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**Mathematical models of coreceptor
usage and a dendritic cell-based
vaccine during HIV-1 infection.**

Tendai Mugwagwa

Master of science

Department of mathematics and applied mathematics

University of Cape Town

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Abstract

While most of Europe is affected by HIV-1 subtype B, sub-Saharan African is dominated by HIV-1 subtype C. Due to costs, most vaccine development is carried out Europe rather than sub-Saharan countries. However since the mechanisms of disease progression in HIV-1 subtype B may be different from those in HIV-1 subtype C, it is interesting to investigate if and how a dendritic cells based vaccine such as the one developed France and tested on Brazilians (Lu et al, Nature; 2004) can be used on individuals in sub-Saharan Africa. To investigate this, mathematical models and sensitivity analysis techniques are used to understand the mechanisms of disease progression in two HIV-1 subtypes. These models are then extended to explore the ways in which the vaccine could be used to treat these different HIV-1 subtypes. It is found that the level of immune activation plays a large role in determining the mechanism of disease progression and can itself be a means to the development of AIDS. Furthermore, it is also shown that the dendritic cells based vaccine could reduce the viral load but not eliminate the virus resulting in a viral rebound. To maintain a low viral load, vaccination would have to be repeated. Unfortunately, repeated vaccination may lead to the overproduction of proinflammatory cytokines resulting in severe side effects however this could be avoided by using a carefully planned treatment schedule. We conclude that the dendritic cells based vaccine can be used in individuals in either subtype B or subtype C region as long as the correct treatment schedule is followed.

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To Judith Mugwagwa and Nneoma Ogbonna

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Chapter 1

Introduction.

1.1 Motivation.

The HIV epidemic causes problems in both industrialised and developing countries. The World Health Organisation estimates that as of 2004, 37.2 million adults and 2.2 million children were living with HIV world wide [1]. Sub-Saharan Africa is currently the worst affected region as it is home to 60% of the world's HIV infected population. Most individuals in this region are infected by HIV-1 subtype C, making it the most prevalent subtype in the world. This subtype is not only associated with high incidence but is also associated with accelerated disease progression hence the high mortality rate in this region [2].

Although there are other HIV-1 subtypes, subtype B and subtype C are the most widespread subtypes. Apart for its predominance in southern and eastern Africa, HIV-1 subtype C is also predominant in India and Nepal while subtype B is common in Europe, the Americas, Japan and Australia.

One of the major differences between these HIV-1 subtypes is their choice of coreceptor usage during the course of disease progression. Coreceptors usage has been associated with different disease progression dynamics [3].

To reduce mortality and morbidity due to AIDS, a number of drugs and interventions have been developed and are still being developed. Although this is encouraging news, most research on drug development has been confined to industrialised nations while very little is being done in the developing nations which are home to 95 % of the world's HIV infected population [1]. Even more disturbing is the fact that, since different world regions harbour different HIV-1 subtypes, there is a danger that the drugs developed for one region may not produce the same effect in another region. There is therefore a need to understand the dynamics of the different viral strains to make suggestions on how drugs developed in industrialised nations may be used to produce the same effect in individuals in developing nations.

Mathematical models provide an alternative way to study the effects of different drugs, a procedure which is otherwise risky or unethical when carried out on patients. Although model animals such as monkeys are at times used, the ethical justification of this practice is controversial [4]. Apart from providing an alternate route that does not violate any rights, mathematical models also provide clinicians with almost instant results on studies that would have required several months or even years when conducted in animals or human beings. Such models have helped clinicians in making the complex choices involved in treating HIV-infected patients.

The broader goal of this thesis is to make use of mathematical models to explore how vaccines developed for individuals infected by HIV-1 subtype B may be used by HIV-1 subtype C infected individuals. To achieve this goal

two mathematical models were developed. The first model was used to:

1. To identify the factors that influence the choice of coreceptor usage during HIV-1 infection.
2. To explain the difference in coreceptor switching frequency in HIV-1 subtype B and HIV-1 subtype C.

On the other hand, the second model was used to:

1. To determine the long term outcomes of the clinical trial by Lu et al [5].
2. To understand the possible effects of a dendritic cell based vaccine therapy on coreceptor switching.

Results from the two models with the help of another model that combines the two were used to infer how the dendritic cells based vaccine may be used in sub-Saharan Africa using structured treatment interruptions without causing adverse side effects.

1.2 Introduction to HIV biology.

HIV is a retrovirus which means that its genome is RNA and is translated into DNA during its life cycle. This translation and completion of the viral life cycle requires a host cell. HIV attaches itself to a target host cell using a CD4 receptor and a coreceptor. Although there are other coreceptors, the CCR5 and CXCR4 coreceptors appear to be the most important for successful viral entry [6, 7, 8, 9, 10]. Figure 1.1 shows diagrammatic representation of the interaction between the virus, target cell receptors and coreceptors. Using these, the virus then gains entry into the target cell where it takes over control of the replication machinery to complete its own life cycle. Target cells of HIV include macrophages, dendritic cells and CD4⁺ T lymphocytes.

Most of these CD4⁺ target cells play a role in the establishment of immune responses against infections. A healthy human adult has about 1000 CD4⁺ T cells per microlitre of blood, but in an infected patient, the CD4 count can drop to lower levels as the immune system also collapses. Currently, if a patient has a CD4 count of below 200 CD4 cells per microlitre, he or she is said to have AIDS.

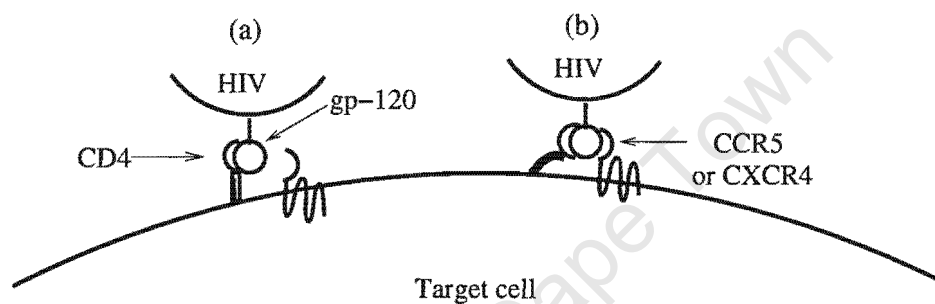


Figure 1.1: A diagrammatic representation of the interaction between viral protein, target cell receptor and coreceptor. (a) represents binding of gp-120 to the CD4 receptor while (b) represents the subsequent binding of gp-120 to a coreceptor which can either be CCR5 or CXCR4 depending on the type of target cell.

Although there is a wide range of immune responses, research in HIV infection has focused largely on the role of T helper cells and CD8 cells. CD8 responses are divided into two.

- (i) Lytic response which is carried out by cytotoxic T lymphocytes (CTLs) which make use of proteins in their cytoplasm such as perforin and granzymes for cell lysis. This is also known as the direct killing response.
- (ii) Non-lytic responses are soluble substances or chemokines secreted by

CD8 cells. These work by either inhibiting HIV replication or inhibiting viral entry into target cells.

The relation between CD8⁺ cells, CD4⁺ cells and HIV has been used to describe the course of disease progression during HIV infection [11].

The dynamics of CD4⁺ and CD8⁺ cells can be used to characterise the different stages of disease progression in HIV infection. Disease progression is divided into three phases summarised in fig (1.2).

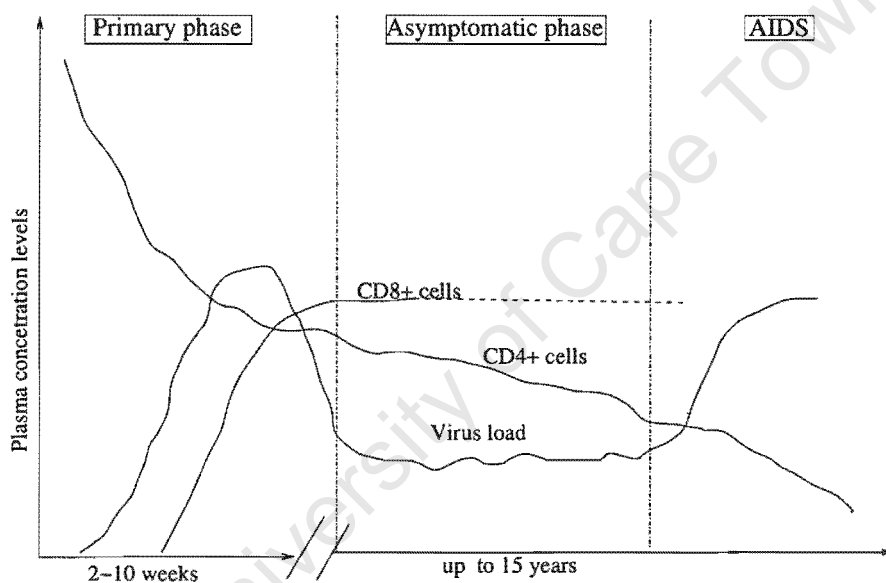


Figure 1.2: A qualitative diagram to show the time course of HIV infection in a typical infected adult.[12, 11]

- *Primary Phase:* During the first few weeks after infection with HIV, patients experience a period of increasing viral load and a decline in CD4⁺ T cells numbers. Flu like symptoms have been associated with this phase [11]. The end of this period coincides with the first signs of a CD8 immune response against HIV.[13]

- *Asymptomatic Phase:* Although there are no visible symptoms present, the replication kinetics of the virus are extremely fast [14, 15, 13]. However, there is little change in the viral load. The CD8 responses are thought to control the virus to low levels but the CD4⁺ T cell numbers continue to decline. The length of this phase may range from a few months to 15 or more years.[11, 13]
- *AIDS:* This is the final stage of the disease. CD4⁺ T cells fall below 200 microlitres and as an overall weakness in the immune system allows opportunistic infections to frequently occur.[13, 11]. Diseases from these infections eventually lead to death.

HIV can exhibit distinct cellular tropisms that have important implications for the viral pathogenesis and disease progression [10]. During the asymptomatic stages of HIV-1 infection, the virus may evolve to show increased tropism for T cells. This phenotypic switch from CCR5 to CXCR4 coreceptor usage has been shown to coincide with the first immunological and clinical signs of chronic disease progression [16, 17, 18, 19].

HIV-1 has been found to predominantly use the CCR5 coreceptor during the primary stage of infection and the asymptomatic phase [20, 16, 21, 22, 10]. The viral strain that uses the CCR5 coreceptor, is known as the R5 strain [22, 6, 7, 8, 9, 10]. It is characterised by a slow replication rate [23], relative acytopathicity and is of the non-synctium inducing (NSI) phenotype. This R5 viral strain infects macrophages and primary T lymphocytes but not CD4⁺ transformed cell lines [10, 9, 21].

The viral strain that makes use of the CXCR4 coreceptor is known as the X4 viral strain [22, 6, 7, 8, 9, 10]. It is characterised by a fast replication rate, a

high degree of T cell killing and shows the syncytium inducing (SI) phenotype. The X4 strain infects T lymphocyte cells [21, 9] but not macrophages [20] and is associated with an accelerated disease progression [24, 13, 8]. Some individuals may retain their use of the CCR5 coreceptor and hence make use of both coreceptors. These individuals show dual tropism [10, 25] and are said to have the R5X4 strain. However, studies have shown that in HIV-1 subtype C infected individuals, the R5X4 strain preferred to use the CXCR4 coreceptor rather than the CCR5 coreceptor [8]. Both the X4 and R5X4 strains show the SI phenotype.

1.3 Thesis organisation.

Having given a brief introduction of the general course of disease progression in HIV infection and the characteristics of different coreceptors, the rest of this thesis is structured as follows.

Chapter 2 gives some background on previous mathematical models and experimental results that have been used to explain the dynamics of HIV infection. In particular, some of the ideas that have been put forward to explain how the cellular immune responses develop and affect the viral load. The chapter also gives an overview on the role of coreceptor usage in disease progression. In the rest of the thesis, these ideas are used to gain a fuller understanding of the mechanisms of disease progression.

Chapter 3 investigates coreceptor switching as a mechanism of disease progression. The chapter seeks to explain the differences in coreceptors usage dynamics in HIV-1 subtype B and subtype C. A mathematical model was

developed to describe the dynamics of two viral strains in the presence of different target cells and different immune responses which turn out to be crucial factors in determining the choice of coreceptor usage. An understanding of these factors will also shed more light on the possible effects of the dendritic cell based vaccine in disease progression.

In the absence of a coreceptor switch as is the case in most HIV-1 subtype C individuals, disease progression would have to be via a different mechanism. In chapter 4, a model was developed to describe how progression to AIDS can result from the impairment CD4 T helper cells and the impairment of the antigen presentation function of dendritic cells. Considering the promising results obtained from a clinical trial on Brazilian subjects with the dendritic cell vaccine [5], the model was extended to include the effect of vaccination. The extended model was then used to investigate the long term outcomes of the clinical trial.

Since Brazil is predominantly affected by HIV-1 subtype B [26], in chapter 5 an investigation is done on how the same vaccine used in Brazil can be used in sub-Saharan African countries to obtain favourable results. In this chapter, suggestions are given on how different treatment schedules may be used in different individuals to gain similar results regardless of the subtype dynamics. These results may be of value to clinicians as drugs and vaccines developed in industrialised countries are made available to the developing countries.

Chapter 2

Literature review

A mathematical model is a simplified representation or replica of the real thing and hence can be used to understand biological systems, a task that can be daunting given the complexity of the system. The application of mathematical models to HIV-1 data has led to a better understanding and prediction of the outcomes of the infection and corresponding immune response [27]. These models have also been used in parameter estimation, hypothesis development and predictions from clinical data to assist policy decisions that affect humans. This chapter is a review of how mathematical models with the help of experimental data have been used to explain disease progression and the eventual development of AIDS. The models provide an explanation for the mechanisms of disease progression, a topic that is highly controversial. Mathematical models that explain three of these mechanisms are also presented here.

