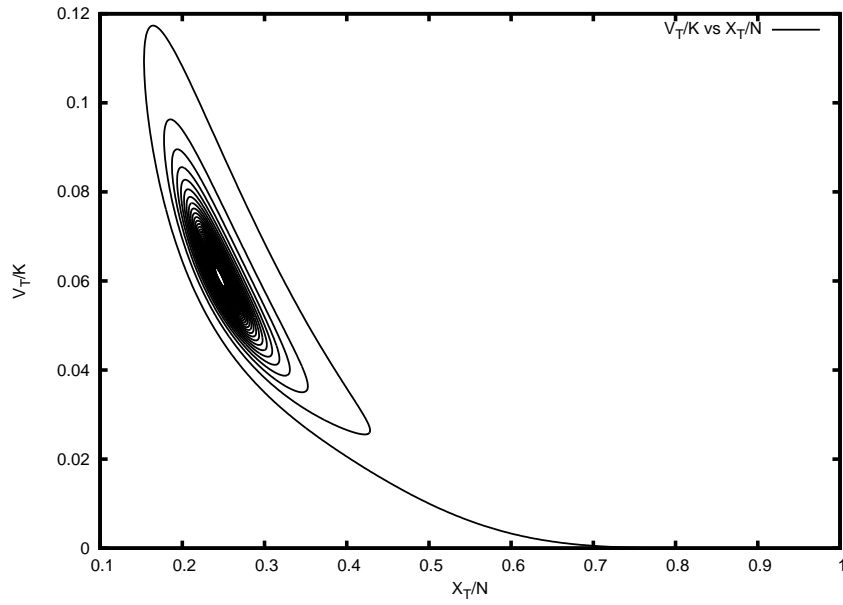
(a)

(b)

Figure 3.6: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$
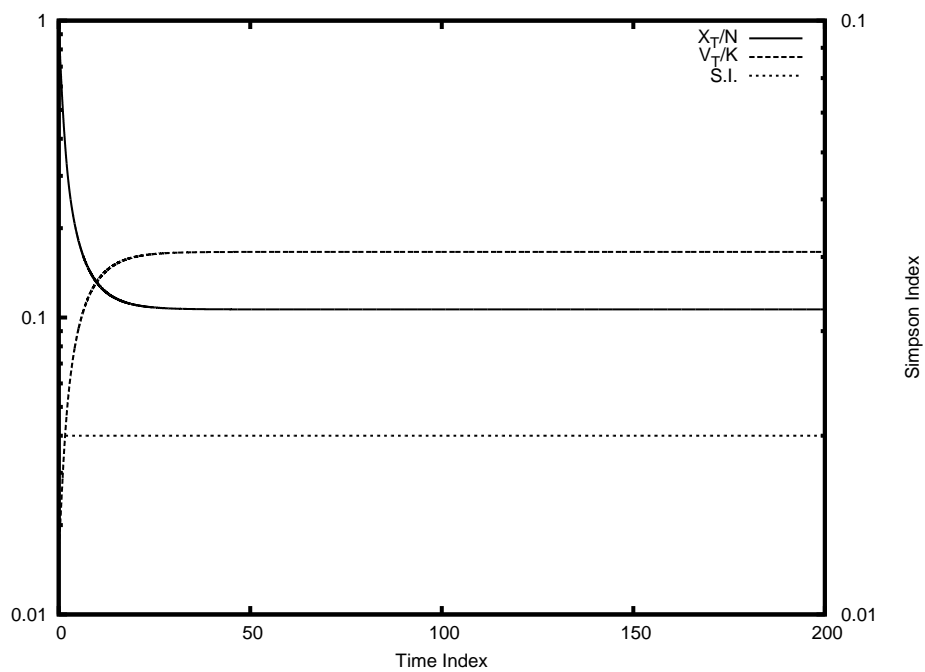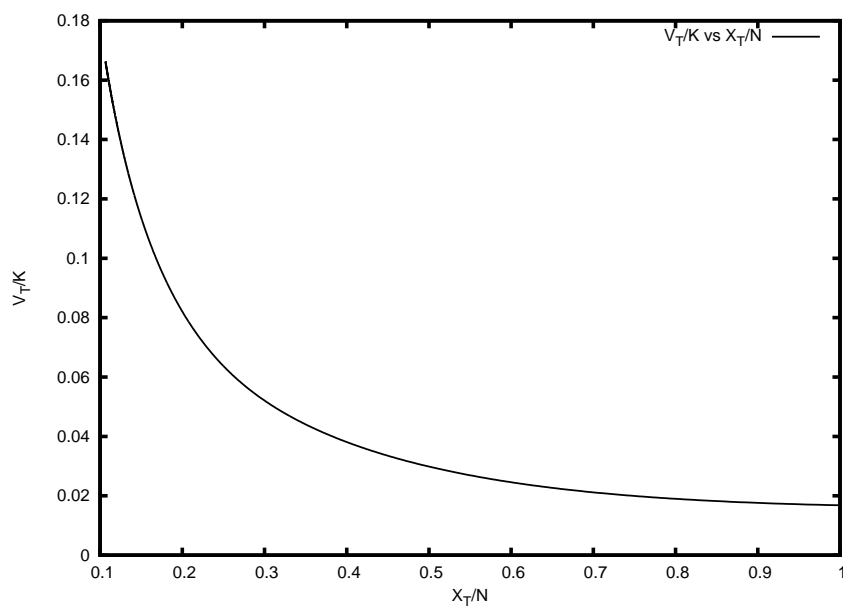
(a)

(b)

Figure 3.7: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$

NM zone, $\tilde{\alpha} = 0.01 < \tilde{\alpha}^*$**.** For NM zone $\tilde{\alpha}^* = 1.92$. If $\tilde{\alpha} < \tilde{\alpha}^*$ the simulation shows the expected stable steady state in $Z = 0.17$, graph 3.8. The initial conditions for V's are $V_T/K(0) = 0.84$, obtained setting $V_\sigma(0) = 0.84 \quad \forall \sigma = 1, \ldots, K$ and $V_\sigma(0) = 0 \quad \forall \sigma = K+1, \ldots, N$. The initial condition $X_T/N(0) = 1$ was obtained simply setting $X_\sigma(0) = 1 \quad \forall \sigma$

NM zone, $\tilde{\alpha} = 1.96 > \tilde{\alpha}^*$**.** In this case the simulation shows a limit cycle because Z_2 becomes locally unstable but the system can explode. If we start near the stationary point Z_2, the system evolves to the limit cycle inside it, graph 3.9. If the initial conditions are far away from Z_2 and Z_0, the system tends to the limit cycle outside it, graph 3.10 .

(a)

(b)

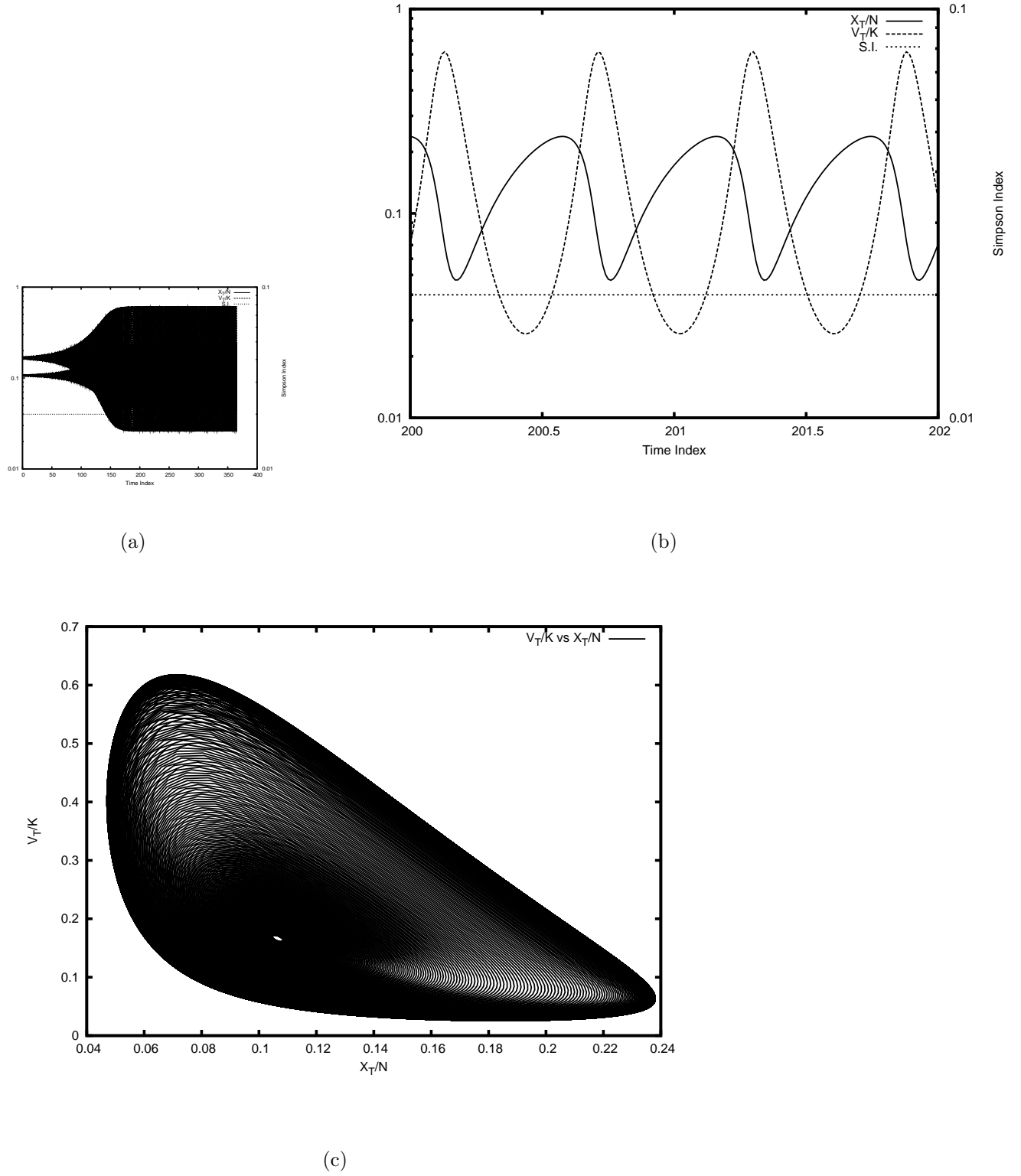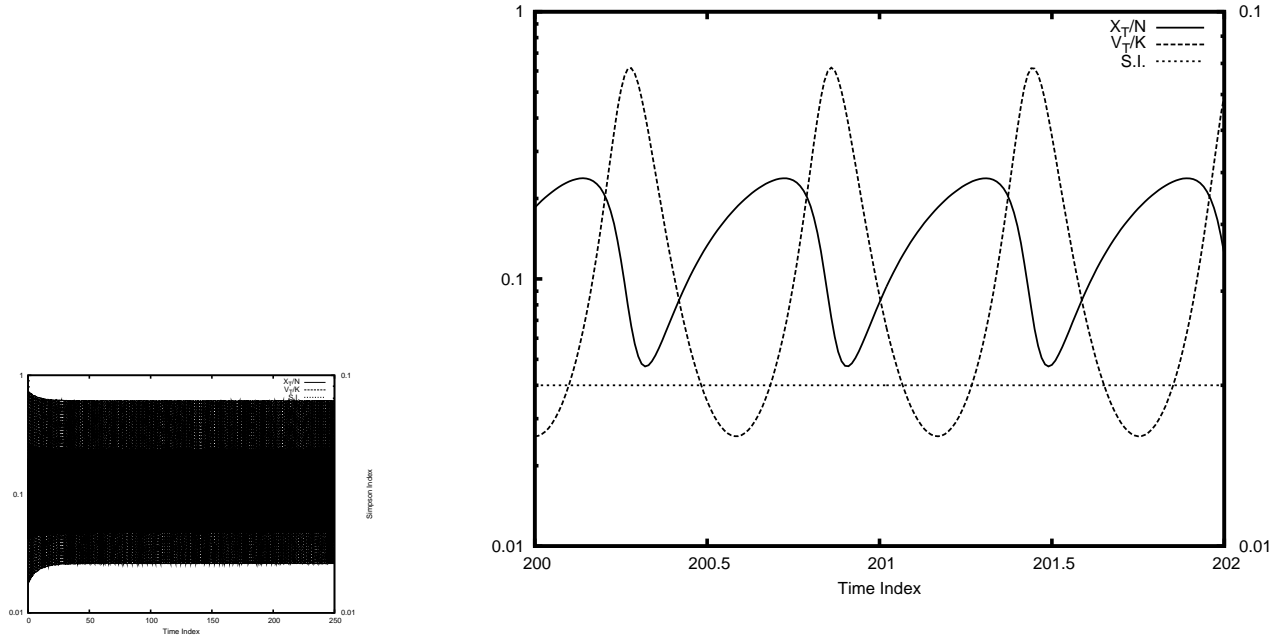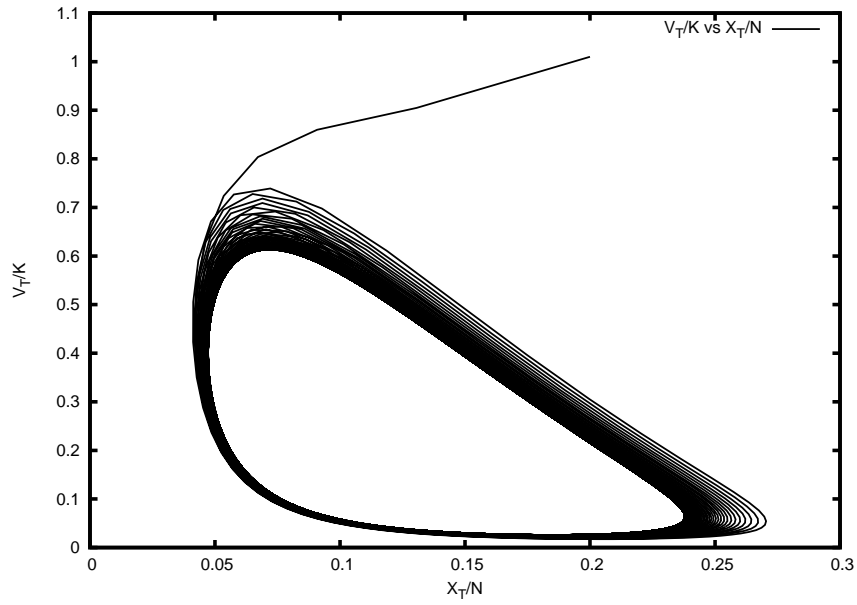Figure 3.8: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$