The mechanisms of disease progression discussed here are generally based on the relationship between the virus, CD8 immune responses and CD4⁺

T cells. There is considerable controversy on the role of CD8 cells in viral control. This controversy arises from experiments such as the one in which CD8 cells were depleted in 6 Simian immunodeficiency virus (SIV) infected macaques and 1 uninfected macaque [28]. A drastic CD8 cell decline in all infected macaques and a subsequent increase in viral load was observed. The infected macaques also showed a subsequent drop in CD4 levels, however unexpectedly, the level also dropped in the uninfected macaque. From further investigations it was concluded that the antibodies used to deplete CD8 cells could induce T cell activation hence increasing the target cells for the virus [28]. The increase in viral load could thus have been due to an increase in target cells and not the absence of a CD8 response, an idea supported by [29].

Despite this controversy, a number of mathematical models have been developed to describe how CD8 cells control the virus during infection [30, 31, 32, 29]. In all the models there is a general consensus that a persistent CD8 immune response is required to maintain low viral load during the asymptomatic phase of disease progression. CD8 cells persist in the form of CD8 memory cells which protects the body against secondary infections [33, 34, 35, 30]. There are however disagreements on whether a persistent antigen or CD4⁺ T helper cells are responsible for maintaining persistent CD8 memory cells [30, 36, 34]. Mathematical models on this have suggested that a cooperation between the mechanisms was essential for viral control depending on the viral kinetics at different disease progression stages [37, 30]. During initial infection stages before an immune response is established, a high viral load compromises the CD4⁺ T helper cells, but the high antigen levels result in the dominance of the antigen-dependent immune response. As this immune response reduces the viral load at the end of the primary

phase, the CD4 T cell pool recovers while the antigen level decreases. At this stage the CD4-dependent response takes over bringing the viral load to even lower levels [37, 30]. Unfortunately at some point both immune responses fail resulting in the development of AIDS. The rest of the chapter looks at some of the mechanisms of this immune collapse and disease progression.

Disease progression due to CD8 memory exhaustion.

Although CD8 cells may persist in the form of CD8 memory, on an encounter with an infected cells they differentiate into CD8 effectors which then respond to the infection by either lysing the infected cell (lytic response) or secreting chemokines that inhibit viral replication or entry into target cells (non lytic response). CD8 effectors however have a shorter lifespan compared to their CD8 memory counterparts. This brings us to the first mechanism of disease progression in which differentiation into CD8 effectors could lead to the depletion and eventual exhaustion of CD8 memory cells. The mathematical model that explained this mechanism suggested that for a lymphocyte infecting virus such as HIV-1, the ability to infect CD4⁺ cells and a fast replication rate are the main factors causing of the decline in CD8 cells [32]. This process was referred to as 'CTL exhaustion'. Higher viral replication rate would lead to decline in CD4 T helper cells. This impairs the development of CD8 memory cells. Furthermore as the viral load increases there is an increase in differentiation of CD8 memory cells into the short lived CD8 effector cells. Both cases will eventually lead to exhaustion of CD8 memory cells and disease progression [32].

Disease progression due to CTL induced pathology.

Although a CD8 immune response is intended to fight against viral infection,

the immune response may cause severe immunopathology as it lyses infected host cells. This is also known as CTL induced pathology [38]. A classic example of this is an experiment in which a mouse infected with *LCMV* remains healthy in the absence of a CTL response [32, 21]. This is due to the non-cytopathic nature of *LCMV*. The presence of an efficient CTL response successfully control the infection, but a less efficient CTL response would lead to a severe immunopathological effect characterised by wasting of the mouse [21]. Such CTL mediated immunopathology has been suggested as a possible reason for the eventual development of AIDS.

A mathematical model was used to examine the properties of CTL induced pathology an its implication for HIV infection [30]. In the model [30], the degree of CTL mediated pathology was defined as the total number of T cells found in the presence of a virus and CTL repsonse. Using the model it was shown that pathology would result from fast viral replication kinetics in the presence of a weak CTL response. The model also suggested that the antiviral activity of neutralising antibodies may be useful in preventing pathology in an individual with an intermediate CTL response.

Although the model [30] suggests the importance of viral inhibitory factors, it does not take into account the non lytic CD8 cell response. Experimental work showed that in mice infected with a slow replicating *Armstrong* strain, the absence of a non-lytic response did not compromise viral control and no CTL induced pathology was observed. On the other hand, mice infected with a faster replicating *Traub* strain, the absence of a non-lytic response resulted in severe tissue damage and wasting of the host [39]. Although the model by Wodarz et al [38], does not account for the two CD8 cell responses, in a different model, Wodarz et al [40] they took this into account and came

up with a similar conclusion. This latter model [40] suggests that in the event of fast replication kinetics, a combination of the lytic and non lytic responses would be required to achieve success and full viral control. The non lytic response would help lower the replication rate while the lytic response reduced the number of infected cell. This model stressed the importance of the individual types of CD8 cell immune responses in viral dynamics, for example the role of the non lytic response in avoiding CD8 cell induced pathology [40]. Furthermore the lytic and non lytic responses have also been shown to control different viral strains with different efficiencies [40, 10].

Disease progression due to coreceptor switching.

In the discussion so far, there seems to be a general consensus that disease progression is associated with a change in viral replication kinetics from slow to fast. This idea has been supported by Connor and Ho [41] who showed that long-term non-progressors harbour only relatively slowly replicating HIV variants. However the models thus far do not explain why it takes such a long time for the development of AIDS. A number of studies have suggested that this lengthy period before AIDS is a result of the slow evolution of the virus from the slow to fast replications rates. Furthermore this change in replication kinetics has been equated to a switch in coreceptor usage, that is, a switch from CCR5 tropism to CXCR4 tropism [38, 42, 13]. It is therefore important to understand the factors that affect this coreceptor switching to understand why disease progression may be slow.

A number of mathematical models have been developed to explain what the driving force in coreceptor switching is [43, 13]. These models take into account the differences in target cell choices and cytopathicities of the R5 and X4 viral strains. They suggest that the gradual weakening of the immune

system due to the cytopathic nature of the virus results in a reduced negative selection pressure on the X4 strain. Since the replication kinetics of the X4 strain are faster than those of the R5 strain, the X4 strain would eventually outcompete the R5 strain [43, 13]. In addition to the role of the immune response in suppressing the X4 strain, the models suggest that the availability of the different target cells is crucial for the switch. On the one hand, Wodarz et al [21] suggested that macrophages provided a refuge for the R5 strain from lytic immune responses hence giving it a replicative advantage over the X4 strain. On the other hand, Callaway et al [13] suggested that a increase in T cells activation due to opportunistic infections during the late stages of infection may provide the X4 strain with a replicative advantage over the R5 strain hence a coreceptor switch.

An understanding of the mechanisms of disease progression is important in the development of antiviral drugs and vaccines. In this chapter we have reviewed some of these mechanisms, however they do not give an account of the differences in disease dynamics in HIV-1 subtype B and subtype C. Furthermore the mathematical models discussed do not take into account the role of antigen presenting cells, such as dendritic cells, in the development of the immune response. This thesis makes use some of these mechanisms to explain the differences in viral dynamics and in addition investigate the effect of a dendritic cell based vaccine in the different HIV-1 subtypes. This study is valuable as drugs and vaccines developed in Europe and other predominantly subtype B regions are made available to sub-Saharan Africa were the virus is predominantly subtype C. em

Chapter 3

Factors affecting the choice of coreceptor usage.

A phenotypic switch, although more common in HIV-1 subtype B than in subtype C individuals, has only been observed in about 50% of HIV-1 subtype B infected patients during the late stages of infection typically after 8 to 10 years of infection [13, 8]. The phenotypic switch is rarely associated with a complete loss of CCR5 usage [19] suggesting that the use of CXCR4 by HIV-1 is not an absolute requirement for development of AIDS [19, 16]. In HIV-1 subtype C, previous studies in India, Ethiopia, Malawi and South Africa [44, 45, 46] showed that there was an almost exclusive use of the CCR5 coreceptor throughout the course of infection. However, recent findings in South Africa [8, 47], suggests that the frequency of an R5 to X4 switch may be higher than previously suggested. These studies [8] argued that results from previous studies [45, 46] may be because the cohorts of patients in the study were in the early stages of HIV-1 infection when the X4 strain is unlikely to emerge. A study in Zimbabwe [47], suggested that SI isolates

observed may have been due to the prolonged anti-retroviral drug exposure. In this study the most common SI isolates was also the R5X4 strain rather than the X4 strain [47] implying that an intermediate stage between the R5 and X4 strains maybe more probable compared to a complete switch. Both studies [47, 8] however provide evidence that a phenotypic switch does occur in subtype C although at a lower frequency than in subtype B.

Mathematical models [13, 43, 38, 21] were also developed to investigate these factors affecting the switch, however these were based on data from HIV-1 subtype B infections. It has been shown that the replication kinetics of HIV-1 subtype B and C are different [48, 49], thus the combination of parameters used in the models may not be completely appropriate to account for the events leading to the absence of a phenotypic switch in subtype C. Since HIV-1 subtype C now accounts for more than half of new infections worldwide and it has the highest prevalence [26, 8; 2], it is therefore of major importance to try and explain the factors that affect the occurrence of this phenotypic switch in subtype C individuals. Switching has also become more relevant as coreceptor inhibiting drugs enter the clinical trials [6, 50, 51, 52] since coreceptor switching may be a route to drug resistance [6].

This chapter investigates whether the difference in choice of coreceptor usage in subtype B and subtype C has a virological, immunological or an environmental basis. Environmental factors refer to host factors affecting the switch time other than the immune response, for example, target cell availability. A mathematical model is developed based on previous models [43, 21, 38]. However the model in this chapter describes the dynamics of two different target cells that are infected by two viral strains, X4 and R5, with the R5 strain evolving towards the X4 strain as a result of selection pressure. Unlike

previous models [43, 21, 38], a CD4 helper dependent CD8 immune response is included. Recent findings are against the involvement of humoral responses in determining coreceptor usage [53, 54] hence this response is not included in the model. It is also assumed that the target cells are infected by the different viral strains at the same rate. This assumption is based on studies in which both the X4 and R5 viral strains were transmitted with comparable efficiency [54]. A model was developed based on these assumptions and is described in the following section.

3.1 Model development.

This model describes the dynamics of two viral strains, the CCR5 using strain (R_5) and the CXCR4 using strain (X_4), each infecting a different and specific target cell. It is assumed that the two target cell populations are macrophages (M) and CD4⁺ T cells (T). The macrophages carry the CCR5 coreceptor while the CD4⁺ T cells carry the CXCR4 coreceptor. Unlike macrophages, CD4⁺ T cells require activation for infection by the virus, hence the CD4⁺ T cells population is divided into resting CD4⁺ T cells (S) and the uninfected but activated CD4⁺ T cells (T). Upon activation the CD4⁺ T cells can then be infected by the X4 viral strain while the macrophages get infected by the R5 strain. Although the R5 strain can also infect CD4⁺ T cells, in these models we do not take this into account. After a successful infection, presentation of antigens by infected cells results in stimulation of a CD8 immune response. The CD8 memory cells (W), which develop in response to the antigen presentation with the help of CD4⁺ T cells, lack the effector function. However, the CD8 memory cells differentiate into

CD8 effector cells (C) which then carry out the immune response against the infection. The CD8 effector cells respond by either lysing infected CD4⁺ T cells or producing chemokines that inhibit the infection of macrophages. This is based on studies that have shown that macrophages are more resistant to TCL lysis compared to CD4⁺ T cells [?]Wod98T). The above processes are described by nine nonlinear ordinary differential equations given by (3.1)-(3.9). Table. (3.1) gives a summary of the variables described in the model:

$$\dot{S} = \lambda - d_s S - gS(T^* + M^*) \quad (3.1)$$

$$\dot{T} = gS(T^* + M^*) - d_T T - \beta T X_4 \quad (3.2)$$

$$\dot{T}^* = \beta T X_4 - d_{T^*} T^* - p T^* C \quad (3.3)$$

$$\dot{M} = \mu - d_m M - \frac{\beta M R_5}{kC + 1} \quad (3.4)$$

$$\dot{M}^* = \frac{\beta M R_5}{kC + 1} - d_{m^*} M^* \quad (3.5)$$

$$\dot{R}_5 = k_{R_5}(1 - e_v) M^* - d_v R_5 \quad (3.6)$$

$$\dot{X}_4 = k_{X_4} T^* + k_{R_5} e_v M^* - d_v X_4 \quad (3.7)$$

$$\dot{W} = r(T^* + M^*) W T - fW(T^* + M^*) - d_w W \quad (3.8)$$

$$\dot{C} = fW(T^* + M^*) - d_c C \quad (3.9)$$

Equations (3.1) and (3.2) describe the dynamics of uninfected resting and activated CD4⁺ T cells, respectively. There is a constant input (λ) of resting CD4⁺ T cells from a source such as the thymus, while the activated CD4⁺ T cells are derived from the resting cells in response to antigen presence ($T^* + M^*$). Loss of these cells may be a result of natural death (d_T). In addition to natural death, infected CD4⁺ T cells (T^*) described by (3.3) are lost by viral induced killing (d_{T^*}) and lysis by CD8 effectors (pT^*C). Equations (3.4) and (3.5) describe the dynamics of the macrophage population which do not require activation [55, 21]. There is a constant input (μ) of macrophages

Table 3.1: A summary of variables used in the model (equations 3.1-3.9).

Variable	Description	Initial value	Units
S	Resting uninfected CD4 ⁺ T cells	1000	cells mm ⁻³
T	Active uninfected CD4 ⁺ T cells	500	cells mm ⁻³
T^*	Infected CD4 ⁺ T cells	0	cells mm ⁻³
M	Uninfected macrophages	1000	cells mm ⁻³
M^*	Infected macrophages	0	cells mm ⁻³
R_5	R5 viral strain	0.001	virions mm ⁻³
X_4	X4 viral strain	0	virions mm ⁻³
W	CD8 memory cells (precursors)	100	cells mm ⁻³
C	CD8 effector cells	0	cells mm ⁻³

also from a source such as the bone marrow. It is assumed that the loss of macrophages is also a result of natural death (d_m) and infection induced killing (d_{m^*}). Unlike for CD4⁺ T cells, the immune response acts in response to macrophage infection by inhibiting entry of the R5 viral strain ($\frac{\beta R_5 M}{kC+1}$) [9].

Of interest in this section are the dynamics of coreceptor usage during the course of HIV-1 infection hence the inclusion of equations (3.6) and (3.7) that describe the dynamics of the R5 and X4 viral strains. The R5 strain infects macrophage cells and not CD4⁺ T cells [10, 21, 6, 56], while the X4 strain infects the CD4⁺ T cells. The viral strain with dual tropism (the R5X4 strain) is included in the X4 strain population as it preferentially

makes use of the CXCR4 coreceptor [8]. The influx of free R5 virus is from infected macrophage cells at a rate k_{R_5} (equation 3.6) and free X4 virus is derived from infected CD4⁺ T cells at a rate k_{X_4} (equation 3.7). To account for the difference in replication rate and cytopathicity between the R5 and X4 strains [21, 56, 57], it is assumed that $k_{X_4} > k_{R_5}$ and $d_{T^*} > d_{m^*}$ (values given in Table. 3.2). Loss from both viral populations is due to viral decay at a rate d_v . During viral replication within the macrophage cell, the R5 strain is allowed to evolve to the X4 strain through mutations. This process is described in equation (3.6) in which a fraction (e_v) of the new virus produced by an infected macrophage are X4 strain.

Lastly, equations (3.8) and (3.9) describe the development and differentiation of the CD8 immune response. The CD8 memory cells (W) proliferate from an initial pool of memory cells in response to the presence of antigens presented by the infected cells ($T^* + M^*$). However infected cells interact with CD4 T helper cells resulting in the development of CD8 memory cells. The CD8 memory cells then differentiate into CD8 effectors, as a result of the presence of antigens but this process does not require CD4 helper cells. The CD8 effectors have the capacity to lyse infected CD4⁺ T cells and inhibit infection of macrophages. In the absence of antigenic stimulation the CD8 memory and effector cells decline at rate d_w and d_c respectively. The model is used to investigate the factors that influence the choice of coreceptor usage during HIV-1 infection. A description and plausible values of parameters in equations (3.1) to (3.9) are given in Table. 3.2, while a schematic representation of the model is given in Fig. (3.1).

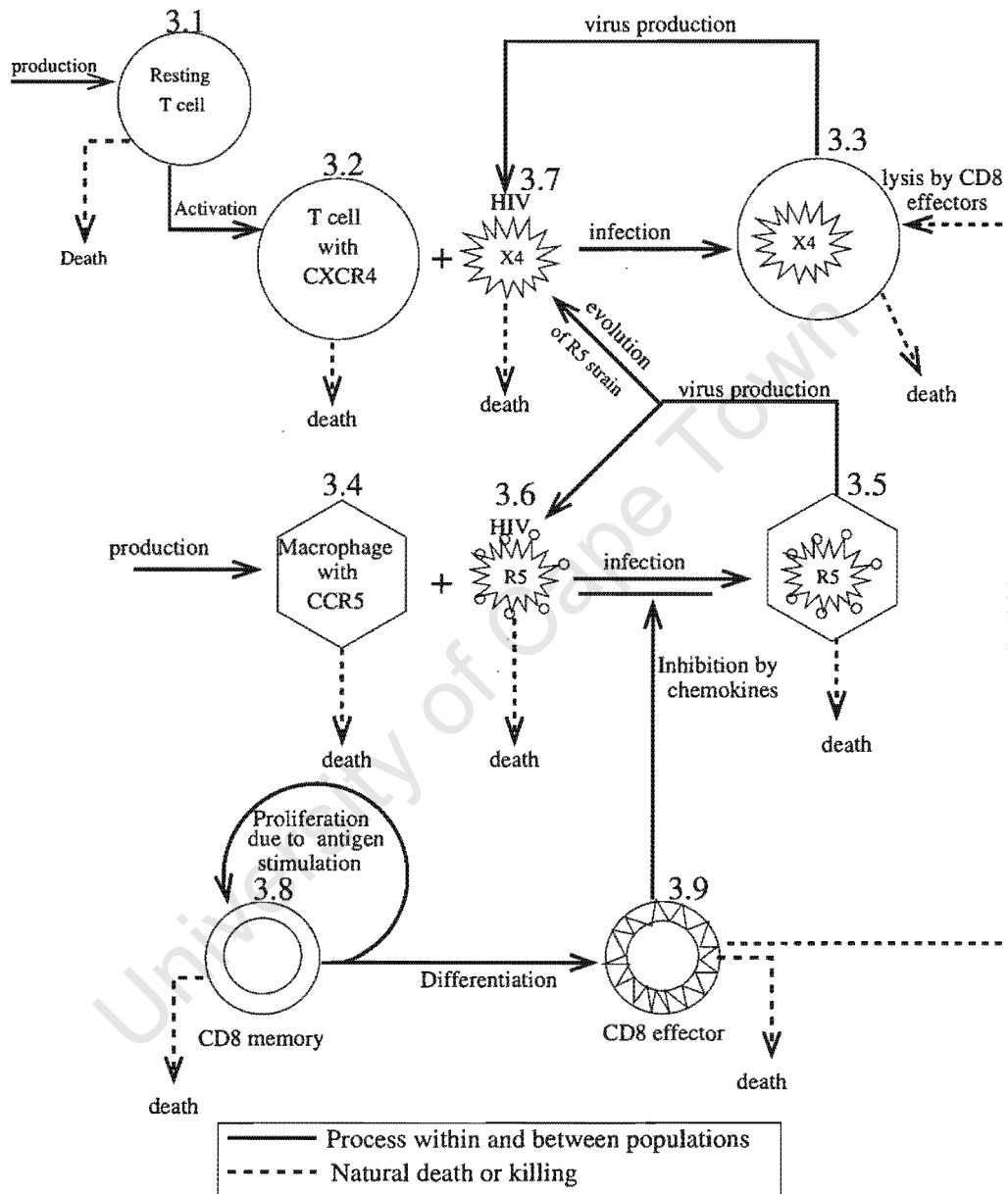


Figure 3.1: A diagrammatic representation of the model. The numbers 3.1 to 3.9 correspond to the equations numbers in the model.

Table 3.2: A summary of parameters used in the model (equations 3.1-3.9).

Parameter	Description	Value	Units
λ	Production rate of resting CD4 ⁺ T cells	1.5	day ⁻¹ mm ⁻³
μ	Production rate of macrophages	5	day ⁻¹ mm ⁻³
g	Activation rate of CD4 ⁺ T cells	0.01	day ⁻¹ mm ³
β	Infection rate for both the R5 and X4 viral strain	$2.4 * 10^{-4}$	day ⁻¹ mm ³
k_{R_5}	Viral production rate by infected macrophages	62.5	day ⁻¹
k_{X_4}	Viral production rate by infected CD4 cells	90	day ⁻¹
e_v	Evolution rate of the R5 strain to the X4 strain	$5.28 * 10^{-5}$	scallar
p	Rate at which CD8 effector cells(CTLs) lyse CD4 ⁺ T cells	0.78	day ⁻¹
k	Efficiency of inhibition of macrophage infection by CD8 effector chemokines	20	cell ⁻¹ mm ³
f	Rate of differentiation of CD8 memory cells into CD8 effectors	0.5	day ⁻¹ mm ³

Table 3.2 – (continued)

Parameter	Description	Value	Units
r	CD8 immune responsiveness	0.005	$\text{day}^{-1} (\text{mm}^3)^2$
d_s	Death rate of resting CD4^+ T cells	0.001	day^{-1}
d_T	Death rate of activated but uninfected CD4^+ T cells	0.01	day^{-1}
d_{T^*}	Death rate of infected CD4^+ T cells including virus induced death	0.24	day^{-1}
d_m	Death rate of uninfected macrophages	0.005	day^{-1}
d_{m^*}	Death rate of infected macrophages including virus induced death	0.03	day^{-1}
d_v	Decay rate of free viral particles	2.4	day^{-1}
d_c	Decay rate of CD8 effectors	0.3	day^{-1}
d_w	Decay rate of CD8 memory cells	0.01	day^{-1}

3.2 Model outcomes.

Using the initial values given in table. (3.1), the model discussed in this paper gives four different outcomes dependent on the values of the infection

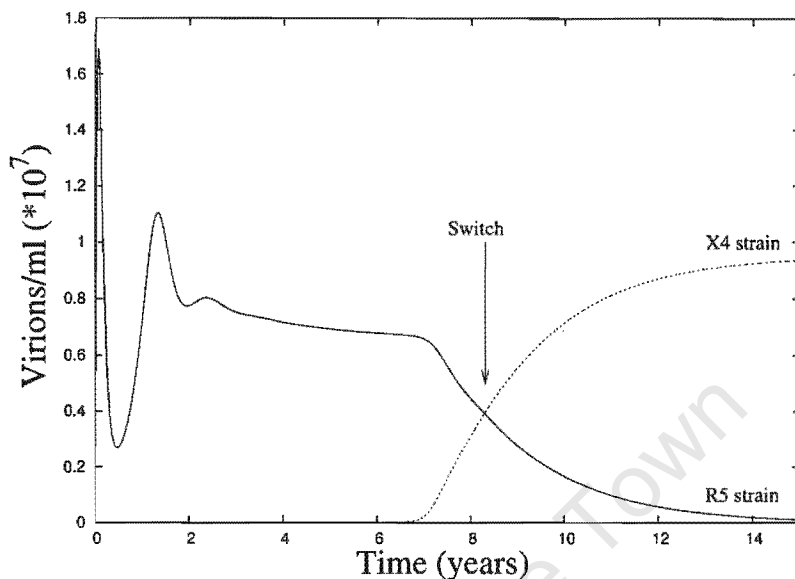


Figure 3.2: *An example of a coreceptor switch (shown by arrow) during a model simulation. The time corresponding to the switch position is the “switch time”. Parameter values as in table. (3.2) with $p=0.5$ and $f=0.1$ and initial values given in table. (3.1).*

rate β summarised in Figure (3.3). Disease progression for 5400 days was simulated and a phenotypic switch was define as the point where the X4 viral population first becomes larger than the R5 viral population (see Fig. 3.2). The “switch time” was thus the time that lapses before this point is reached. It was found that when the infection rate is very low ($\beta < \beta_1$) the virus fails to establish an infection since the basic reproduction number, R_0 , for the R5 strain is less than one. The basic reproduction number is defined as the number of R5 viral particles that are produced as a result of a single R5 viral particle at the beginning of an infection and is given by $R_0 = \frac{\beta \mu k_{R_5} (1 - e_v)}{d_m \mu d_m d_v}$, hence $\beta_1 = \frac{d_m \mu d_m d_v}{\mu k_{R_5} (1 - e_v)}$. Since there is no infection, the CD8 memory also decays due to the absence of stimulation by antigens.

If however $\beta > \beta_1$, the virus successfully establishes an infection and an immune response is also stimulated. The presence of the R5 strain allows for the emergence of the X4 strain, but whether or not a switch occurs depends on how large β is. If $\beta_1 < \beta < \beta_2$ where $\beta_2 \approx 1.55 * 10^{-4} \text{ day}^{-1} \text{ mm}^3$, the X4 strain emerges but remains at very low levels. In this range of β , the infection rate, is not fast enough to counteract the effect of the immune response on the X4 strain, hence its suppression. Furthermore, the replacement number for the R5 strain, ψ , is always greater than one however as it decreases to approaches 1, the R5 viral population reaches an equilibrium (\hat{R}_5), about 3000 days post infection. On the contrary the replacement number for the X4 strain (ϕ) remains below 1 and the population reaches an equilibrium (\hat{X}_4), as its replacement number increases and approaches 1. The replacement number is the number of virions produced from an infected target cell at any given time during the infection. For the R5 strain, $\psi = \frac{\beta k_{R_5} (1 - e_v) M}{d_m * d_v (kC + 1)}$ whereas for the X4 strain, $\phi = \frac{\beta k_{X_4} T}{d_v (d_{T^*} + pC)}$.

As β increases so that $\beta > \beta_2$, there exists a time interval $[t_1, t_2]$ such that $\psi < 1$ and $\phi > 1$. During this time the R5 viral population gradually decays from \hat{R}_5 to a lower equilibrium R'_5 and the X4 viral population increases from \hat{X}_4 to new a detectable equilibrium X'_4 . As the two viral strains settle at the new equilibria, $\psi \rightarrow 1$ and $\phi \rightarrow 1$. In this range of β the two viral strains coexist, a condition which is equivalent to dual tropism, that is the existence of the R5X4 strain. The presence of a phenotypic switch during the simulation time (5400 days) depends on whether $[t_1, t_2]$ falls completely within $[0, 5400]$. For $\beta_2 < \beta < \beta_3$, $[t_1, t_2]$ does not entirely lie in the simulation range $[0, 5400]$ hence $X_4 < R_5$ and no switch is observed during the simulation. However, if $\beta > \beta_3$, where $\beta_3 \approx 1.6 * 10^{-4} \text{ day}^{-1} \text{ mm}^3$, then $[t_1, t_2]$ lies entirely within $[0, 5400]$. In this case a phenotypic switch is observed ($X_4 > R_5$) as R5

strain goes to zero while the viral population becomes dominated by the X4 strain as shown in Fig. (3.2). However due to the high cytopathicity of the X4 strain and increased infection rate, the phenotypic switch results in depletion of CD4⁺ T cells .

If $\beta > \beta_4$ where $\beta_4 \approx 3.22 \times 10^{-3} \text{ day}^{-1} \text{ mm}^3$, then the CD4⁺ T cells are depleted below the critical value, $T < f/r$. A low value of CD4⁺ T cells and high viral load then leads to CD8 cell exhaustion [32]. CD8 cells that are produced may also be overwhelmed by the fast viral replications kinetics resulting in their exhaustion [58, 59, 31]. In the absence of an immune response, the growth of the X4 population is then limited by the availability of CD4⁺ T cells . CD8 exhaustion may also result in a negative CD8 population hence simulations of the model were done for $\beta_1 < \beta < \beta_4$ to ensure that the outcome of the model has a non-trivial and biologically plausible meaning.

3.3 Model analysis.

3.3.1 Methodology of model analysis.

Plackett-Burman sensitivity analysis methodology.