(a)

(b)

(c)

Figure 3.9: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$

(a)

(b)

(c)

Figure 3.10: (a) Time evolution of V_T/K and X_T/N; (b) Zoom of graph (a) (c)Evolution in the plane $(X_T/N, V_T/K)$

3.2.2   Numerical Study of the Model with $\varepsilon \neq 0$

Until now, analitical study of the model with $\varepsilon \neq 0$ hasn't yet given a better understanding of the system. So we run many numerical simulations in order to have some information on the behavior of the system in this case. Depending on the distribution of v_σ at the initial conditions, the simulations indicate that the virus can either grow and compact in a single strain σ (graph 3.11), 3.12), if it is concentrated, or be killed otherwise. The very important thing is that the NM zone, when $\tilde{\alpha} > \tilde{\alpha}^*$, and the distribution of the virus is concentrated, seems to have a stable stationary point, graph 3.13 with the same parameters as the previous simulations.

Despite increasing ε the simulation show the same behavior for the system. This can do essentially to the fact that non active X cells can growth as the active ones. When virus mutate it find a X_σ that as already the right volume. In other word, we need a system in which the actives can growth more than the non active ones. In the next section we will try to modify the model in this way.
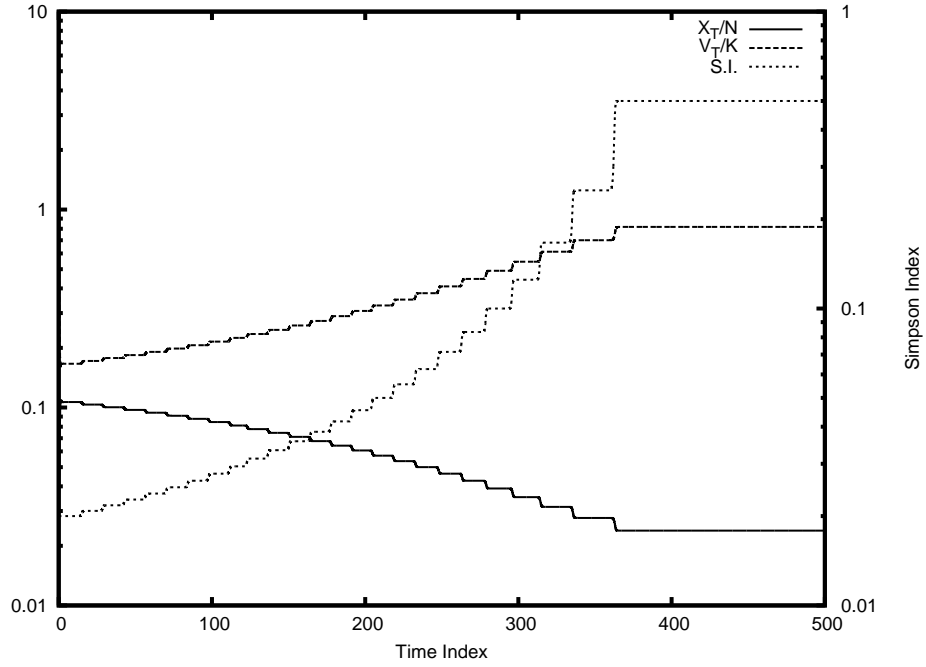
(a)
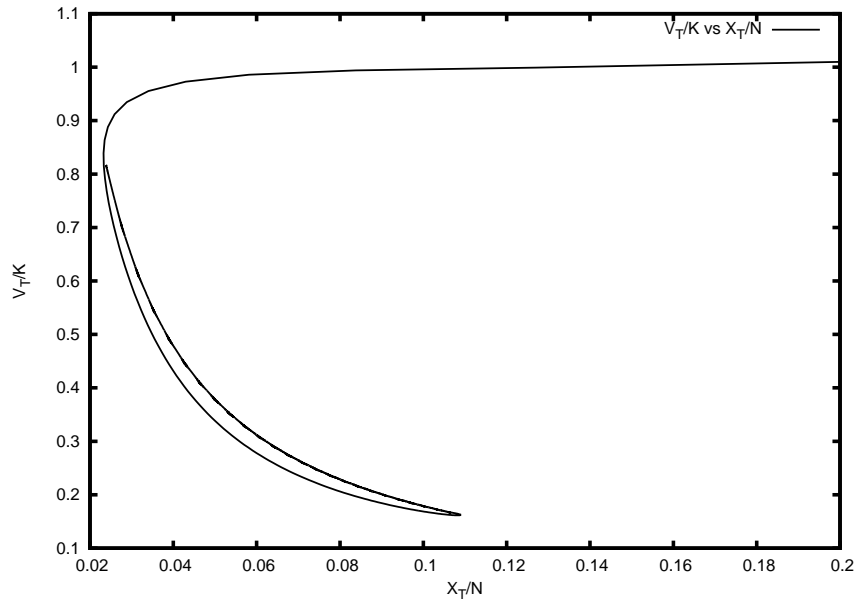
(b)

Figure 3.11:   (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$. Note that isn't a projection of trajectory

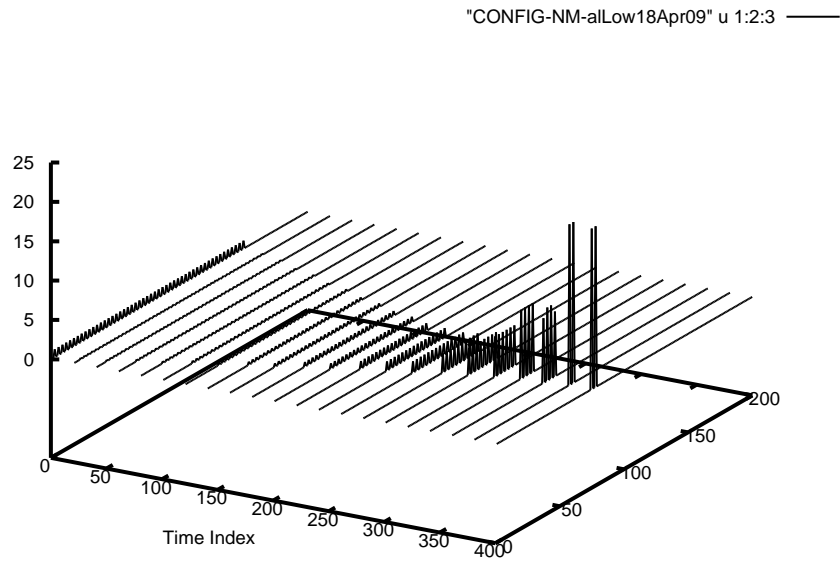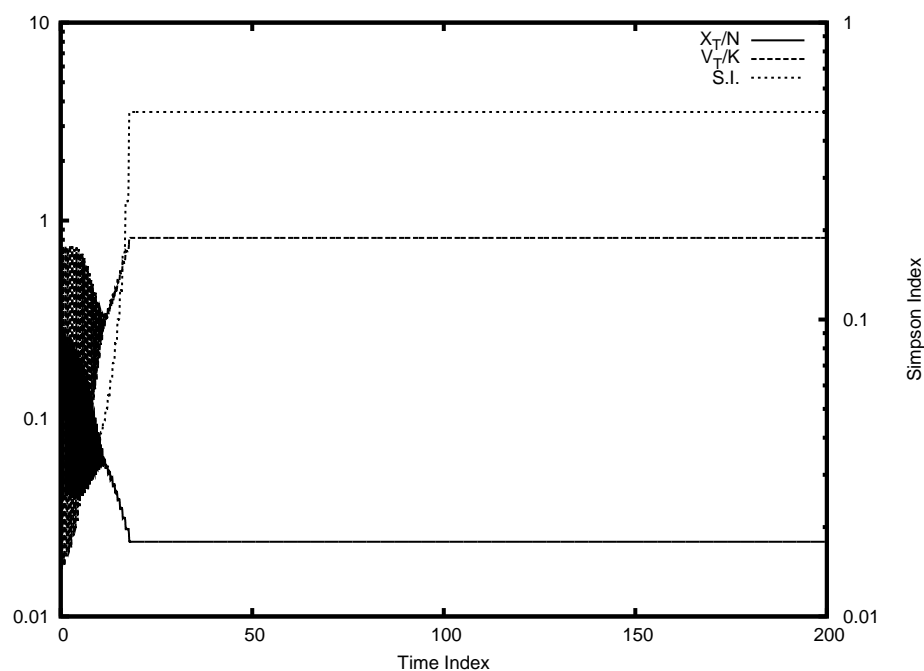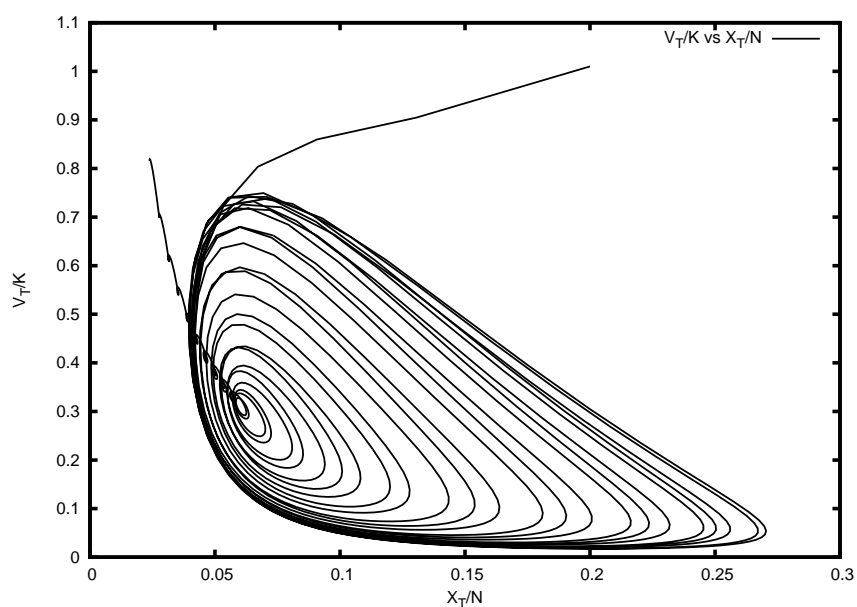"CONFIG-NM-alLow18Apr09" u 1:2:3 ———