Sensitivity analysis illuminates the relative importance of a model's parameters in bringing about outcomes [60]. The Plackett-Burman technique for sensitivity analysis (PBSA) was employed to investigate the importance of different parameters in the model outcomes. This technique allows for a simultaneous investigation of all parameters, as well as acquiring information about two-way parameter interactions by means of a relatively small number

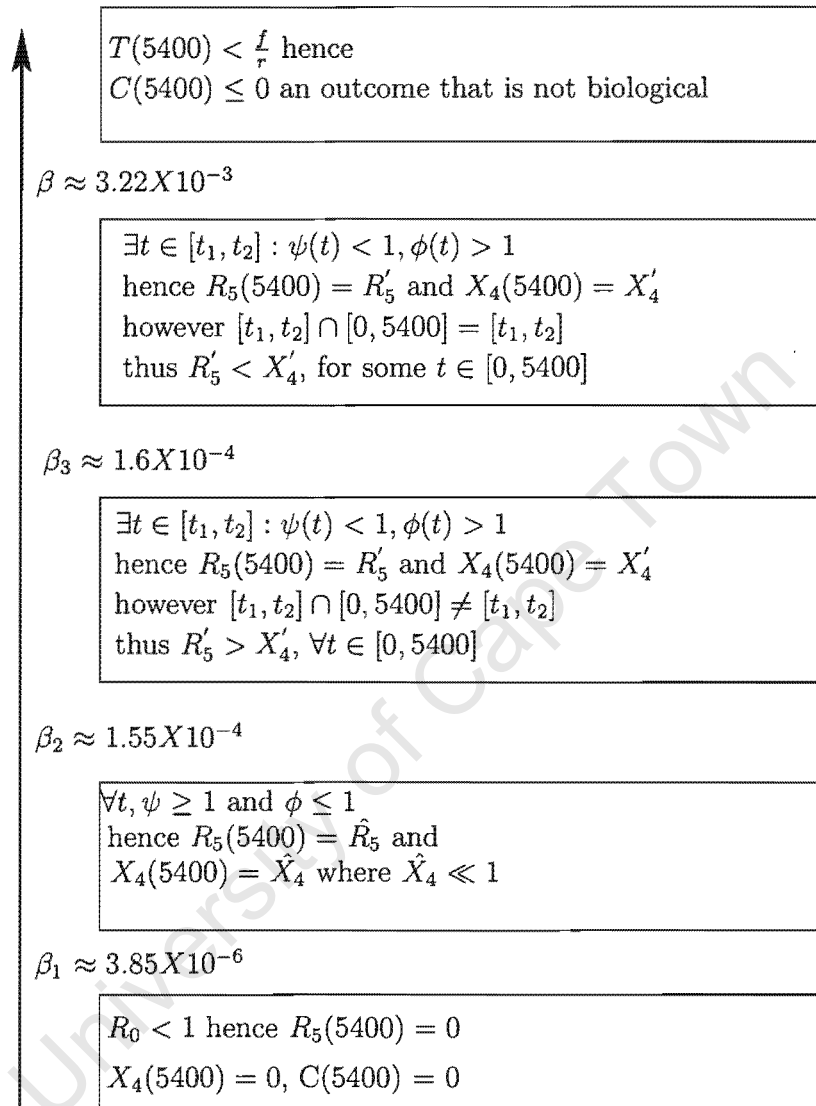


Figure 3.3: A summary of the model outcomes based on the value of β .

of scenarios [61]. This method enables the estimation of effects with the same accuracy as if “attention had been concentrated on varying a single component throughout” [61, 62] using twice as many scenarios as parameters. An overview of the procedures of the method is:

1. Determine parameters to be tested and select upper and lower parameter values.

The interval between the lower and upper values do not need to be the same for all parameters, but each parameter value should be biologically plausible.

2. Find the appropriate Plackett-Burman pattern.

Patterns are strings of pluses and minuses, which are available in the original paper [61]. The designs are two-level fractional designs for studying $k = N - 1$ variables, where N is a multiple of 4 [62]. The number of signs in the list is one less than the design size(d) and the design size should be larger than the number of parameters.

3. Create a Plackett-Burman sensitivity analysis matrix.

The matrix is an array of signs. The top row is the PB pattern found previously. The next $d-2$ rows are generated by all cyclic permutations of this pattern. Append the last row of minuses. The matrix at this point has d rows. Another set of d rows with exactly opposite signs to the first d rows form the rest of the matrix. The final matrix thus has $2d$ rows in which the last row has all pluses

4. Run the prescribed scenarios.

A scenario is a simulation of the model with a particular set of parameter values. Each row of the PBSA matrix corresponds to a scenario of the model and each column represents a parameter. The columns are ordered exactly as in the list of parameters in step 1. If the number of parameters is less than the number of columns then ignore the extra columns. A negative sign is associated with the lower values of the

parameter while the positive sign is associated with the upper value of the parameter. A total of $2d$ scenarios are run and results are recorded.

5. Calculate the effect of each parameter on the model outcome .

The effect of a parameter is defined to be the change in response produced by a change in the level of the parameter. This is called the main effect because it refers to the primary parameter of interest [62]. To determine the main effect of a particular parameter, average all the plus outputs and subtract from the average of all minus outputs.

6. Calculate the effect of two-way interactions of paired parameters.

Similarly as in (3), the matrix consists of plus and minus signs. However, in this case the signs for a particular pair of parameters found by multiplying the signs of the individual parameters. The matrix has C_2^n (n choose 2) columns and each column represents a pair of parameters. After finding the matrix, the scenarios are then run as in (4) and the interaction effect is calculated as in (5). Finally, the effect of augmenting the two parameters on the target outcome is the average of the sum of the individual effects and the interaction effect.

7. Interpret results.

3.3.2 Results of model analysis.

Having identified the different outcomes of the model, further analysis of the model is now restricted to the case when $\beta_3 < \beta < \beta_4$, a region in which the model predicts a possible coreceptor switch. A sensitivity analysis and computer based numerical simulations are used to investigate the effect of

different parameters on the switch time. Since there are many results from the PBSA, I decide to limit my analysis to only those with magnitude is greater than 365 days.

The reasons why individuals infected with HIV-1 subtype C should switch their viral phenotype less frequently than those in subtype B imply either a restriction in the host favouring the expression of a phenotype over another or, alternatively differences in the biological characteristics of these viruses [48, 6]. In the light of this, it was investigated how host factors including immunological factors and viral factor may result in a difference in coreceptor switch frequency in HIV-1 subtype B and C.

How host factors influence the switch time.

It has been suggested that the emergence of the X4 strain depends on its environment [63, 64, 47, 53]. Host factors such as the nature and strength of the immune response, availability of target cells, and coinfection with other pathogens, all contribute to creating such an environment that influences the emergence of the X4 strain. The following sections investigate how coreceptor switching depends on these factors.

The role of the immune response.

Previous models on coreceptor switching [13, 43, 38, 21] suggested that a weak CD8 immune responses results in the emergence of the X4 strain. A further investigation was done on whether it is the individual or combined effect of the lytic and non-lytic immune response that results in a coreceptor switch. From the PBSA results, it was found that increasing the rate of CTLs killings (p), exerted a greater effect on the switch time as compared to the effect of increasing the efficiency of chemokine inhibition (k). The

PBSA results also predicts that such an increase of p by 10%, increases the switch time by 677 days while a 10% increase in k reduces the switch time by 424 days meaning the switch occurs earlier. The increase in the chemokine inhibition increases the selection pressure on the R5 strain and gives the X4 strain a selective advantage. On the other hand the increase in the CTL killings gives the R5 strain a selective advantage as the target cells for the X4 strain get depleted due to its cytopathicity and the lytic effect of the immune response. However increasing the two parameters at the same time results in an increase in switch time by 270 days.

Most paired interactions involving the immune response parameters had greater effects on the switch time as compared to the individual effects of the parameters. An example is the interactions that involved increasing k by 10% which resulted in a significant decrease in the switch time as shown in appendix (A.2). Increasing k increases the selection pressure exerted on the R5 strain, while the second parameter either gives the X4 strain a selective advantage or exerts more negative pressure on the R5 strain. Parameter that gave the X4 strain a selective advantage included, k_{X_4} , g , β and λ while those that further suppressed the R5 strain included d_{m^*} and d_m . Interactions that involved increasing of p also had a similar result trend as all interactions resulted in an increase in switch time (see appendix. (A.2)).

For immune response parameters, d_w , d_c , f and r , the result of an interactions depended largely on the second parameter involved. Although previous models [13, 43, 38, 21] and experiments showed a relation between the emergence of the X4 strain and the decline of the immune system [16, 17, 18], it was found that this was not always the case. One example is increasing the proliferation rate of CD8 memory cells (r) together with the viral production

rate of the X4 strain (k_{X_4}) resulted in a decrease in switch time despite an increase in the equilibrium value of CD8 memory cells. These results imply that the efficiency and type of the immune response rather than the number of CD8 cells produced are greater determinants of switch time.

The role of availability target cells.

Since HIV-1 requires host cells for replication, the survival of a given viral strain depends on the availability of the corresponding target cells. Using the PBSA it was found that two of the individual parameters that had significantly large effects on the switch time were the death rate of uninfected macrophages (d_m) and the production rate of the uninfected macrophages (μ). An increase of 10 % on d_m and a similar increase on μ , resulted in a decrease in switch time of 651 days and an increase in switch time of 426 days respectively. An increased availability of target cells for the R5 strain gives it a replicative advantage over the X4 strain thereby increasing the switch time. Similarly for the X4 strain, a decrease in switch is predicted when the availability of CD4⁺ T cells is increased by reducing the death rate of uninfected CD4⁺ T cells (d_T) and when the production rate (λ) of CD4⁺ T cells is increased, however the magnitude of the effect was not significant (see appendix. (A.1)). Other individual parameters affecting CD4⁺ T cells availability (g and d_s) had effects whose magnitude was not significant.

Interactions that involved the parameters that affect the availability of target cells ($\lambda, d_m, \mu, d_T, g$ and d_s), resulted in higher and significant effects on the switch time as compared to their individual parameter effects. All paired interactions involving an increase in the death rate of macrophages (d_m), T cell activation (g) or production rate of resting CD4 cells (λ) resulted in a decrease in the switch time regardless of the second parameters. These results

emphasise that role of the life span of macrophages and the influx of CD4⁺ T cells in determining the switch time. For the rest of the parameters that influence target cell availability, μ , d_T and d_s , outcomes of paired interactions were influenced by the second parameter involved rather than the former.

To further investigate the role of target cells in determining the switch time, computer based simulations were used to find the effect of different initial conditions on the coreceptor switch. The model predicted a slight decrease in switch time when the initial concentration of CD4⁺ T cells was increased from 0 to about 200. However an increase above 200 resulted in generally the same switch time (Fig. 3.4). This result implies that the initial concentration of CD4⁺ T cells has little effect on the switch time except at very low levels. For macrophages it was found a constant decline in switch time as the initial concentration was increased from 0 to about 600. On the other hand, for an initial concentration of macrophages greater than 600, a slight increase in switch time was predicted (Fig. 3.4). Compared to the effects from the PBSA results, the change in switch time as a result of changing the initial number of target cells, was not significant. The largest change was observed for the CD4⁺ T cells : the difference between the highest and lowest switch time was about 300 days.

How viral factors influence the switch time.

Having looked at the factors that create an environment for a coreceptor switch, an investigation was done on how viral properties may affect the switch time. The R5 and X4 viral strains have been shown to have different replication rates and cytopathicity [21, 56, 57, 10, 54]. These differences constitute the viral factors that are possible determinants of coreceptor usage [6]. In the model, the infection rate (β) and viral production rate (k_{R_5} and

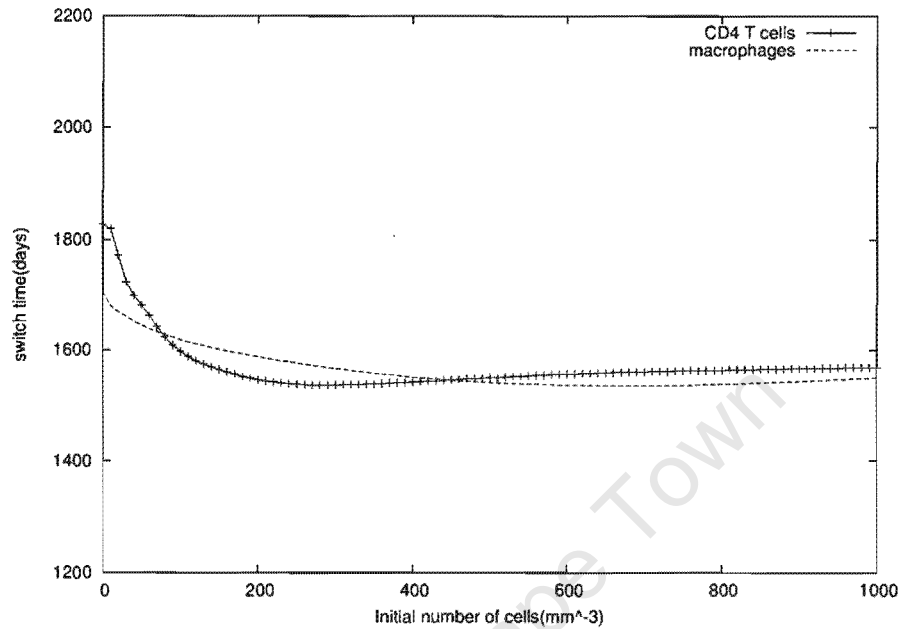


Figure 3.4: Dependence of the switch time on the initial value target cells . Parameter values are given in table. (3.2) and other initial values given in table. (3.1)

k_{X_4}) were chosen as the determinants of the viral replication kinetics. The PBSA and model simulations were then used to infer the effect of these three parameters and the cytopathicities of the individual viral strains (d_{m^*} and d_{T^*}) on the switch time (see results in appendix. (A.1 and A.2)).

The role of viral replication kinetics.

When compared with all the other parameters in the model, the viral production rate for the X4 strain, k_{X_4} had the greatest individual effect on the switch time with an increase of 10% resulting in a decrease in switch time of 903 days. Surprisingly, this is more than two fold the magnitude of the effect of increasing k_{R_5} (viral production rate of R5) by the same factor.

Similar to factors discussed before, paired interaction involving viral production parameters resulted in effects greater than the individual effects. It was found that interactions that involved k_{X_4} dominated the interaction pairs that had the highest effect among all the other interaction pairs. Interactions that involved k_{X_4} and the immune system or target cells have already been discussed. It is however interesting to note that an increase in k_{X_4} and any other parameter always resulted in a decrease in switch time. For example, a 10% increase in k_{X_4} and λ resulted in a decrease in switch time by 652 days, despite the fact that while k_{X_4} augmented the replication of the X4 strain, λ gave R5 a replicative advantage. On the other hand, most interactions involving an increase in k_{R_5} did not have effects of significant magnitude.

Since the analysis is restricted to $\beta_2 < \beta < \beta_4$, an investigation was done on how a gradual increase in the infection rate from β_2 to β_4 affected the switch time. It was found a sharp decline in the switch time as values of β were increased from β_2 to β_3 . For $\beta_2 < \beta < \beta_3$ no switch was predicted however if $\beta > \beta_3$ then a switch is possible. Above β_3 , increasing β does not have a major impact on the switch time (results shown in Fig. 3.5)). From this it was concluded that β plays a major role in determining the presence or absence of a switch rather than determining the time when the switch occurs. This is in agreement with the result from the PBSA which showed that increasing the infection rate (β) by 10 %, decreases the switch time by only 22 days.

Although the individual effect was insignificant, the interaction of β and other parameters such as d_m, μ, λ, k and k_{X_4} had significant effects on the switch time. However the outcome of the interaction depended on which viral strain's replication was augmented by the second parameter. The greatest

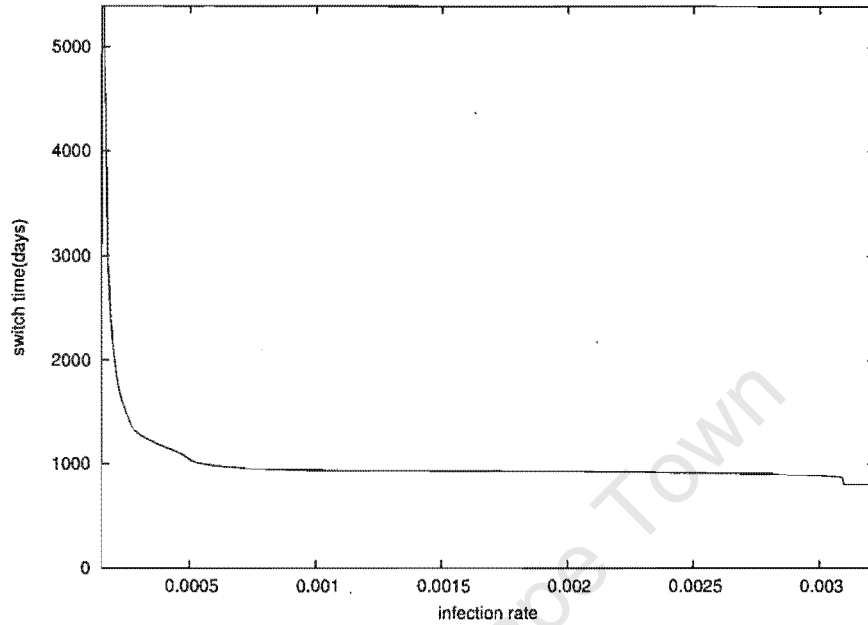


Figure 3.5: A diagram showing the effect of increasing the infection rate in the range $\beta_3 < \beta < \beta_4$. Parameter values as in table. (3.2) except for β which is varied here. Initial values given in table. (3.1).

effect was from an interaction between β and d_m which resulted in an increase in switch time of 880 days. An interesting outcome was from the interaction between β and k_{R_3} (result not shown), which resulted in an increase in switch time of 218 days while an increase in β and k_{X_4} resulted in a decrease in switch time of 628 days. This has an implication on the effect of drugs on coreceptor usage. A combination of a reverse transcriptase inhibitor and protease inhibitor would be equivalent to reducing the infection rate and viral production rate, in this case such drugs would result in an increase in switch time if it mainly targets the X4 strain. This is supported by a study [47] which showed a correlation between antiretroviral treatment (reverse transcriptase

inhibitor) and the emergence of the X4 strain. In vitro studies [53] have shown adaptive mutations within the CCR5 coreceptor rather than a switch to CXCR4 usage when an R5 viral strain was cultured in the presence of a CCR5 specific inhibitor.

The role of viral cytopathicity.

Since the X4 viral strain has been documented to be more cytopathic compared to the R5 strain [21, 56, 57, 10, 54], it was assumed that $d_T > d_{m^*}$. From the PBSA, it was found that the individual effect of d_{m^*} and not d_T had a significant effect on the switch time. A 10 % increase in d_{m^*} resulted in a decrease in switch time of 379 days. In the case of macrophages, an increase in death rate of infected cells would increase the selection pressure on the R5 strain by depleting the viral strain's source hence leading to the emergence and successful domination of the X4 strain. For CD4⁺ T cells the selection pressure exerted by d_T was not strong enough to result in a significant result.

A number of paired interactions involving cytopathicity produced a significant effect on the switch time. Interactions involving d_{m^*} all resulted in a decrease in switch time despite the effect of the second parameter. An example is the effect of increasing d_{m^*} and r which resulted in a decrease in switch time by 1114 days. On the contrary, the outcome of paired interactions involving d_T depended on the effect of second parameter. Previous models [38] have predicted that increasing cytopathicity results in a reduction in the viral equilibrium value. The PBSA result therefore implies that such an outcome can be obtained with the R5 strain rather than the X4 strain.

The role of the evolution rate.

Given that the X4 strain evolves from the R5 strain, one would have thought that the evolution rate would be one of the factors that will have the greatest effect on the switch time. However from the PBSA results it was found that it was not the case. The individual effect of e_v was insignificant, however when interacting with other parameters, e_v yielded significant effects on the switch time. The outcome of the interactions depended largely on the second parameter, for example the largest effect came from the interaction between e_v and p which resulted in an increase in switch time of 1194 days. In this case the selection pressure exerted on the X4 strain by increasing p over shadowed the increase in evolution from R5 to X4 that resulted from increasing e_v . From this, and the results from other interactions, it was concluded that e_v only allows for the emergence of the X4 strain, however survival of the strain depends on the effect of the second interaction parameter on its replication kinetics. The role of e_v is also consistent with the idea that X4 is only a few mutations away from R5 hence evolution rate is not a limiting factor in this case.

3.4 Chapter summary and conclusion.

This chapter investigates how host and viral factors influence coreceptor usage in HIV-1 infection. Two viral strains which infect two different target cells are assumed. In addition two types of CD8 mediated immune responses, namely CTLs and chemokines are also considered. The rationale of this being that each target cell has a different role to play in the establishment and progression of HIV-1 infection and is affected by the two types of CD8 immune responses with different efficiency [54, 53]. Previous models have

suggested that a persistent immune response suppresses the emergence of the X4 strain. This chapter goes on to show that the different components of this persistent immune response would have different effects on the switch time. Increased CTL killing would result in a delay in a coreceptor switch while an increased rate of inhibition by the non lytic response would have the opposite effect.

While CTLs suppress the X4 strain, enhanced X4 viral kinetics promote an early occurrence of a coreceptor switch. However experimental data has shown that subtype C X4 strain replicates to the same degree as subtype B on CD4⁺ T cells [48]. This implies that factors other than the X4 production rate are responsible for the difference in coreceptor usage in subtype C and B. Possibly slower R5 viral kinetics which exerts a negative pressure on the R5 strain may account for the delayed switch in subtype C as shown in this chapter, but other studies [46] have ruled out a relation between viral replicative capacity and biological phenotype. This means that the replication kinetics of the two strains may be the same but other host factors influencing the replication capacity of the virus by altering its habitat.

This chapter further emphasises the role of macrophages in choice of coreceptor usage. It was shown that the life span of macrophages both infected and healthy is crucial in determining the switch time. This result is supported by studies that have shown that resistance of macrophages to CTL killings and their prolonged life span allows them to create a harbour for the virus [53, 54, 65, 21]. A difference in availability of macrophages cells in subtype C and B is however yet to be show experimentally.

Although the model predicts the important role played by CTLs in determining coreceptor usage, the model also predict a similar role for chemokine

inhibition. It would be therefore useful to compare the result of the model on role of chemokine with experimental studies as done for CTLs. This would make it clearer whether the overall CD8 level or the individual components of the immune system are necessary for determining coreceptor usage. It has been noted in experiments [54] and previous models [13, 43, 38, 21] that a lower CD8 levels would create conditions that favoured X4 replication as observed in subtype B, however since the X4 strain is known to be more cytopathic than the R5 strain, an increase in the X4 strain would therefore lead to impairment of the immune system due to a decline in CD4⁺ T cells . The question thus still remains whether the emergence of X4 viral strain is the result of or the cause of immune failure [54].

Immune activation as a cause of a coreceptor switch absence.

Having established that slight variation in parameter values may lead to large changes in the switch time, the question now arises “how can such variations occur in different patients”? A number of studies have suggested that different levels of immune activation in both HIV-1 infected and uninfected individuals may contribute to variations in switch time [46, 63, 53, 8, 66, 67]. In one such study [53, 63] on Italians and Ugandans, Ugandan residents had higher immune activation characterised by high CCR5 levels compared to Italian residents. This was attributed to the relatively high levels of immune activation found in Ugandan residents particularly due to parasitic infections. Tuberculosis (TB) has been identified as the major opportunistic infection associated with HIV-1 infected patients in countries where HIV-1 subtype C is most prevalent [46, 8, 66]. The infection is associated with increased expression of CCR5 since the Mycobacterium tuberculosis (MTB) bacilli infects cells of the monocyte-macrophage lineage [46]. Due to its target cells,

TB has been associated with the dominance of the R5 strain, however infections such as cryptococcal meningitis in HIV-1 infected African patients have been shown to particularly favour the X4 viruses [8, 46]. It is therefore important to note that whether immune activation results in persistence of the R5 strain or not, depends on type of infection that caused the immune activation.

Apart from infections, immune activation in African subjects has also been attributed to environmental conditions, poor hygienic conditions and dietary limitations [64, 63, 53, 8, 66, 67]. For this reason immune activation is not only associated with HIV-1 subtype C infection, but has also been observed in HIV-1 uninfected individuals in regions where HIV-1 subtype C circulates [53, 63, 8, 66, 67]. This finding suggests that the initial number of target cells may affect the choice of coreceptor usage later during the course of infection. However from the model simulations (Figure(3.1 and 3.2)), the initial number of both uninfected CD4⁺ T cells and macrophages did not have a significant effect on the switch time. However an increase in the production rate of macrophages resulted in a significant delay in the switch time. The latter result implies that the availability of target cells throughout the infection rather than at during initial infection is a more plausible explanation of the delay or absence of a coreceptor switching in HIV-1 subtype C infection. Immune activation may thus result in a delay in switch time in HIV-1 subtype C by increasing the availability of CCR5 target cells.

Studies on immune activation have showed increased levels of tumour necrosis factors alpha TNF- α , gamma interferon INF- γ , IL-10 and NF- κ B production due to the increased level of cytokine production [68, 46, 64, 63, 67]. These cytokines have also been proposed as possible mechanisms through which

coinfection with TB in HIV-1 subtype C infected individuals results in increased viral levels [46]. TNF- α induces T cell apoptosis and in the presence of IL-10 it has been shown to enhance virion production from acutely infected macrophages [68, 64]. The two are thought to stimulate AP-1 and NF- κ B, which increase R5 viral replication [68]. The role of IL-10 in augmenting viral replication is in addition to its role in down regulating the anti-virus specific cell-mediated immune response [68, 64]. Lastly INF- γ together with IL-10, promote the expression of CCR5 on target cells [64].

Although the role of cytokines is not explicitly included in the model, based on the effect of the different cytokines, variations in levels of TNF- α , IL-10, INF- γ in the model would be equivalent to variations in parameter values such as death rate of uninfected CD4⁺ T cells. Paired interactions of these factors (d_T and k_{R_5} , d_T and μ , μ and k_{R_5}) all resulted in delayed coreceptor switching when the parameters were increased. In a three way interaction involving increasing the parameters k_{R_5} , μ and d_T , model simulations predicted the absence of a switch. The results of the pairwise and three way interaction affirm the role of variation in environmental (host) factors in determining the switch time.

While some studies in coreceptor usage in subtype C point out a strong relation between the dominance of the CCR5 coreceptor usage and an increased immune activation [64, 67, 54], other studies suggest a link between suppression of the X4 strain and the immune response [46, 69, 63, 53, 8, 66, 67]. Both sets of studies support the view that environmental (host) factors and/or immunological factors rather than virological properties influence coreceptor switch patterns [63]. Assuming that increased immune activation associated with HIV-1 subtype C infected individuals also results in a persistent im-

immune response due to increased levels of antigens, then immune activation results in a delay in switch time in two ways, (i) the presence of a prolonged immune response that selectively suppresses the X4 strain [54] or (ii) the action of $\text{TNF-}\alpha$, IL-10 and $\text{INF-}\gamma$ which augment the expression of the CCR5 coreceptor, increase viral replication and regulates the immune system [46, 68, 64, 63, 67]. From the latter explanation, the role of virological factors as immune activation alters the viral replication kinetics cannot be completely ruled out.

Apart from the effects of immune activation mentioned above, the model suggests that HIV-1 subtype C infected individuals may have a high number of CTLs and lower concentrations of CCR5 inhibiting chemokines which both lead to a delay in switch time. Whether this difference in immune system composition exists between HIV-1 subtype C and subtype B infected individuals is a subject for further investigation. It would be relevant to measure the levels of CCR5 chemokines and CTLs and find whether they are associated with coreceptor usage.