Figure 3.12: Evolution of the distribution of the virus in σ

(a)

(b)

Figure 3.13:   (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$, but isn't a trajectory

3.3 Second purpose of a Model

As explained in the previous section, the model 3.2.1, describes an immune system that has already the right density of cells to respond to an infection. To modelize an immune system that has the ability to increase only its specific parts involved in immune responses, we modify the equation for the X's, such that Λ becomes a $\Lambda(V_\sigma)$:

$$\begin{aligned}
\dot{X}_\sigma &= \Lambda \frac{1 + NV_\sigma}{N(1 + V_\sigma)} - X_\sigma \left(\alpha V_T + \alpha_1 V_\sigma + \mu_X \right) & (3.3.1) \\
\dot{V}_\sigma &= V_\sigma \left(\alpha X_T + \alpha_2 X_\sigma - \mu_V \right) + \varepsilon \left(\Delta V \right)_\sigma
\end{aligned}$$

Applying the following scaling of variables and parameters to the model 3.3.1

- $t' = \mu_X t$

- $\varepsilon' = \varepsilon/\mu_X$, $\alpha' = \alpha/\mu_X$, $\Lambda' = \Lambda/\mu_X$, $A = \alpha_1/\alpha$, $B = \alpha_2/\alpha$, $C = \mu_V \mu_X/(\alpha \Lambda)$

- $X'_\sigma = X_\sigma \mu_X/\Lambda$, $V'_\sigma = \alpha/\mu_X V_\sigma$

we obtain

$$\begin{aligned}
\dot{X}'_\sigma &= \frac{\alpha' + NV'_\sigma}{N(\alpha' + V'_\sigma)} - X'_\sigma \left(V'_T + AV_\sigma + 1 \right) & (3.3.2) \\
\dot{V}'_\sigma &= \Lambda' \alpha' V'_\sigma \left(X'_T + BX'_\sigma - C \right) + \varepsilon \left(\Delta V \right)_\sigma
\end{aligned}$$

3.3.1 Study of the model with $\varepsilon = 0$

As in section 3.2.1, under the hypothesis $\varepsilon = 0$ and supposing that only for $\sigma = 1, \ldots, K$ V'_σ are $\neq 0$ and equal to Z, so $V'_T = KZ$, there are K equations of the form $\dot{X}'_\sigma = \frac{\alpha' + NV'_\sigma}{N(\alpha' + V'_\sigma)}$ and $N - K$ of the form $\dot{X}'_\sigma = 1/N - X'_\sigma (KZ + 1)$ for the X's.

Under these hypotheses, we can reduce the system 3.3.2 to a 3-dimensional system (X_a, X_{na}, Z) of the form:

$$\begin{aligned}
\dot{X}_a &= \frac{\alpha' + NZ}{N(\alpha' + Z)} - X_a \left((K + A)Z + 1 \right) \\
\dot{X}_{na} &= 1/N - X_{na} \left(KZ + 1 \right) & (3.3.3) \\
\dot{Z} &= \alpha' \Lambda' Z \left((K + B)X_a + (N - K)X_{na} - C \right)
\end{aligned}$$

Stationary Points

The stationary points are solutions of the following system:

$$
\begin{aligned}
0 &= \frac{\alpha' + NZ^*}{N\left(\alpha' + Z^*\right)} - X_a^*\left((K+A)Z^* + 1\right) \\
0 &= 1/N - X_{na}^*\left(KZ^* + 1\right) \\
0 &= \alpha'\Lambda'Z^*\left((K+B)X_a^* + (N-K)X_{na}^* - C\right)
\end{aligned}
$$

So, neglecting the suffix *, at stationary $X_a = \frac{\alpha' + NZ}{N(\alpha' + Z)((K+A)Z+1)}$ and $X_{na} = (N(KZ+1))^{-1}$. We assume $A > -K$ because we don't want divergence of X_a at stationary point for $Z \geq 0$.

We find an equation for Z:

$$
\alpha'\Lambda'Z\left(\frac{(\alpha' + NZ)(K+B)}{N(\alpha' + Z)\left((K+A)Z+1\right)} + \frac{N-K}{N(KZ+1)} - C\right) = 0
$$

as expected, $Z = 0$ gives the solution of non-infectious state $(X_a, X_{na}, Z) = (1/N, 1/N, 0)$ For $Z \neq 0$ we find a third order equation in Z, that corresponds to the equation:

$$
C = \frac{(\alpha' + NZ)(K+B)}{N(\alpha' + Z)\left((K+A)Z+1\right)} + \frac{N-K}{N(KZ+1)} \tag{3.3.4}
$$

In order to find the positive value of Z at stationary point, we study C thought as a function of Z, $C = C_N(Z)$.

Existence of stationary points with approximation $N \to \infty$

Considering the biological aspect, we are interested in a situation in which HIV virus infects the host with a low number of variants. So, we apply the approximation $N \to \infty$. This gives to $C_\infty(Z) = \frac{Z^2((K+A)+K(K+B))+Z(1+\alpha'(K+A)+(K+B))+\alpha'}{(\alpha'+Z)(KZ+1)(1+Z(K+A))}$.
Let us now study the properties of $C_\infty(Z)$.

$$
\begin{aligned}
C_\infty(0) &= 1 \\
\lim_{Z \to \infty} C_\infty(Z) &= 0 \\
\frac{dC_\infty(Z)}{dZ}\Big|_{Z=0} &= \frac{K+B}{\alpha'} - K > 0 \qquad iff \ \ K+B > \alpha'K
\end{aligned}
$$

The intersections of $C_\infty(Z)$ with Z axis are given by the solution of the equation
$Z^2((K+A)+K(K+B)) + Z(1+\alpha'(K+A)+(K+B)) + \alpha' = 0$ that is of
the type $Z^2 - (Z_1+Z_2)Z + Z_1 Z_2 = 0$ where $Z_1 + Z_2 = -\frac{1+\alpha'(K+A)+(K+B)}{(K+A)+K(K+B)}$ and
$Z_1 Z_2 = \frac{\alpha'}{(K+A)+K(K+B)}$.
While $\alpha' > 0$, if $(K+A)+K(K+B) > 0$, that means $(K+B) > -(K+A)/K$,
$Z_1 Z_2 > 0$. $sign(Z_1+Z_2) = -sign(1+\alpha'(K+A)+(K+B))sign(Z_1 Z_2)$ and
$1+\alpha'(K+A)+(K+B) > 0$ iff $(K+B) > -1-\alpha'(K+A)$.

Summarizing:

$K+B < \alpha'K$ This is the case $C'_\infty(0) < 0$.

If $K+B < -(K+A)/K$, $Z_1 < 0$ and $Z_2 > 0$. So the qualitative behavior
of $C_\infty(Z)$ is shown in graph 3.14. This shows that under these conditions there
exist only a value for the stationary point $Z \neq 0$ iff $0 \leq C < 1$, that is the NM
zone where $C = 1/R_0$.

If $K+B > -(K+A)/K$, while
$sign(Z_1+Z_2) = -sign(1+\alpha'(K+A)+(K+B))sign(K+B+(K+A)/K)$,
we distinguish two cases:

  $-(K+A)/K > -1-\alpha'(K+A)$
  This condition implies $K+B > -1+\alpha'(K+A)$ and is verified if $0 < K <
  A/2(\sqrt{1+4/(\alpha'A)} - 1)$. This gives to $Z_1 + Z_2 < 0$ and, as shown in graph
  3.15, an infectious stationary point exists iff we are in NM zone.