Although immune activation explains the variations in parameter resulting in variations in switch time, it has also been suggested as the cause of accelerated disease progression [67, 64]. While individuals in subtype B regions are thought to progress to AIDS faster after a coreceptor switch, increased immune activation in subtype C individuals is thought to cause accelerated disease progression by increasing susceptibility of target cells to HIV-1 infection, stimulating viral replication and altering cell mediated immunity [68, 67].

It can be concluded that the delay or absence in a coreceptor switch in HIV-1 subtype C infection is a result of variations in parameters due to increased

immune activation. While these factors result in a delay rather than absence of a switch, it is possible that the low frequency of the X4 strain among individuals residing where HIV-1 subtype C is most prevalent, is a result of the shorter natural history of the individuals that does not allow sufficient time for an R5 to X4 switch to occur [46]. Although the impact that this lack of coreceptor switch may have on transmission and pathogenesis of HIV-1 subtype C is unknown [46], understanding the factors that influence it may be of importance as vaccines targeted on coreceptors and immune boosting vaccines enter the clinical trials.

University of Cape Town

Chapter 4

The role of dendritic cells in viral dynamics

Stimulation of an immune response requires viral antigen to interact with CD8 memory cells. Nucleated cells are capable of processing and presenting foreign antigens to solicit an immune response. Unlike other nucleated cells, dendritic cells (DCs) can present antigen processed in other cells. During HIV-1 infection, DCs which are mainly found in the periphery can easily obtain antigen then migrate to the lymphoid organs where they initiate secretion of cytokines and a subsequent immune response [70].

A foreign antigen can be presented in two ways depending on its source. In the event of direct presentation, a replicating virus may infect a DC or other CD4⁺ T cells, its antigens are processed within the host cell and are presented on the major histocompatibility complex class I (MHC-I). MHC-I can be recognised by CD8 memory and effector cells which in turn lyse the antigen bearing cell. This interaction can also result in proliferation of CD8

cells. On the other hand, in the event of cross presentation, DCs obtain antigen processed in other infected cells such as CD4⁺ T lymphocytes. This antigen can also come from apoptotic and necrotic cells and is presented on MHC-II. Unlike MHC-I, MHC-II is only recognised by CD4⁺ T helper cells. The interaction between CD4⁺ T helper cells and MHC-II antigen presenting DCs is required for the development of CD8 memory cells from naive CD8 cells. These provide protection against re-emergence of HIV-1 from latent or chronic sites of infection, or by new infection from outside the host individual [33, 71, 35].

Table 4.1: *The table summarises some of the cytokines produced during the development of an immune response, their sources and function.*

Cytokine	Source	Function
IL-2	CD4 T helper cells	enhances proliferation and differentiation of T cells
IL-12	DCs and macrophages	upregulation of costimulatory molecules, activate CD8 effectors
INF- γ	CD4 T helper cells and CD8 memory cells	DC maturation, enhances MHC-I and MHC-II expression, inhibit viral replication
TNF- α	CD4 T helper cells, DCs and macrophages	enhances MHC-I expression

Although the details of the processes that result in the development of CD8 memory are not clear, it is thought the interaction between CD4 helper cells and MHC-II antigen presenting DCs results in their activation and up-regulation of costimulatory molecules such as B-7, CD40 or CD40L [33, 72, 73]. These activated DCs have the ability to make use of the MHC class I

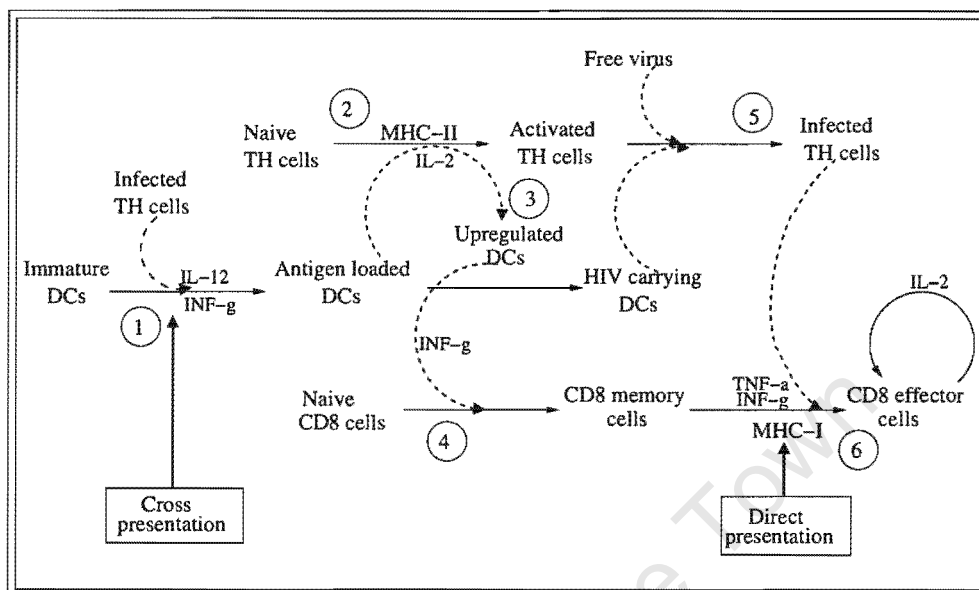


Figure 4.1: A schematic representation of antigen presentation routes. The different stages are explained in box 4.1.

restricted antigen to stimulate naive CD8 cells into CD8 memory cells and CD8 effector cells [33, 74, 73]. In addition to upregulation of costimulatory molecules, the interaction between DCs and CD4 T helper cells also results in secretion of cytokines which also play a role in development of the immune response. A summary of some of these cytokines, their source and function is given in Table (4.1). CD8 memory cells are not directly involved in the response against an infection, however they differentiate into CD8 effectors upon a direct encounter with infected cells through the MHC-I restricted antigen. Fig. (4.1) shows a schematic representation of direct and cross presentation of antigens.

Box 4.1: A summary of antigen presentation routes i.e. cross presentation and direct presentation as shown in fig 4.1.

(1) Immature DCs take up antigen from other $CD4^+$ infected cells including those that have died due to apoptosis and necrosis. This already processed antigen is then presented on MHC-II. This is known as cross presentation. IL-12 that is secreted by DCs helps in upregulation of costimulatory molecules that are required from interaction between antigen loaded DCs and $CD4^+$ T helper cells. INF-g in this case promotes DC maturation.

(2) Antigen loaded DCs through MHC-II and costimulatory molecules interacts with $CD4^+$ T helper cells resulting in the differentiation of naive $CD4^+$ T lymphocytes into activated $CD4^+$ T helper cells. Both activated and naive $CD4^+$ T cells produce IL-2 which enhances proliferation of these cells and other T cells.

(3) This interaction in (2) also results in further upregulation of antigen DCs allowing them to make use MHC-I restricted antigen.

(4) The upregulated DCs through MHC-I interact with naive CD8 cells resulting in their differentiation into CD8 memory cells. This interaction is promoted by INF-g which enhances MHC-I expression on DCs.

(5) Activated $CD4^+$ T cells can be infected by free virus or virus bound to DCs through DC-sign. These cells process antigen from the virus and present it on MHC-I.

(6) This presentation on MHC-I is enhanced by the presents of INF-g and TNF-a. The infected cells then interact with CD8 memory cells resulting in their differentiation into CD8 effector cells which in turn kill the antigen bearing cells. This is known as direct presentation.

It has been shown that CD8 cells are required to control of the viral load [28]. However the chronic stage of HIV infection is associated with the failure to mount such an immune response against the virus. While some studies have suggested that HIV-1 leads to impairment of the immune system by reducing the number of CD4⁺ T helper cells present [75, 76], other studies have attributed this loss of immunity to the impairment of DC function [5, 77, 78]. Although it is not clear how HIV-1 impairs the DC function, the following mechanisms have been proposed. (1) HIV-1 infection of DCs may impair the production of cytokines resulting in poor proliferative rates of the immune system [78], (2) HIV-1 may impair the ability of DCs to stimulate T cells by producing HIV-gp120 which inhibits IL-2 production and T cell signalling [77] and (3) the presence of a high and persistent viral load during the chronic stage causes hyper-inflammation which results in the overmaturation of DCs due to increased cytokine levels such as INF- γ [79]. As Dcs mature they lose their antigen presentation function.

In this chapter, a mathematical model is developed which describes the role of cross presentation and direct presentation during HIV-1 infection. Although simplified, the model captures the different role of direct and cross presentation in development of different CD8 cells. The model explains how immune dysfunction can be a result of both an impaired DC function as well as impaired CD4⁺ T help. The model is extended to investigate the short term and long term effect of a therapeutic DC based vaccine.

4.1 Model Development

The model describes the dynamics of HIV-1 infection in the presence of different antigen presenting cells, DCs and CD4+ T lymphocytes. It contains seven variables: uninfected CD4 T helper cells(T), infected CD4 T helper cells(T^*), free virus (V), DCs (A), antigen loaded DCs (A^*), CD8 memory cells (W) and CD8 effector cells (C). The model equations are given as follows.

$$\dot{T} = \lambda - d_T T - \beta_1 TV - \beta_2(1-x)A^*T \quad (4.1)$$

$$\dot{T}^* = \beta_1 TV + \beta_2(1-x)A^*T - d_{T^*} T^* - pCT^* \quad (4.2)$$

$$\dot{V} = \alpha d_{T^*} T^* - d_v V \quad (4.3)$$

$$\dot{A} = \phi - d_A A - hAT^* \quad (4.4)$$

$$\dot{A}^* = hAT^* - d_{A^*} A^* \quad (4.5)$$

$$\dot{W} = fTA^*W - qT^*W - d_w W \quad (4.6)$$

$$\dot{C} = qT^*W - d_c C \quad (4.7)$$

This model assumes that uninfected CD4 T helper cells are produced at a rate λ , die at a rate d_T , and become infected by free virus at a rate $\beta_1 V$. The interaction between uninfected CD4 T helper cells and activated DCs may result in infection of the former at a rate $\beta_2(1-x)$. This infection is mediated via DC-sign which allows DCs to transport HIV from peripheral regions of the body to CD4+ T lymphocytes without themselves being infected [80, 81]. The fraction of activated DCs carrying the virus is $1-x$. The infected cells die at a rate d_{T^*} or are killed through lysis by CD8 effectors at a rate pC . DCs are produced at a rate ϕ die at a rate d_A . Since DCs are inefficiently infected by HIV [80, 81], it is assumed that they only cross present the antigen from infected cells at a rate hT^* . This process does not require apoptosis

or necrosis of infected cells [82]. The antigen loaded DCs die at a rate d_{A^*} . A CD8 memory whose proliferation is a result of the interaction between antigen loaded DCs and T helper cells at a rate fTA^* is considered. The CD8 memory cells die at a rate d_w or differentiate into CD8 effector cells as a result of direct presentation of antigen by infected CD4 T helper cells at a rate qT^* . In the absence of antigen stimulation, CD8 effectors decay at a rate d_c which is higher than the death rate of CD8 memory cells.

Assuming that the model describes the dynamics of HIV-1 during the chronic stage, the following initial condition (table. 4.2) were used.

Table 4.2: A summary of variables used in the model.

Variable	Description	Initial value	Units
T	uninfected CD4 ⁺ T helper cells	750	cells mm ⁻³
T^*	Infected CD4 ⁺ T helper cells	0.007	cells mm ⁻³
V	viral load	3.65	RNA copies mm ⁻³ * 10 ⁴
A	unactivated DCs	15	cells mm ⁻³
A^*	activated (antigen loaded) DCs	0.02	cells mm ⁻³
W	CD8 memory cells (precursors)	950	cells mm ⁻³
C	CD8 effector cells	8.25	cells mm ⁻³

Note: I assume that the model simulations start from a the asymptomatic phase of infection

Parameter	value	Parameter	value
λ	8.6 day ⁻¹ ml ⁻¹	d_{T^*}	0.42 day ⁻¹
ϕ	0.125 day ⁻¹ ml ⁻¹	d_v	3.1 day ⁻¹
β_1	0.00105 day ⁻¹ ml ⁻¹	d_A	0.008 day ⁻¹
β_2	0.05 day ⁻¹ ml ⁻¹	d_{A^*}	0.06 day ⁻¹
x	0.9	d_c	0.085 day ⁻¹
p	50 day ⁻¹ mm ³	d_w	0.009 day ⁻¹
α	3800	d_d	0.0085 day ⁻¹
h	0.01 day ⁻¹	r	0.03
f	0.00063 day ⁻¹	T_{start}	300 days
q	0.1 day ⁻¹	d_T	0.01 day ⁻¹

Table 4.3: Parameter values used in model simulations.

4.2 Model outcomes

The model has three outcomes which are described in this section. Fig 4.2 shows two of these outcomes.

- Equilibrium 1: No infection and no immune response established. This is a trivial outcome and is given by (4.8)

$$\begin{aligned}
 \hat{T}_1 &= \frac{\lambda}{d_T} \\
 \hat{A}_1 &= \frac{\phi}{d_A} \\
 \hat{T}^*_1 &= \hat{V}_1 = \hat{A}^*_1 = \hat{W}_1 = \hat{C}_1 = 0
 \end{aligned} \tag{4.8}$$

- Equilibrium 2: Infection may be established but the immune system fails to control it leading to the unchecked viral growth and a decline of CD4 helper cells to low levels. The virus however does reach an equilibrium but at very high viral level as T goes to zero. This is referred to as the progression case

and is given by (4.9).

$$\begin{aligned}
 \hat{T}_2 &= \frac{\lambda - \hat{T}^*_{2} d_{T^*}}{d_T} \\
 \hat{T}^*_{2} &= \frac{-Z_1 + \sqrt{Z_2^2 - 4Z_1 Z_3}}{2Z_1} \\
 \hat{V}_2 &= \frac{k d_{T^*} \hat{T}^*_{2}}{d_v} \\
 \hat{A} &= \frac{\phi}{d_A + h \hat{T}^*_{2}} \\
 \hat{A}^*_{2} &= \frac{h \phi \hat{T}^*_{2}}{d_{A^*} (d_A + h \hat{T}^*_{2})} \\
 \hat{W}_2 &= 0 \\
 \hat{C}_2 &= 0
 \end{aligned} \tag{4.9}$$

where $Z_1 = d_A h \beta_1 j_1 d_{T^*}$

$$Z_2 = d_{A^*} d_A \beta_1 j_1 d_{T^*} + h d_{A^*} (d_{T^*} d_T - \beta_1 \lambda j_1) + h \phi d_{T^*} \beta_2 (1 - x)$$

$$Z_3 = d_{A^*} d_A (d_{T^*} d_T - \beta_1 \lambda j_1) - h \phi \lambda \beta_2 (1 - x)$$

$$j_1 = \frac{k d_{T^*}}{d_v}$$

note that \hat{T}^*_{2} is real and positive if $z_2^2 > 4z_1 z_3$

• Equilibrium 3: An infection may also be established but in this case the immune system manages to control the virus at low levels but does not eliminate the virus. This is referred to as the non-progression case and is given by (4.10).

$$\begin{aligned}
 \hat{T}_3 &= \frac{q \hat{T}^*_{3} + d_w}{f A^*} \\
 \hat{V}_3 &= \frac{k d_{T^*} \hat{T}^*_{3}}{d_v}
 \end{aligned}$$

$$\begin{aligned}
\hat{A} &= \frac{\phi}{d_A + h\hat{T}_3^*} \\
\hat{A}_3^* &= \frac{h\phi\hat{T}_3^*}{d_{A^*}(d_A + h\hat{T}_3^*)} \\
\hat{W}_3 &= \frac{d_c\hat{C}_3}{q\hat{T}_3^*} \\
\hat{C}_3 &= \hat{T}_3^* \frac{\beta_1\hat{V}_3 + \beta_2(1-x)\hat{A}_3^*}{p\hat{T}_3^*} - \frac{d_{T^*}}{p}
\end{aligned} \tag{4.10}$$

where \hat{T}_3^* is a positive and real solution of $f(T^*) = 0$

with $f(T^*) = \mathbf{a}_1\hat{T}_3^{*3} + \mathbf{a}_2\hat{T}_3^{*2} + \mathbf{a}_3\hat{T}_3^* + \mathbf{a}_4 = 0$

where $\mathbf{a}_1 = q\beta_1 d_{A^*} h j_1$

$\mathbf{a}_2 = q\beta_1 d_{A^*} d_A j_1 + h d_{A^*} (d_T q + \beta_1 j_1 d_w) + \beta_2 (1-x) h \phi q$

$\mathbf{a}_3 = d_{A^*} d_A (d_T q + \beta_1 j_1 d_w) + d_w d_T d_{A^*} h - f \lambda h \phi + \beta_2 (1-x) h \phi d_w$

$\mathbf{a}_4 = d_w d_T d_{A^*} d_A$

4.2.1 Analysis of equilibrium points

The question now arises, for what parameter values are the different equilibria stable. To answer this question, bifurcation diagrams generated by XPPAUTO were used to determine stability of the equilibria. The following subsections look at how different parameters affect the stability of the equilibrium outcomes of the model. It has been suggested that the ratio of cross presentation to direct presentation is a measure of whether immunity against an infection can be established or not [83]. In the model this ratio of cross presentation to direct presentation is given by $\frac{fT_{A^*}}{qT^*}$. If this ratio is relatively high a persistent immune response (CD8 memory) can be estab-

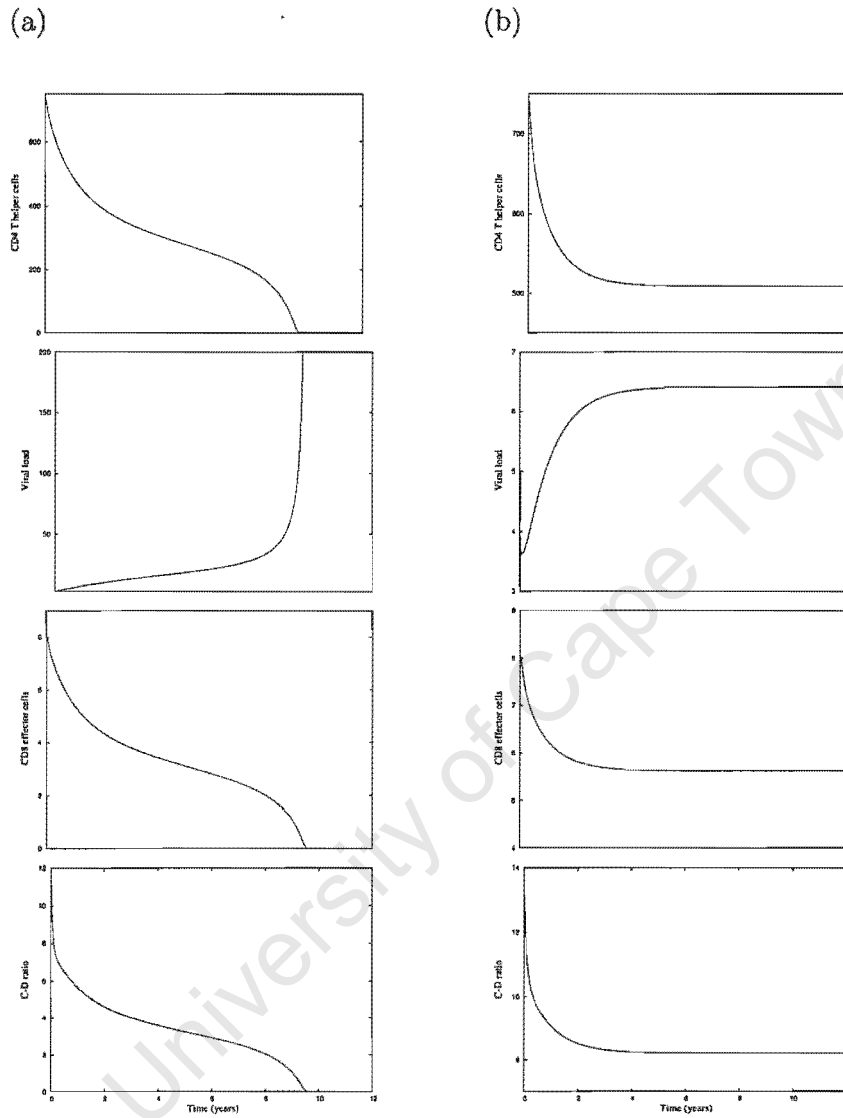


Figure 4.2: *Model outcomes. (a) The progression case in which the system goes to equilibrium 2. (b) The non progression case in which the system goes to equilibrium 3. parameter values given in table (4.3) but in (a) $h=0.072$.*

lished. However if the ratio is low, then the immune response will eventually collapse leading to the unchecked growth of the virus. The DC functionality

and availability of CD4⁺ T helper cells is also considered as it may affect the equilibria stability by influencing the ratio of cross presentation to direct presentation.

The role of DC function in determining model outcome.

When the rate of antigen uptake (h) is low, the ratio of cross presentation to direct presentation is low hence CD8 memory cells fail to persist. For such low values of h equilibrium 2 is stable while equilibrium 3 is unstable (fig. 4.3). As h increases above a certain threshold, equilibrium 2 loses its stability and equilibrium 3 becomes stable. In this case the ratio of cross presentation to direct presentation becomes relatively higher allowing for the establishment of a CD8 memory pool. A similar result was obtained by Wodarz et al [83] in their model of the dynamics of viral induced cancer tumours.

The role of h in determining the outcome of the model is in agreement with the suggestion that HIV impairs the antigen presentation function of DCs resulting in loss of viral control [77, 84, 78]. Despite the efficient presentation of antigen by DCs, low CD8 memory proliferation rates (f) result in failure to establish and maintain a persistent immune response. While this parameter reflects the interaction between DCs and the antigen carrying cell, the parameter f reflects the interaction between the activated DC, T helper cells and naive CD8 cells. The proliferation rate parameter f is also an indicator of the interaction rate between antigen loaded DCs and T helper cells that should result in activation of both and up-regulation of costimulatory molecules required for CD8 memory development [74, 85]. A low value of f thus implies an impaired interaction or signalling between DCs and T helper cells. Studies have suggested that such a poor proliferative response to antigens in HIV infection may not be due to the absence of antigen specific cells

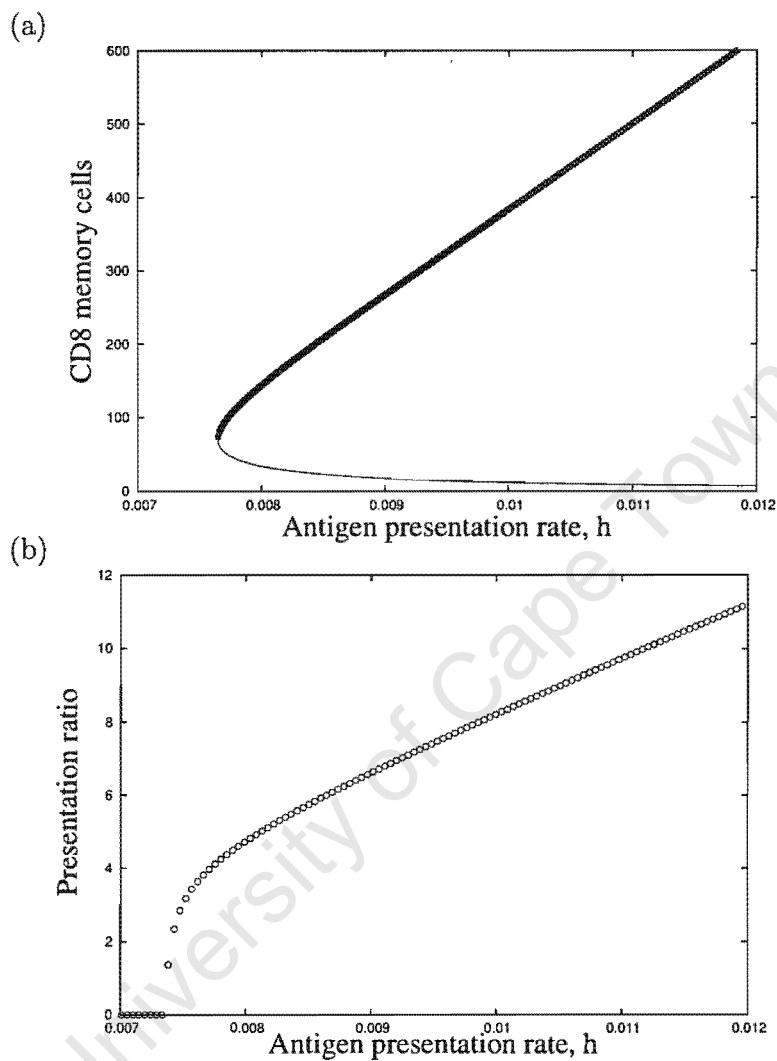


Figure 4.3: *Dependence of CD8 memory cell equilibrium levels on the antigen presentation rate, h . The bold line represents the case when equilibrium 3 is stable and dashed line when it is unstable. (b) Dependence of cross presentation to direct presentation ratio on the antigen presentation rate. Parameter values are given in Table (4.3).*

but rather a direct result of viremia [77].

Given that CD8 memory does develop, the differentiation rate to CD8 effector cells, q would have to be in a certain range to avoid CD8 memory exhaustion. This is largely a result of the short life span of CD8 effector cells [32]. If q is large the long lived memory cells rapidly differentiate into CD8 effectors which have a shorter lifespan. Continued differentiation of CD8 memory cells eventually leads to exhaustion of this cells population thus equilibrium 2 becomes stable. Although differentiation into CD8 effectors may seem to work against the maintenance of a persistent CD8 memory pool, the process is essential in preventing the accumulation of CD8 memory cells and the subsequent overproduction of proinflammatory cytokines such as interferon-gamma (IFN- γ). However a balance has to be maintained to prevent exhaustion of the CD8 memory cells.

The role of CD4⁺ T helper cells in determining the model outcome.

The level of CD4⁺ T helper cells has been shown to depend largely on the cytopathicity of the virus [38]. When the cytopathicity of the virus is low, the level of CD4⁺ T helper cells is higher and a CD8 memory is successfully established. However as the cytopathicity increases, impairment of CD4⁺ T helper cells also increases resulting in the eventual collapse of the immune response. For such higher values of d_{T^*} , equilibrium 3 is unstable while equilibrium 2 is stable as the ratio of cross presentation to direct presentation is low (fig. 4.4). Similar behaviour is observed with variations in the infection rate (β). The model predicts that high infection rates result in increased CD4 T helper cells impairment due to an increase in viral load. As the viral load increases with increasing β , direct presentation increases on the other hand, cross presentation decreases due to the cytopathic effect of the virus on CD4⁺ T helper cells. This all results in a decrease of the ratio of cross

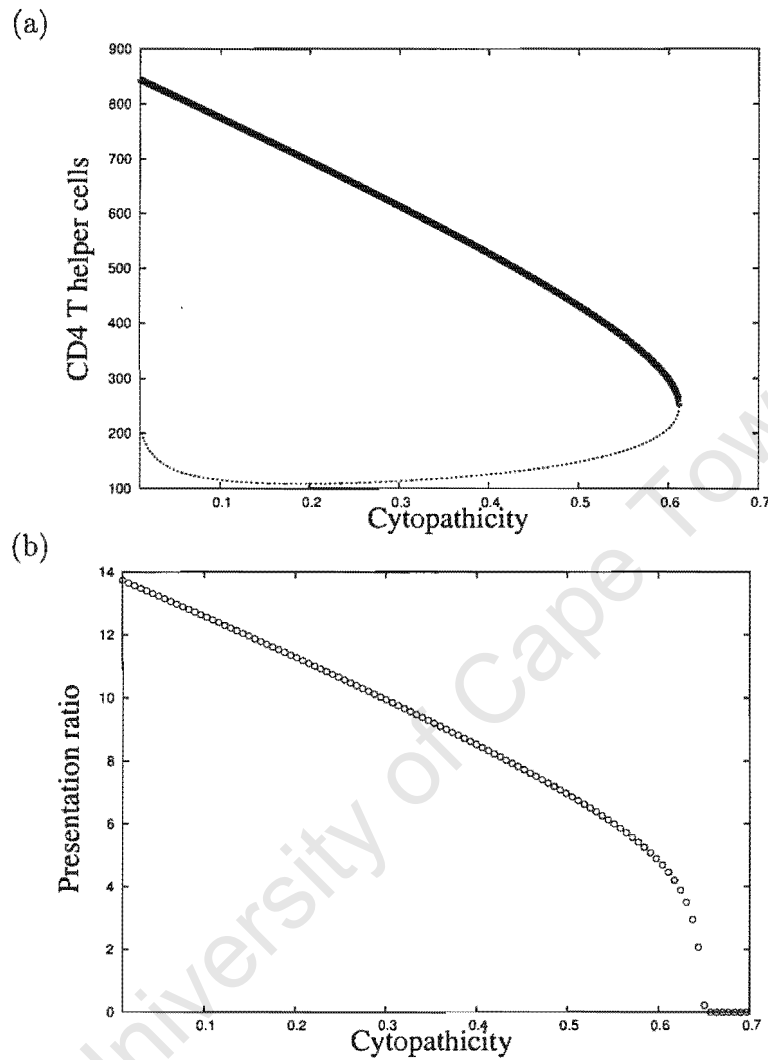


Figure 4.4: (a) Dependence of $CD4^+$ T helper cell equilibrium on the cytopathicity of the virus, d_{T^*} . The bold line represents the case when equilibrium 3 is stable and thin line when it is unstable. (b) Dependence of cross presentation to direct presentation ratio on the cytopathicity of the virus. Parameter values are given in Table (4.3).

presentation to direct presentation hence immune failure. Previous models on CD8 memory development have also such a relationship between memory

development and the infection rate [30, 76, 32]

The following section analyses the dynamics of the model after a DC based vaccine is introduced during the chronic stage. Since the vaccine is made in vitro, it is assumed that the resulting antigen loaded DC cells may be more efficient in cross presentation to CD4⁺ helper cells in vivo. Also under consideration is the possibility that the vaccine influences the ratio of cross presentation to direct presentation hence shifting the dynamics of the model from equilibrium 2 to equilibrium 3 thus regaining viral control.