  $-(K+A)/K < -1-\alpha'(K+A)$
  This condition is verified if $K > A/2(\sqrt{1+4/(\alpha'A)} - 1)$. So it is possible
  to have the condition $-(K+A)/K < K+B < -1-\alpha'(K+A)$. In this
  case there exist $Z_1, Z_2 > 0$ and, as shown in graph 3.16, there are 2 or 3
  infectious stationary states for $C > 0$.
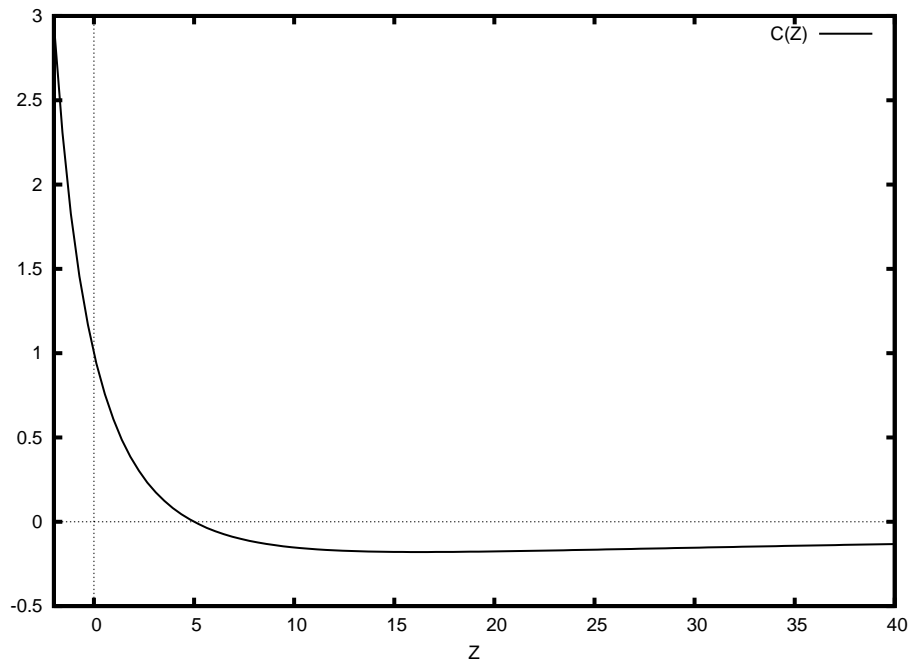
Figure 3.14: Qualitative behavior of $C_\infty(Z)$ under conditions $K + B < \alpha' K$ and $K + B < -(K + A)/K$
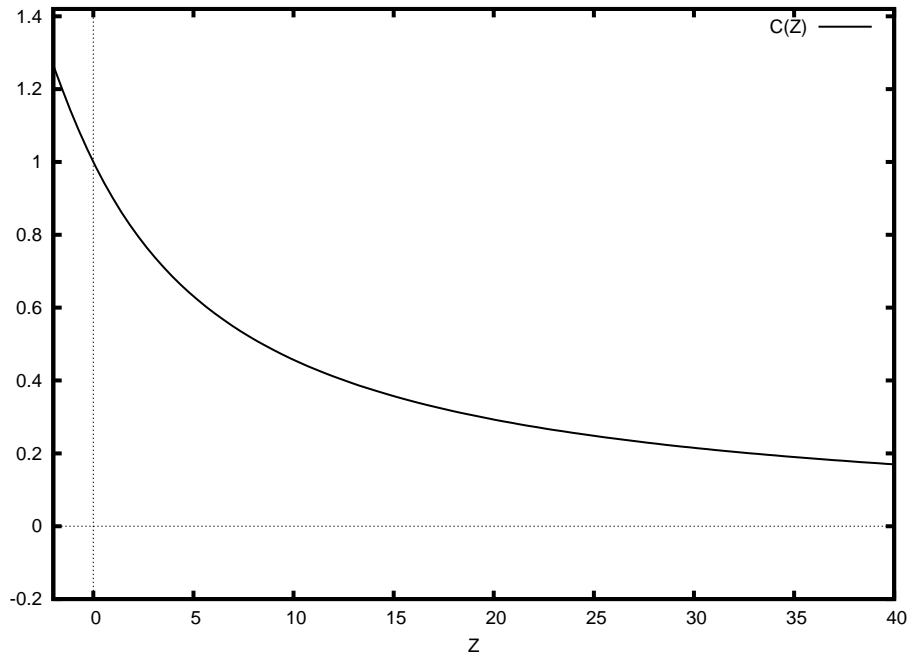
Figure 3.15: Qualitative behavior of $C_\infty(Z)$ under conditions $K + B < \alpha'K$ and $K + B > -(K + A)/K > -1 - \alpha'(K + A)$
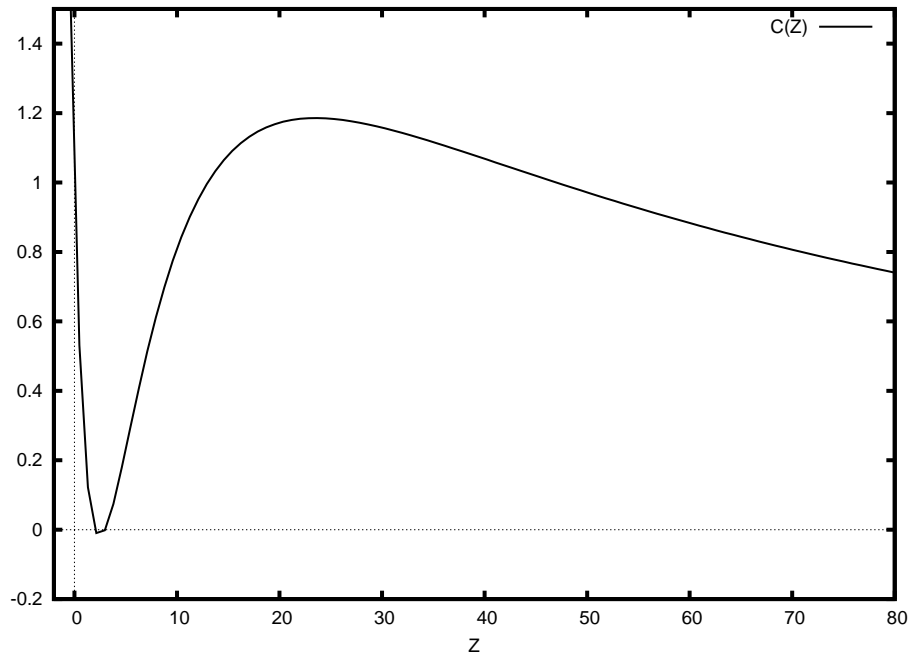
Figure 3.16: Qualitative behavior of $C_\infty(Z)$ under conditions $K + B < \alpha'K$ and $-(K + A)/K < K + B < -1 - \alpha'(K + A)$
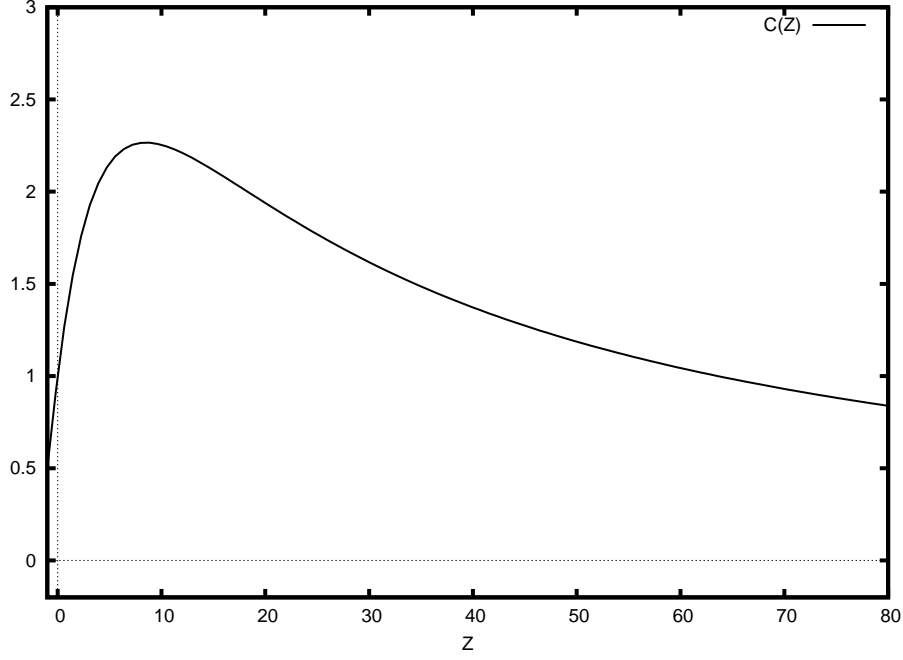
Figure 3.17: Qualitative behavior of $C_\infty(Z)$ under condition $K + B > \alpha'K$

$K + B > \alpha'K$ This is the case $C'_\infty(0) > 0$.

In this case $K + B > -(K + A)/K$ and $-1 - \alpha'(K + A)$ and $Z_1, Z_2 < 0$. So for $C > 1$ there are two positive stationary solutions, while, for $C < 1$, there is only one. The qualitative behavior of $C_\infty(Z)$ is shown in graph 3.17.

3.3.2 Numerical Study of the Model with $\varepsilon \neq 0$

As just an example, we show that in this second model we obtain the opposite behavior than the first one. In fact, in this case numerical simulation shown that virus can expand in all the sites σ, see graphs 3.18, 3.19
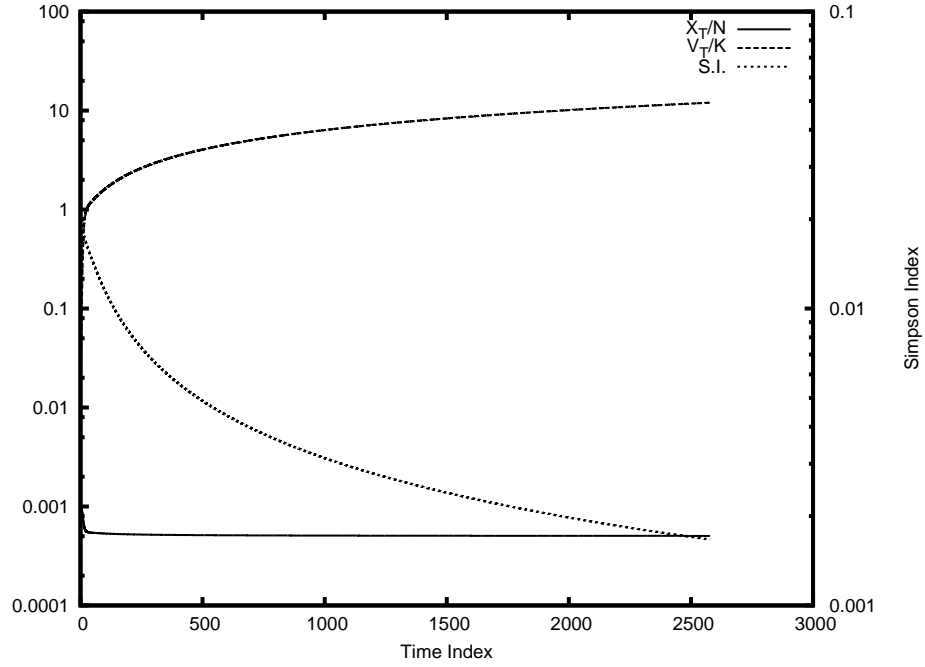
(a)
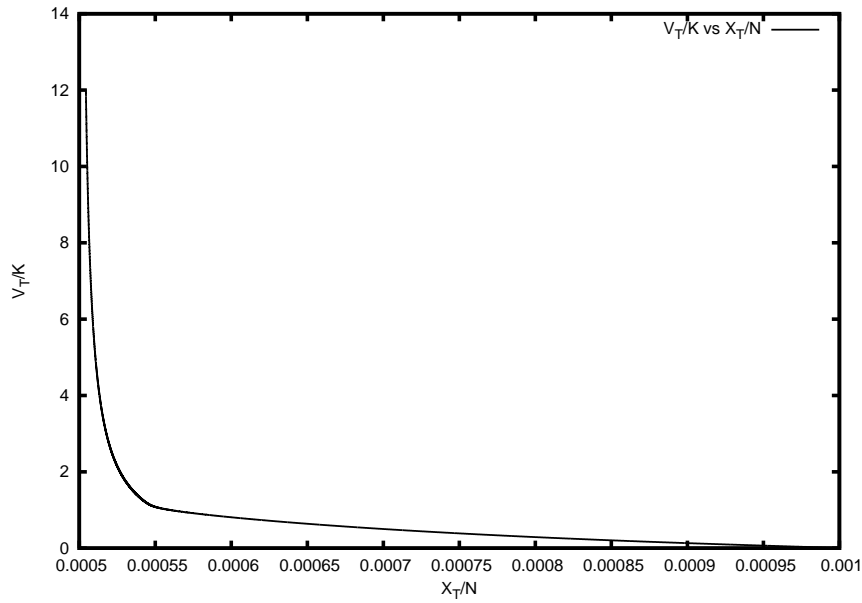
(b)

Figure 3.18: (a) Time evolution of V_T/K and X_T/N; (b) Evolution in the plane $(X_T/N, V_T/K)$. Note that isn't a projection of trajectory

Figure 3.19: Evolution of the distribution of the virus in σ

3.4   General Model

The two previous model can be view as a particular cases of this general one:

$$
\begin{aligned}
\dot{X}_\sigma &= \Lambda(V_\sigma) - X_\sigma(1 + AV_\sigma + V_T) \\
\dot{V}_\sigma &= \omega V_\sigma(BX_\sigma + X_T - C)
\end{aligned}
\tag{3.4.1}
$$

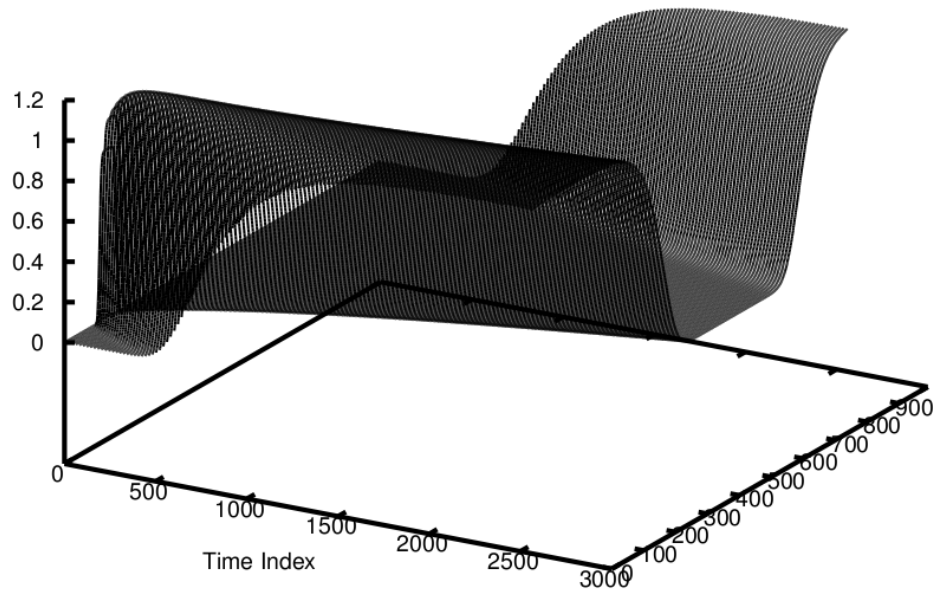where $1 \le \sigma \le N$, $V_T = \sum_\sigma V_\sigma$, $X_T = \sum_\sigma X_\sigma$, and $\Lambda(v)$ is a continuous inceasing function on $\mathbb{R}^+$ with a finite limit. We set $\lambda_0 = \Lambda(0)$ and $\lambda_\infty = \lim_{v \to +\infty} \Lambda(v)$.

Even in this general model, we consider the particular solutions with K identical non zero strain densities. We set

$$
\begin{aligned}
1 \le \sigma \le K : &\qquad V_\sigma = V, &\qquad X_\sigma = X_a \\
\sigma > K : &\qquad V_\sigma = 0, &\qquad X_\sigma = X_{na}
\end{aligned}
$$

so that $V_T = KV$ and $X_T = KX_a + (N - K)X_{na}$.
We get:

$$
\begin{aligned}
\dot{X}_a &= \Lambda(V) - X_a(1 + (K + A)V) \\
\dot{X}_{na} &= \lambda_0 - X_{na}(1 + KV) \\
\dot{V} &= \omega V((B + K)X_a + (N - K)X_{na} - C)
\end{aligned}
\tag{3.4.2}
$$

Since at equilibrium, both X_a and X_{na} go to zero when $V \to \infty$, $\dot{V} < 0$ for V large enough, and the stationary solutions are bounded. If $\Lambda(v)$ is continuous in 0, the stability of the $V = 0$ solution is determined by the ratio $R_0 = \frac{(N+B)\lambda_0}{C}$: If $R_0 > 1$, this solutiuon is unstable and the model is of Nowak-May type (there is at least one non zero solution). If $R_0 < 1$, $V = 0$ is locally stable.

The transition point between these two regimes can be crossed in two different settings:

$B \approx -N$ (large cyto-toxicity) or $\lambda_0 = O(\frac{1}{N})$ (finite volume).

Note that for $B + K < 0$, we have at equilibrium

$$
V_T = KV = \frac{(N - K)\lambda_0}{C - (B + K)X_a} - 1 \le \frac{N\lambda_0}{C}
\tag{3.4.3}
$$

so that the total number of viruses is bounded independently of K and the larger value is obtained for a large K ($< -B$) value (in the limit of large N).

In the finite volume case, $\lambda_0 = O(\frac{1}{N})$ and $B << N$, one of the effects of mutations might be the change in sign of $B + K$ which may turn specific T-cells into a positive growth factor for the viruses. Indeed, for $K > -B$, V_T can exceed $\frac{N\lambda_0}{C}$ ($\approx R_0$ for $B << N$) , but then it is bounded by

$$V_T \leq \frac{\lambda_0}{C}((B + K)\frac{\lambda_\infty}{\lambda_0} + (N - K)) \tag{3.4.4}$$

which may be very large for $\frac{\lambda_\infty}{\lambda_0}$ of order N.

Chapter 4

Nowak-May like models

In the previous section we presented a purpose of a new general basic model, neglecting some, but secondary, biological aspects. This is principally due to the fact that the mechanisms and the dynamics of infection and immune responses are too complex to be modelized with a well mathematically analyzable model. In this chapter we present a model that contain more biological aspect, like different immune system cells, memory, as a tool of modelling different biological aspect of complex HIV-immune system interactions. We start to do this whit a deeper considerations of the biological facts related to HIV tropism, immune system dynamics and HIV escape from immune responses.

Immune System Dynamics, Immune Responses to HIV and HIV Mutations and Tropism

Human immune system can be divided into 2 principal component: innate immune system and adaptive immune system. The innate immune system provides an immediate, but non-specific response. Innate immune systems are found in all plants and animals. However, if pathogens successfully evade the innate response, vertebrates possess a second protection, the adaptive immune system, which is activated by the innate response. Here, the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount

faster and stronger attacks each time this pathogen is encountered.HIV infect cells of the innate component especially the phagocytic cells (macrophages, neutrophils and dendritic cells) and cells of the adaptive component especially CD4$^+$ T cells[3]. HIV enters target cells through interactions between the viral glycoproteins (gp120 and gp41), the cellular receptor CD4[1], and a co-receptor, most often CCR5($R5$) or CXCR4 ($X4$) [25]. Over the course of infection, HIV mute into different strains and the adaptive immune systems needs different specific responses to fight them. In addiction, the co-receptor usage of the HIV virus changes from a preference for $R5$ to a preference for $X4$ in $\simeq 60\%$ of infected individuals. $R5$-using viruses are often present in the early phase of infection, whereas $X4$-using viruses usually become detectable only at later stages. The broadening of co-receptor usage to include CXCR4 is associated with accelerated loss of CD4 cells and faster progression to AIDS [26]. The mechanisms responsible for virus co-receptor switch during the course of infection are still unclear. Several hypotheses have been proposed that may explain the late appearance of X4 viruses [11]. The transmission-mutation hypothesis suggests that R5 viruses are preferentially transmitted and gradually mutate into X4 viruses, whereas the target-cell-based hypothesis emphasizes that a gradual shift in the availability of CCR5- and CXCR4-expressing cell populations is responsible for the appearance of X4 viruses. Finally, the immune system-based hypothesis suggests that X4 viruses are better recognized by the immune system and subsequently suppressed. X4 populations may emerge as a consequence of gradual immune system dysfunction[27].

Considering all the above facts we need to develop a model in which we distinguish between healthy macrophages,representing the phagocytic cells, and those infected by $X4$ or $R5$ virus populations, different immune specific cells ($X4$ or $R5$) and memory/immunization effect. We have also to insert the mutation $X4 < - > R5$ and the mutations due to the viral escape from immune system responses. We resume all this fact in a graphic model in figure 4.1

We model the multiplicity of the immune responses by considering a periodic space of N different specific responses T_σ, $\sigma = 1, \ldots, N$, in this case periodic-

---

[1]Cluster of differentiation 4 (CD4) is a glycoprotein expressed on the surface of T helper cells (CD4+ T), regulatory T cells, macrophages, and dendritic cells.
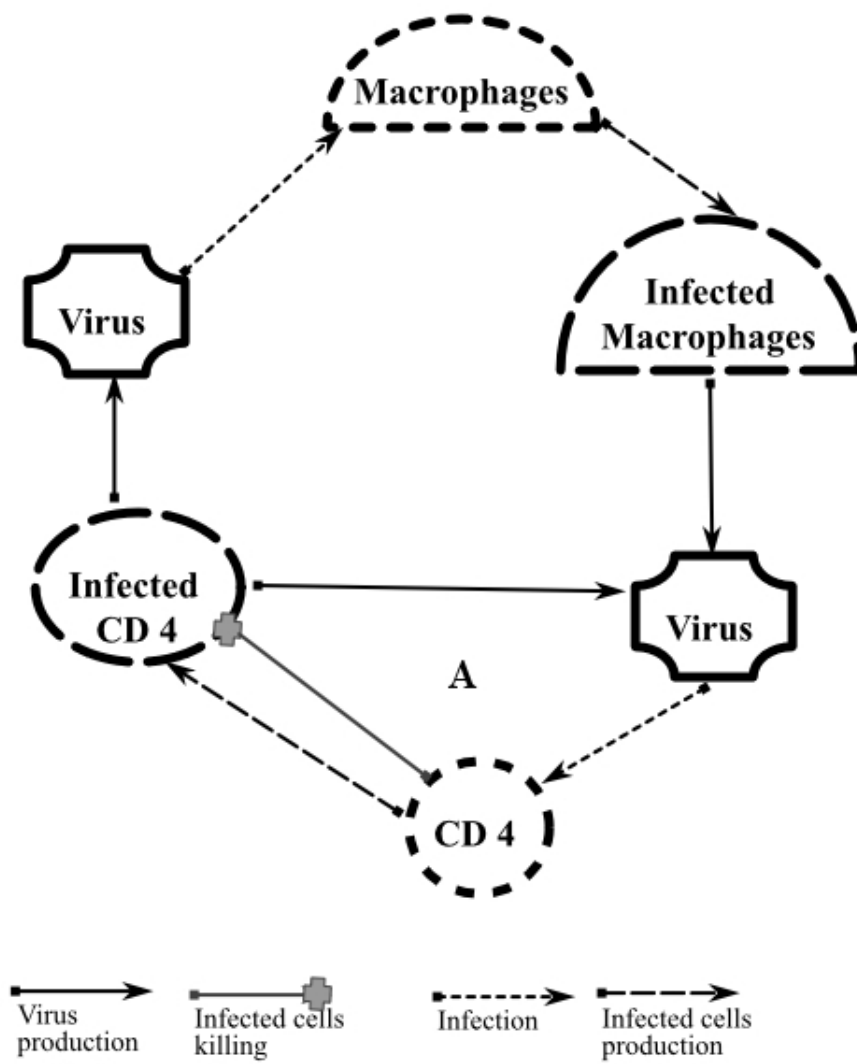
Figure 4.1: Schematic representation of HIV-immune system dynamics

ity means $T_0 = T_N$, $T_{N+1} = T_1$ and so on. Each T_σ kills the infected cells, Y_σ, with the same index σ, which generate the corresponding viral sub-population V_σ. The mutation term contributes to the evolution of Y_σ's like a discrete diffusion term, $\alpha\varepsilon(\Delta V)_\sigma$, where $(\Delta V)_\sigma = V_{\sigma+1} + V_{\sigma-1} - 2V_\sigma$. In our model, "immunization/memory" term is inserted in this way: after a successful response T_σ to an infection ($V_\sigma = Y_\sigma = 0$), the decay term in the equation of T_σ is proportional to it until T_σ is sufficiently slow ($T_\sigma < (1 + \nu)(T_T)/N$, $\nu > 0$, $T_T = \sum_{\sigma=1}^{N} T_\sigma$) and it decays proportional to $(T_T)/N$. When T_σ becomes smaller than $(T_T)/N$ its decay returns proportional to T_σ. This ensure that the immunization effect is present only when it represent a small fraction of the immune system. In other word "the memory of infection" is maintained only if the immune system has the resources to responds to other infections. We also include a term of carrying capacity for production of T's stimulated by the Y's that is proportional to $T_\sigma \left(Y_\sigma^{R5} + Y_\sigma^{X4}\right)\left(1 - \frac{T_\sigma}{T_{MAX}}\right)\Theta\left(1 - \frac{T_\sigma}{T_{MAX}}\right)$. Θ is the Heaviside function, $\Theta(z) = 1$ when $z < 0$, otherwise $\Theta(z) = 0$.

Resuming:

- The T's has a death term like this $-\mu_T \frac{T_T}{N} D\left(\frac{NT_\sigma}{T_T}\right)$. $D(z)$, $z \in [0, N]$, should satisfy:

    - if $1 \leq z \leq z_0$, $D(z) = 1$,

    - otherwise $D(z) = z$

    (see figure 4.2, where $z_0 = 5$).

- The T's has a growth term like this $\Lambda + FT_\sigma Y_\sigma \left(1 - \frac{T_\sigma}{T_{MAX}}\right)\Theta\left(1 - \frac{T_\sigma}{T_{MAX}}\right)$

We set the parameters of the model independent from σ. So we may introduce this part of the model, resembling the Nowak-May one, with virus mutations and a more complex immune system dynamics, represented by the A cycle in figure 4.1.
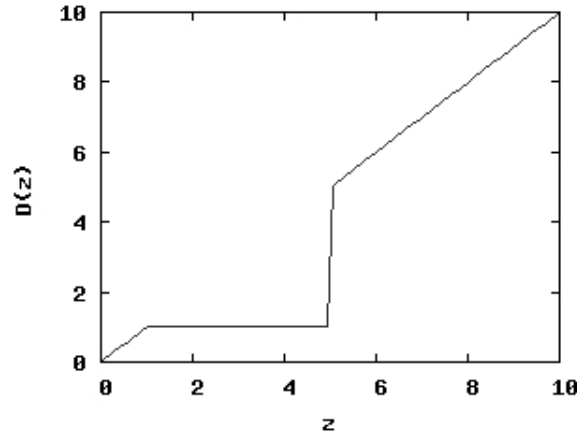
Figure 4.2: Behavior of $D(z)$ for $z_0 = 5$

$$
\begin{aligned}
\dot{T}_\sigma &= \Lambda + FT_\sigma Y_\sigma \left(1 - \frac{T_\sigma}{T_{MAX}}\right) \Theta \left(1 - \frac{T_\sigma}{T_{MAX}}\right) - T_\sigma \alpha_V V_T - \mu_T T_T D \left(\frac{NT_\sigma}{T_T}\right) \\
\dot{Y}_\sigma &= T_T \left(V_\sigma + \epsilon_2 (\Delta V)_\sigma\right) - Y_\sigma \left(\mu_T + \beta T_\sigma\right) \\
\dot{V}_\sigma &= kY_\sigma - \mu_V V_\sigma
\end{aligned}
$$

We simply test the immune system structure. The initial conditions are $T_1 = 0.9$, $\forall \sigma \neq 1$ $T_\sigma = 10^{-4}$, $\forall \sigma$ $Y_\sigma = V_\sigma = 0$. In figure 4.3 we show the memory effect on T_1 population.

Figure 4.3: Behavior of the complete immune system $(-\,-\,-)$ and T_1 specific subpopulation

Introducing the healthy population of macrophages, M_0, we need to distinguish between $R5$ and $X4$ populations of infected macrophages, M^{R5} and M^{X4}, respectively. So we have two type of mutations, $X4 \leftrightarr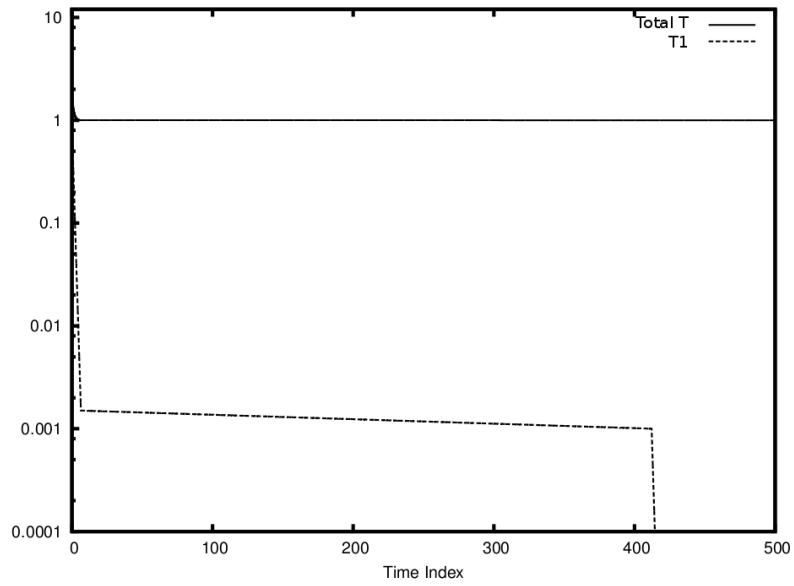ow R5$ and between σ genotypes whit mutation coefficients ϵ_1 and ϵ_2, respectively. Now T's, Y's and V's are also labeled by $X4$ or $R5$ in this way $T_\sigma \Rightarrow T_\sigma^{R5}$ and so on. Now we can write the complete model for the figure 4.1

$$
\begin{aligned}
\dot{M_0} &= \Lambda - M_0 \left(\mu_{M_0} + \alpha_{MR5} V_T^{Y,R5} + \alpha_{MX4} V_T^{Y,X4} \right) \\
\forall \sigma &= 1, \dots, N \\
\dot{M_\sigma^{R5}} &= M_0 \left((1-\epsilon_1)\alpha_{MR5} \left(V_\sigma^{Y,R5} + \epsilon_2 (\Delta V)_\sigma^{Y,R5} \right) + \epsilon_1 \alpha_{MX4} \left(V_\sigma^{Y,X4} + \epsilon_2 (\Delta V)_\sigma^{Y,X4} \right) \right) - \mu_{M_{R5}} M_\sigma^{R5} \\
\dot{M_\sigma^{X4}} &= M_0 \left((1-\epsilon_1)\alpha_{MX4} \left(V_\sigma^{Y,X4} + \epsilon_2 (\Delta V)_\sigma^{Y,X4} \right) + \epsilon_1 \alpha_{MR5} \left(V_\sigma^{Y,R5} + \epsilon_2 (\Delta V)_\sigma^{Y,R5} \right) \right) - \mu_{M_{X4}} M_{R5}^{X4} \\
\dot{T_\sigma^{R5}} &= \Lambda_{R5} + F_{R5} T_\sigma^{R5} \left(Y_\sigma^{R5} + Y_\sigma^{X4} \right) \left(1 - \frac{T_\sigma^{R5}}{T_{MAX}^{R5}} \right) \Theta \left(1 - \frac{T_\sigma^{R5}}{T_{MAX}^{R5}} \right) \\
&\quad - T_\sigma^{R5} \alpha_{VR5} \left(V_T^{Y,R5} + V_T^{M,R5} \right) - \mu_{T_{R5}} T_T^{R5} D \left(\frac{NT_\sigma^{R5}}{T_T^{R5}} \right) \\
\dot{T_\sigma^{X4}} &= \Lambda_{X4} + F_{X4} T_\sigma^{X4} \left(Y_\sigma^{X4} + Y_\sigma^{R5} \right) \left(1 - \frac{T_\sigma^{X4}}{T_{MAX}^{X4}} \right) \Theta \left(1 - \frac{T_\sigma^{X4}}{T_{MAX}^{X4}} \right) - T_\sigma^{X4} \alpha_{VX4} \left(V_T^{Y,X4} + V_T^{M,X4} \right) \\
&\quad - \mu_{T_{X4}} T_T^{X4} D \left(\frac{NT_\sigma^{X4}}{T_T^{X4}} \right) \\
\dot{Y_\sigma^{R5}} &= T_T^{R5} (1-\epsilon_1) \left(V_\sigma^{Y,R5} + V_\sigma^{M,R5} + \epsilon_2 \left((\Delta V)_\sigma^{Y,R5} + (\Delta V)_\sigma^{M,R5} \right) \right) \\
&\quad + \epsilon_1 T_T^{X4} \left(V_\sigma^{Y,X4} + V_\sigma^{M,X4} + \epsilon_2 \left((\Delta V)_\sigma^{Y,X4} + (\Delta V)_\sigma^{M,X4} \right) \right) - Y_\sigma^{R5} \left(\mu_{T_{R5}} + \beta_5 T_\sigma^{R5} + \beta_4 T_\sigma^{X4} \right) \\
\dot{Y_\sigma^{X4}} &= T_T^{X4} (1-\epsilon_1) \left(V_\sigma^{Y,X4} + V_\sigma^{M,X4} + \epsilon_2 \left((\Delta V)_\sigma^{Y,X4} + (\Delta V)_\sigma^{M,X4} \right) \right) \\
&\quad + \epsilon_1 T_T^{R5} \left(V_\sigma^{Y,R5} + V_\sigma^{M,R5} + \epsilon_2 \left((\Delta V)_\sigma^{Y,R5} + (\Delta V)_\sigma^{M,R5} \right) \right) - Y_\sigma^{X4} \left(\mu_{T_{X4}} + \beta_5 T_\sigma^{R5} + \beta_4 T_\sigma^{X4} \right) \\
\dot{V_\sigma^{M,R5}} &= k_{M_{R5}} M_\sigma^{R5} - \mu_{V_{R5}} V_\sigma^{M,R5} \\
\dot{V_\sigma^{Y,R5}} &= k_{Y_{R5}} Y_\sigma^{R5} - \mu_{V_{R5}} V_\sigma^{Y,R5} \\
\dot{V_\sigma^{M,X4}} &= k_{M_{X4}} M_\sigma^{X4} - \mu_{V_{X4}} V_\sigma^{M,X4} \\
\dot{V_\sigma^{Y,X4}} &= k_{Y_{X4}} Y_\sigma^{X4} - \mu_{V_{X4}} V_\sigma^{Y,X4}
\end{aligned}
$$

After the assumption $\mu_{M_0} = \mu_{M_{R5}}$, $\Lambda_{R5} = \Lambda_{X4}$, $F_{R5} = F_{X4} = F$, $T_{MAX}^{R5} = T_{MAX}^{X4} = T_{MAX}$, several scaling of variables and parameters and keeping the same symbols, the system can be rewritten:

$$
\begin{aligned}
\dot{M}_0 &= 1 - M_0\left(1 + \alpha_{MR5}V_T^{Y,R5} + \alpha_{MX4}V_T^{Y,X4}\right) \\
\forall \sigma &= 1,\dots,N \\
\dot{M}_\sigma^{R5} &= M_0\left((1-\epsilon_1)\alpha_{MR5}\left(V_\sigma^{Y,R5} + \epsilon_2(\Delta V)_\sigma^{Y,R5}\right) + \epsilon_1\alpha_{MX4}\left(V_\sigma^{Y,X4} + \epsilon_2(\Delta V)_\sigma^{Y,X4}\right)\right) - M_\sigma^{R5} \\
\dot{M}_\sigma^{X4} &= M_0\left((1-\epsilon_1)\alpha_{MX4}\left(V_\sigma^{Y,X4} + \epsilon_2(\Delta V)_\sigma^{Y,X4}\right) + \epsilon_1\alpha_{MR5}\left(V_\sigma^{Y,R5} + \epsilon_2(\Delta V)_\sigma^{Y,R5}\right)\right) - \mu_{M_{X4}}M_{R5}^{X4} \\
\dot{T}_\sigma^{R5} &= 1 + FT_\sigma^{R5}\left(Y_\sigma^{R5} + Y_\sigma^{X4}\right)\left(1 - \frac{T_\sigma^{R5}}{T_{MAX}}\right)\Theta\left(1 - \frac{T_\sigma^{R5}}{T^{MAX}}\right) \\
&\quad - T_\sigma^{R5}\left(V_T^{Y,R5} + V_T^{M,R5}\right) - \mu_{T_{R5}}T_T^{R5}D\left(\frac{NT_\sigma^{R5}}{T_T^{R5}}\right) \\
\dot{T}_\sigma^{X4} &= \Lambda_{X4} + F_{X4}T_\sigma^{X4}\left(Y_\sigma^{X4} + Y_\sigma^{R5}\right)\left(1 - \frac{T_\sigma^{X4}}{T_{MAX}^{X4}}\right)\Theta\left(1 - \frac{T_\sigma^{X4}}{T_{MAX}^{X4}}\right) - T_\sigma^{X4}\left(V_T^{Y,X4} + V_T^{M,X4}\right) \\
&\quad - \mu_{T_{X4}}T_T^{X4}D\left(\frac{NT_\sigma^{X4}}{T_T^{X4}}\right) \\
\dot{Y}_\sigma^{R5} &= T_T^{R5}(1-\epsilon_1)\left(V_\sigma^{Y,R5} + V_\sigma^{M,R5} + \epsilon_2\left((\Delta V)_\sigma^{Y,R5} + (\Delta V)_\sigma^{M,R5}\right)\right) \\
&\quad + \epsilon_1 T_T^{X4}\left(V_\sigma^{Y,X4} + V_\sigma^{M,X4} + \epsilon_2\left((\Delta V)_\sigma^{Y,X4} + (\Delta V)_\sigma^{M,X4}\right)\right) - Y_\sigma^{R5}\left(\mu_{T_{R5}} + \beta_5 T_\sigma^{R5} + \beta_4 T_\sigma^{X4}\right) \\
\dot{Y}_\sigma^{X4} &= T_T^{X4}(1-\epsilon_1)\left(V_\sigma^{Y,X4} + V_\sigma^{M,X4} + \epsilon_2\left((\Delta V)_\sigma^{Y,X4} + (\Delta V)_\sigma^{M,X4}\right)\right) \\
&\quad + \epsilon_1 T_T^{R5}\left(V_\sigma^{Y,R5} + V_\sigma^{M,R5} + \epsilon_2\left((\Delta V)_\sigma^{Y,R5} + (\Delta V)_\sigma^{M,R5}\right)\right) - Y_\sigma^{X4}\left(\mu_{T_{X4}} + \beta_5 T_\sigma^{R5} + \beta_4 T_\sigma^{X4}\right) \\
\dot{V}_\sigma^{M,R5} &= k_{M_{R5}}M_\sigma^{R5} - \mu_{V_{R5}}V_\sigma^{M,R5} \\
\dot{V}_\sigma^{Y,R5} &= k_{Y_{R5}}Y_\sigma^{R5} - \mu_{V_{R5}}V_\sigma^{Y,R5} \\
\dot{V}_\sigma^{M,X4} &= k_{M_{X4}}M_\sigma^{X4} - \mu_{V_{X4}}V_\sigma^{M,X4} \\
\dot{V}_\sigma^{Y,X4} &= k_{Y_{X4}}Y_\sigma^{X4} - \mu_{V_{X4}}V_\sigma^{Y,X4}
\end{aligned}
$$

As previously said this model is intended for numerical simulations and predictions and it can be used only if the most part of parameters is estimated by biological and clinical data. Until now we don't have this estimations, but the model is intended as framework of numerical modelling and test clinical hypotheses. We remark that this model is to be used only if the most part of parameters is already done.

Conclusions

Widely used models for the viral dynamics in vivo, particularly HIV, are based on the model of Nowak-May[17]. We have seen how the simple introduction of a period of immune response to this model is not sufficient to generate a suitably dynamics, more adeguate to the HIV's one. Note that we have extensively reported in appendix A the calculations for the stability of stationary points, not present in the literature. We have therefore developed in the following sections two proposals for a new basic model for the dynamics of HIV that are not only a generalization of the Nowak-May one, but also contain an improved biological modeling (immune system, mutations).

In both proposals, we have verified that there is a zone of stability for the infection equivalent to that of Nowak-May, which corresponds to a highly cytopathic virus ($C < C_0$). A new picture emerges here: we can identify a region of parameters where the virus is less cytopathic ($C_0 < C < C_1$), but where it still manages to survive. In this area, in the case of the first model (equations 3.2.2) we also show a Hopf bifurcation. All the calculations have been validated by numerical simulations of the system.

Finally in the last section of Chapter 3 we define a general model in which the two previous are special cases.

In Chapter 4 we have presented a proposal for a numerical model as a tool to analyze or estimate particular parameters of HIV infection. This represents, from a biological point of view, a more refined model (mutations, tropism, macrophages, CD4), but is usable only if there is a refined estimate of almost all the parameters used. This is because the model contains many parameters and, in a certain range, one can get the desired dynamics, without knowing if the parameters have the right (biological) order of magnitude.

The model in Chapter 4 is a tool to test specific hypotheses on the effect of a drug or on the estimation of a parameter of viral dynamics. We don't present numerical results because the estimated parameters of the model, in collaboration with the department of Microbiology and Experimental Medicine of this University (Prof. Carlo Federico Perno), have not yet been finalized.

This work has hopefully contributed to a refinement of the HIV infection modeling and also to the debate on a better understanding of the HIV-Immuno competition.

Appendix A

Scaling and stationary points calculations of NM-like Models

A.1 Nowak-May basic Model

The starting model is:

$$\begin{aligned}
\dot{x} &= \Lambda - x(\mu_x + \alpha v) \\
\dot{y} &= \alpha v x - \mu_y y \\
\dot{v} &= ky - \mu_v v
\end{aligned}$$

Scaling

Taking $\mu_x = \mu_y = \mu$ and scaling time t, $t' = \mu t$,

$$\begin{aligned}
\dot{x} &= \Lambda' - x(1 + \alpha' v) \\
\dot{y} &= \alpha' v x - y \\
\dot{v} &= k'y - \mu'_v v
\end{aligned}$$

where $constant' = constant/\mu$.

It is also possible to scale Λ' and α':

$$\begin{aligned}
\dot{x}' &= 1 - x'(1 + v') \\
\dot{y}' &= v'x' - y' \\
\dot{v}' &= k''y' - \mu'_v v'
\end{aligned}$$

where:

- $Z' = Z/\Lambda' = Z\mu/\Lambda$ for $Z = x, y$

- $v' = \alpha' v = v\alpha/\mu$

- $k'' = k'\Lambda'\alpha' = k\Lambda\alpha/\mu^3$,

Recalling all variables and parameters without quotes, we get:

$$
\begin{aligned}
\dot{x} &= 1 - x(1 + v) \\
\dot{y} &= vx - y \\
\dot{v} &= ky - \mu_v v
\end{aligned}
\tag{A.1.1}
$$

Stationary Points

From the system 3.1.1, the equations for stationary points are:

$$
\begin{aligned}
1 - x(1 + v) &= 0 \\
vx - y &= 0 \\
ky - \mu_v v &= 0
\end{aligned}
$$

A.2 Nowak-May with immune system responses

The starting model is:

$$
\begin{aligned}
\dot{x} &= \Lambda - x(\mu_x + \alpha v + Fy) \\
\dot{c} &= Fxy - c(\mu_c + \alpha v) \\
\dot{y} &= \alpha v(x + c) - y(\mu_y + \beta_x x + \beta_c c) \\
\dot{v} &= ky - \mu_v v
\end{aligned}
$$

Scaling

Taking $\mu_x = \mu_c = \mu_y = \mu$ and scaling time t, $t' = \mu t$

$$
\begin{aligned}
\dot{x} &= \Lambda' - x(1 + \alpha'v + F'y) \\
\dot{c} &= F'xy - c(1 + \alpha'v) \\
\dot{y} &= \alpha'v(x + c) - y(1 + \beta'_x x + \beta'_c c) \\
\dot{v} &= k'y - \mu'_v v
\end{aligned}
$$

where $constant' = constant/\mu$.

It is also possible to scale Λ' and α':

$$
\begin{aligned}
\dot{x}' &= 1 - x'(1 + v' + F''y') \\
\dot{c}' &= F''x'y' - c'(1 + v') \\
\dot{y}' &= v'(x' + c') - y'(1 + \beta''_x x' + \beta''_c c') \\
\dot{v}' &= k''y' - \mu'_v v'
\end{aligned}
$$

where:

- $Z' = Z/\Lambda' = Z\mu/\Lambda$ for $Z = x, c, y$

- $v' = \alpha'v = v\alpha/\mu$

- $F'' = F'\Lambda' = F\Lambda/\mu^2$, $\beta''_i = \beta'_i\Lambda' = \beta_i\Lambda/\mu^2$ for $i = x, c$

- $k'' = k'\Lambda'\alpha' = k\Lambda\alpha/\mu^3$,

Recalling all variables and parameters without quotes, we studied:

$$
\begin{aligned}
\dot{x} &= 1 - x(1 + v + Fy) \\
\dot{c} &= Fxy - c(1 + v) \\
\dot{y} &= v(x + c) - y(1 + \beta_x x + \beta_c c) \qquad\text{(A.2.1)} \\
\dot{v} &= ky - \mu_v v
\end{aligned}
$$

stationary points

The equations for stationary points of system A.2.1 are:

$$
\begin{aligned}
1 - x(1 + v + Fy) &= 0 \\
Fxy - c(1 + v) &= 0 \\
v(x + c) - y(1 + \beta_x x + \beta_c c) &= 0 \\
ky - \mu_v v &= 0
\end{aligned}
$$

Stability of healthy state

The first stationary point is the healthy state $(x^{eq}, c^{eq}, y^{eq}, v^{eq}) = (1, 0, 0, 0)$ and the associated characteristic equation for the eigenvalues T, $p(T) = 0$, is:

$$
\begin{vmatrix}
-1 - T & 0 & -F & -1 \\
0 & -1 - T & F & 0 \\
0 & 0 & -1 - \beta_x - T & 1 \\
0 & 0 & k & -\mu_v - T
\end{vmatrix} = 0 = -(T + 1)^2 \left[k - (1 + \beta_x + T)(\mu_v + T) \right]
$$

$$
T_{1,2} = -1 < 0 \text{ and } T_3 = -1/2 \left[(1 + \beta_x + \mu_v) + \sqrt{(1 + \beta_x - \mu_v)^2 + 4k} \right] < 0.
$$

$T_4 = -1/2 \left[(1 + \beta_x + \mu_v) - \sqrt{((1 + \beta_x - \mu_v)^2 + 4k)} \right] < 0$, that means that healthy state is locally stable, iff $R_0 < 1 + \beta_x$.

Existence and stability of infectious state

If $\bar{v}^{eq} \neq 0$ the second equilibrium point is $(\bar{x}^{eq} = (1 + \bar{v}^{eq} + F/R_0 \bar{v}^{eq})^{-1}, \bar{c}^{eq} = \frac{F\bar{v}^{eq}}{R_0(1 + \bar{v}^{eq})(1 + \bar{v}^{eq} + F/R_0 \bar{v}^{eq})}, \bar{y}^{eq} = \bar{v}^{eq}/R_0, \bar{v}^{eq})$ where the equation for $\bar{v}^{eq}$ is

$$
J_2(R_0)(\bar{v}^{eq})^2 + J_1(R_0)\bar{v}^{eq} + J_0(R_0) = 0 \tag{A.2.2}
$$

where:

- $J_2(R_0) = 1 + F/R_0$

- $J_1(R_0) = 1 + \beta_x + F(1/R_0 - 1) + \beta_c F/R_0 + (1 - R_0)$

- $J_0(R_0) = \beta_x + 1 - R_0$

For $F = 0 = \beta_c$, $c^{eq} = 0$ the equation A.2.2 is reduced to:

$$(\bar{v}^{eq})^2 + [2 + \beta_x - R_0)]\bar{v}^{eq} + \beta_x + 1 - R_0 = 0 \qquad (A.2.3)$$

and just one positive solution exists, $\bar{v}^{eq} = R_0 - \beta_x - 1$, iff $R_0 > \beta_x + 1$.
Stability is given by the existence of all negative solutions for eigenvalues equation
$p(T) = 0$

$$\begin{vmatrix} -(1 + \bar{v}^{eq} + T) & 0 & 0 & -\bar{x}^{eq} \\ 0 & -(1 + \bar{v}^{eq} + T) & 0 & -\bar{c}^{eq} \\ \bar{v}^{eq} - \beta_x + \bar{y}^{eq} & \bar{v}^{eq} & -(1 + \beta_x \bar{x}^{eq} + T) & \bar{x}^{eq} + \bar{c}^{eq} \\ 0 & 0 & k & -\mu_v - T \end{vmatrix} = 0$$

using $k = \mu_v R_0$

$$\begin{vmatrix} -(R_0 - \beta_x + T) & 0 & 0 & \frac{1}{\beta_x - R_0} \\ 0 & -(R_0 - \beta_x + T) & 0 & 0 \\ R_0 - \beta_x + (\beta_x + 1)(\beta_x/R_0 - 1) & R_0 - \beta_x - 1 & -\left(\frac{R_0}{R_0 - \beta_x} + T\right) & \frac{1}{R_0 - \beta_x} \\ 0 & 0 & \mu_v R_0 & -\mu_v - T \end{vmatrix} = 0$$

$$\frac{R_0 \mu_v}{R_0 - \beta_x}(T + R_0 - \beta_x)\left\{T + (1 + \beta_x)\left(1 - \frac{\beta_x}{R_0}\right)\right.$$
$$\left. + \left[R_0\left(1 - \frac{\beta_x}{R_0}\right) + T\right]\left(1 + \frac{T}{\mu_v}\right)\left[1 + T\left(1 - \frac{\beta_x}{R_0}\right)\right]\right\} = 0$$

$T_1 = \beta_x - R_0 < 0$ iff $R_0 > \beta_x$
$p(T)$ is reduced to

$$T + AB = \left(1 + \frac{T}{\mu_v}\right)(T + R_0 B)(1 + BT)$$

where: $A = 1 + \beta_x > 0$ and $B = 1 - \beta_x/R_0 > 0$.
One can see, for example graphically, that $T_2, T_3 < 0$ and $T_4 < 0$ iff $R_0 > A$.
Finally we can conclude that the infectious state, for $\beta_c = F = 0$, exists and is
stable iff $R_0 > 1 + \beta_x$

Bibliography

[1] N. Bellomo. *Modeling complex living systems. A kinetic theory and stochastic game approach.* Modeling and Simulation in Science, Engineering and Technology. Birkhauser., 2008.

[2] N. Bellomo, M. Chaplain, and E. De Angelis, editors. *Selected Topics on Cancer Modelling Genesis - Evolution - Immune Competition - Therapy.* Birkhauser-Springer, 2008.

[3] D.M. Knipe, P.M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B.Roizman, and S.E. Straus. *Fields Virology 4th ed.* Lippincott, Williams and Wilkins, 2001.

[4] D.C. Chan, D. Fass, J.M. Berger, and P.S. Kim. Core structure of gp41 from the hiv envelope glycoprotein. *Cell*, 89:263–273, 1997.

[5] F. Clavel, and A.J. Hance. Medical progress: Hiv drug resistance. *N.E. J. Med.*, 350:1023–1035, 2004.

[6] M. Peeters. *HIV Sequence Compendium*, chapter Recombinant HIV sequences: their role in the global epidemic. Los Alamos National Laboratory, Los Alamos, 2000.

[7] WHO. Intrem who clinical staging of hiv/aids and hiv/aids case. definitions for surveillance. Technical report, World Health Organization, 2005.

[8] A. S. Perelson, and P.W. Nelson. Mathematical analysis of hiv-1 dynamics in vivo. *Siam Review*, 41:3–44, 1999.

[9] A. S. Perelson. Modelling viral and immune system dynamics. *Nat Rev Immunol*, 2:28–36, 2002.

[10] R.M. Ribeiro, M.D. Hazenberg, A.S. Perelson, and M.P. Davenport. Naive and memory cell turnover as drivers of ccr5 to cxcr4. tropism switch in human immunodeficiency virus type 1: Implications for therapy. *J. Virology*, 80(2):802–809, Jan 2006.

[11] R.R. Regoes and S. Bonhoeffer. The hiv coreceptor switch: a population dynamical perspective. *Trends in Microbiology*, 13:269–277, 2005.

[12] M.A. Nowak, and C.R.M. Bangham. Population dynamics of immune response to persistent viruses. *Science*, 272:74–79, 1996.

[13] M. A. Nowak, and D. Wodarz. Mathematical models of hiv pathogenesis and treatment. *BioEssays*, 24:1178–1187, 2002.

[14] A.M. Jeffrey, X. Xia, and I.K. Craig. When to initiate hiv therapy: A control theoretic approach. *IEEE TRANS. BIOM. ENG.*, 50(11):1213–1220, November 2003.

[15] B.M. Adamsa, H.T. Banksa, M. Davidiana , Hee-Dae Kwona, H.T. Trana, S.N. Wynnea, E.S. Rosenberg. Hiv dynamics: Modeling, data analysis, and optimal treatment protocols. *J. Comp. App. Mat.*, 184:10 – 49, 2005.

[16] Ji Hyun Ko, Won Hee Kim, and Chung Choo Chung. Optimized structured treatment interruption for hiv therapy and its performance analysis on controllability. *IEEE TRANS. BIOM. ENG.*, 53(3):380–386, MARCH 2006.

[17] M.A. Nowak, and R.M. May. *Virus dynamics: Mathematical principles of Immunology and Virology.* New York, NY: Oxford University Press, 2000.

[18] D.S. Callaway, and A.S. Perelson. Hiv-1 infection and low steady state viral loads. *Bull. Math. Bio.*, 64:29–64, 2002.

[19] S. M. Blower, A. N. Aschenbach, H. B. Gershengorn, and J. O. Kahn. Predicting the unpredictable: Transmission of drug-resistant hiv. *Nature Medicine*, 7:1016–1020, 2001.

[20] L. Rong, Z. Feng, and A.S. Perelson. *Mathematical Modeling of Biosystems*, chapter Mathematical modeling of HIV-1 infection and drug therapy, pages 87–131. Springer-Verlag, 2008.

[21] D. Wodarz, and A.L. Lloyd. Immune responses and the emergence of drug-resistant virus strains in vivo. In *Proceedings of the Royal Society*, 2003.

[22] A. V. M. Herz, S. Bonhoeffer, R. M. Anderson, R. M. May, and M. A. Nowak. Viral dynamics in vivo: Limitations on estimates of intracellular delay and virus decay. *Proc. Natl. Acad. Sci. USA*, 93:7247–7251, 1996.

[23] L.M. Wein, S.A. Zenios, and M.A. Nowaj. Dynamic multidrug therapies for hiv: A control theoretic approach. *J. theor. Biol.*, 185:15–29, 1997.

[24] I.M. Rouzine, A. Rodrigo, and J.M. Coffin. Transition between stochastic evolution and deterministic evolution in the presence of selection: General theory and application to virology. *Microb. Mol. Bio. Rev*, 65(1):151Â–185, March 2001.

[25] E.A. Berger. Hiv entry and tropism: the chemokine receptor connection. *AIDS*, 11(Suppl. A):S3–S16., 1997.

[26] G. Scarlatti, E. Tresoldi, A. Bjorndal, R. Fredriksson, C. Colognesi, H.K. Deng, M.S. Malnati, A. Plebani, A.G. Siccardi, D.R. Littman, E.M. Fenyo, P. Lusso. In vivo evolution of hiv-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nature Medicine*, 3:1259–1265, 1997.

[27] M. Mild, J. Esbjornsson, E.M. Fenyo, P. Medstrand. Frequent intrapatient recombination between human immunodeficiency virus type 1 r5 and x4 envelopes: Implications for coreceptor switch. *Journal of Virology*, 81:3369–3376, 2007.

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