4.3 Extension of model to include vaccination.

This section considers a therapeutic vaccine similar to that used by Lu et al [5]. The model (equation (3.1)) is extended by introducing an equation describing the dynamics of in vitro antigen loaded DCs, A_d . Since the vaccine is in the form of live DCs, it is assumed that after its administration, the effect of the vaccine decays at a rate d_d as the DCs die. Assuming that the in vitro antigen loaded DCs have a similar function as the in vivo antigen loaded DCs, the term fTA^*W in equation (3.1) is replaced by $fT(A^* + A_d)W$. The extended model is thus given by

$$\begin{aligned}
 \dot{T} &= \lambda - d_T T - \beta_1 TV - \beta_2(1-x)A^*T \\
 \dot{T}^* &= \beta_1 TV + \beta_2(1-x)A^*T - d_{T^*} T^* - pCT^* \\
 \dot{V} &= kd_{T^*} T^* - d_v V \\
 \dot{A} &= \phi - d_A A - hAT^* \\
 \dot{A}^* &= hAT^* - d_{A^*} A^*
 \end{aligned} \tag{4.11}$$

$$\begin{aligned}\dot{W} &= fT(A^* + A_d)W - qT^*W - d_w W \\ \dot{C} &= qT^*W - d_c C \\ \dot{A}_d &= H(t) - d_d A_d\end{aligned}$$

where

$$H(t) = r\delta(t - T_{\text{start}} + \text{interval}(i - 1))$$

r refers to efficiency of the vaccine,

t_{start} refers to time of first injection,

interval refers to the time between any two injections

i refers to the injection number.

4.3.1 Extended model results.

Single therapy results

A simulation of the clinical trial by Lu et al [5] is carried out. In the clinical trial, the vaccine was injected into chronically infected individuals three times at two week intervals and the individuals were monitored for one year. It is found that at a vaccine efficiency of 0.03, the model fits the viral data well (Fig. 4.5). From the first injection, the viral load decreases and reaches a minimum about 7 months later. Similar to the findings of Lu et al [5], the viral reduction was correlated with an increase in the T helper cell and CD8 memory populations (Fig. 4.6). Since the major source of IL-2 is CD4⁺ T helper cells, I assumed that the level of IL-2 expression in CD4⁺ T cells observed during the clinical trial is a reflection of the amount of CD4⁺ T helpers cells. On the other hand the level of IFN- γ expression in CD8 cells reflects the amount of CD8 memory cells [5]. Both CD4⁺ T helper cells and

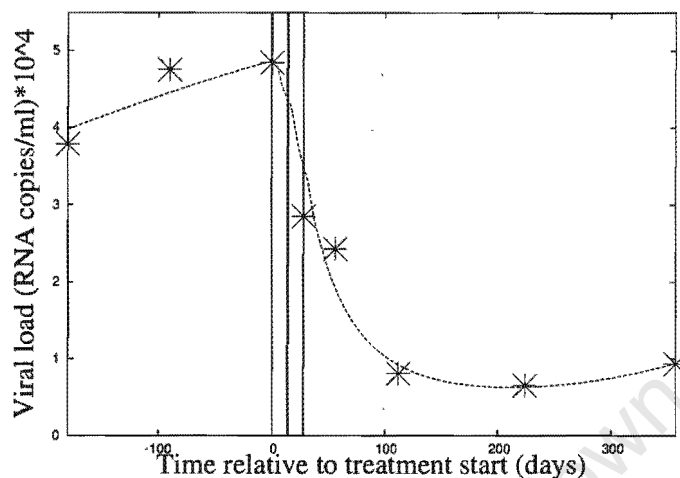


Figure 4.5: A fit of the model to HIV RNA data [5]. The data is indicated by * while the curve indicates the model solution and the vertical lines represent the times when the vaccine is administered during the treatment phase. Parameter values are given in Table (4.3)

CD8 memory cells increase from the first injection however while the peak of CD8 memory cell coincides with the minimum viral load, the CD4⁺ T helper cell peak occurs a few months later. Although these are encouraging results, of interest are the long term outcomes of the clinical trial.

Long term outcomes of single therapy.

Using the parameter values that fit the clinical trial data, the long term outcome of the vaccine are investigated. Simulations are done for both the non-progression and progression cases. In both cases, a viral rebound to pretreatment levels is predicted (Fig. 4.7). This rebound coincides with the vanishing of the in vitro antigen loaded DCs and a decline in CD8 memory cells numbers. This can be attributed to the in vitro antigen loaded DCs that temporarily stimulate the immune response, however, the effect of the

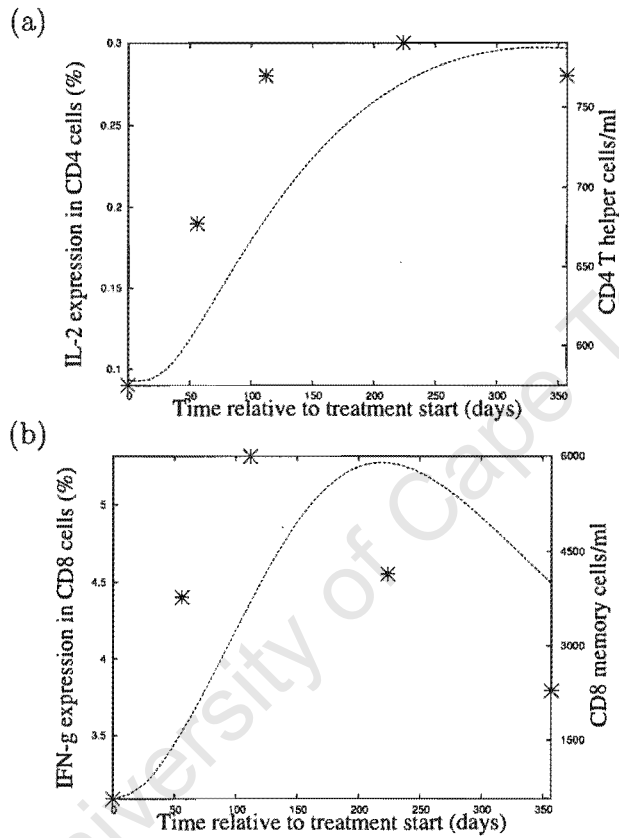


Figure 4.6: Model simulation for CD4 T helper cell (a) and CD8 memory cells (b) plotted with percentage IL-2 expression of CD4 cells data and percentage IFN- γ expression on CD8 cells data respectively [5]. The data is indicated by * while the curve indicates the model solution. Parameter values are given in Table (4.3)

vaccine diminishes after the in vitro antigen loaded DCs die. Such behaviour was observed in clinical trials with HAART but the viral rebound was also attributed to the presence of viral reservoirs in latently infected cells [86]. In the case of a DC vaccine, latently infected cells may be activated from their quiescent state by the increased expression of cytokines, particularly IL-2 [86] that is reported in the clinical trial [5], resulting in a new pool of free virus. As the immune response declines to the pretreatment level, it fails to control the reemerging virus hence a rebound to pretreatment levels.

While the initial model outcome depends on variations in parameter values, the introduction of the in vitro loaded DCs fails to alter these parameter values resulting in the absence of a shift in equilibria. To achieve immunity restoration or improvement, treatment would have to alter parameter values such as the infection rate (β), the rate of antigen uptake (h), or the rate of CD8 proliferation (f) in the absence of the vaccine. A decrease in the infection rate could move the outcome of the model from the progression to the non-progression case and hence the use of a protease inhibitor as a potent adjuvant for the vaccine to gain a favourable result [79]. A similar shift in equilibria could be achieved if the antigen uptake and presentation in DCs was improved by the presence of the vaccine (Fig. 4.3). This would therefore require that the vaccine be able to reverse the impairment of the APC function of DCs that resulted from HIV infection [77]. Although the model predicts a viral rebound, results show that for the progression case, the vaccine delays the onset of unchecked viral growth. This is equivalent to delaying the onset of AIDS.

The influence of treatment initiation.

This subsection investigates the factors affecting the performance of vac-

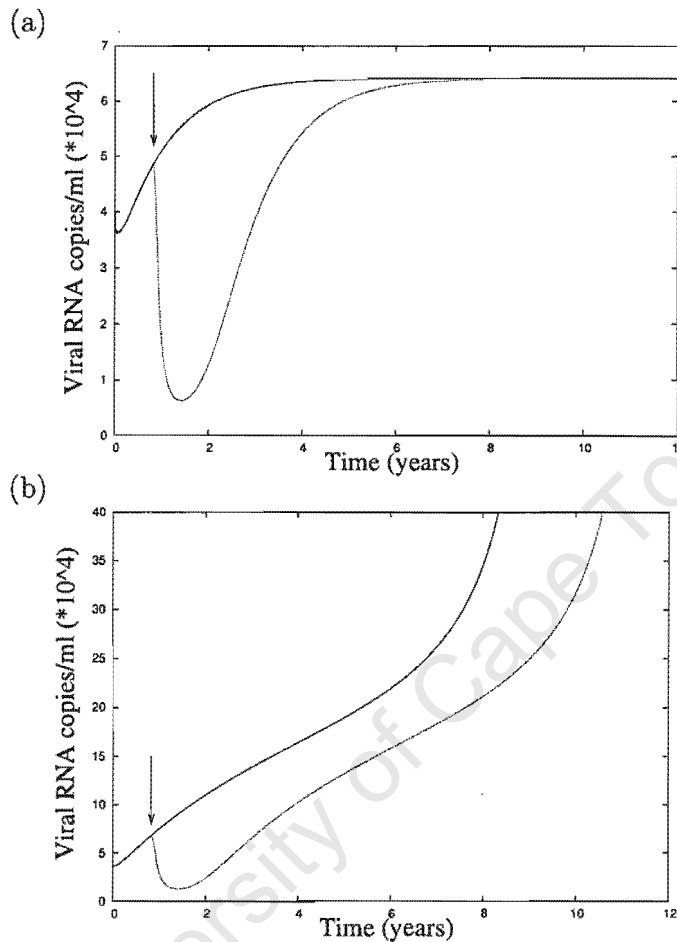


Figure 4.7: Comparison of long term outcomes of vaccination (solid curve) compared with no vaccination (dashed curve) assuming the non-progression case (a) and the progression case (b). Time of treatment initiation is shown by the arrow. (a) In both cases, after vaccination the viral load decreases to reach a minimum after about 7 months after which the virus begins to increase until it eventually reaches the pretreatment level. The vaccine does not only fail to eliminate the virus but also fails to alter the outcome of the infection as both systems converge to their respective no vaccination equilibria. Nonetheless, in (b) the vaccine does delay the onset of unchecked viral growth. Parameter values are given in Table (4.3) with $h = 0.0072$ for (b).

ination. Performance of the vaccine is defined as the extent to which it suppresses the virus and/or shifts the time that the system returns to its pretreatment equilibrium. As is the case with HAART, it is found that earlier administration of the vaccine resulted in higher percentage viral suppression levels in both the non-progression and progression cases of infection (Fig. 4.8(a)). This is due to the presence of a stronger immune response during this period. Such conditions at the start of treatment result in a stronger although temporal boost of the immune system and the subsequent high viral reduction.

On the other hand, later treatment eventually resulted in a total failure of the vaccine due to the immune dysfunction at these times. Although the maximum viral suppression shown in Fig. 4.8(a) generally decreases with increasing T_{start} , before a threshold of T_{start} is reached, the rate of this decrease is low. During this time the performance of the vaccine although decreasing, does not vary much. The level of CD8 cells available is roughly at the same level hence the performance of the vaccine during this period of time does not vary by a large margin. Since the delay in unchecked viral growth value is in addition to T_{start} itself, roughly linear relation is found. Increasing the initiation time thus results in an increase in the delay in the onset of unchecked viral growth¹ not necessarily due to improved vaccine performance but due to the increasing T_{start} . If however T_{start} is above a threshold, due to increased impairment of CD4⁺ T helper cells, the vaccine eventually fails to avert the time of unchecked viral growth. This result implies that during the late chronic stage of infection, the initiation time of treatment does not improve the overall performance of the vaccine.

¹Note: The onset of unchecked viral growth is defined as the time when the number of CD8 memory cells first reaches zero.

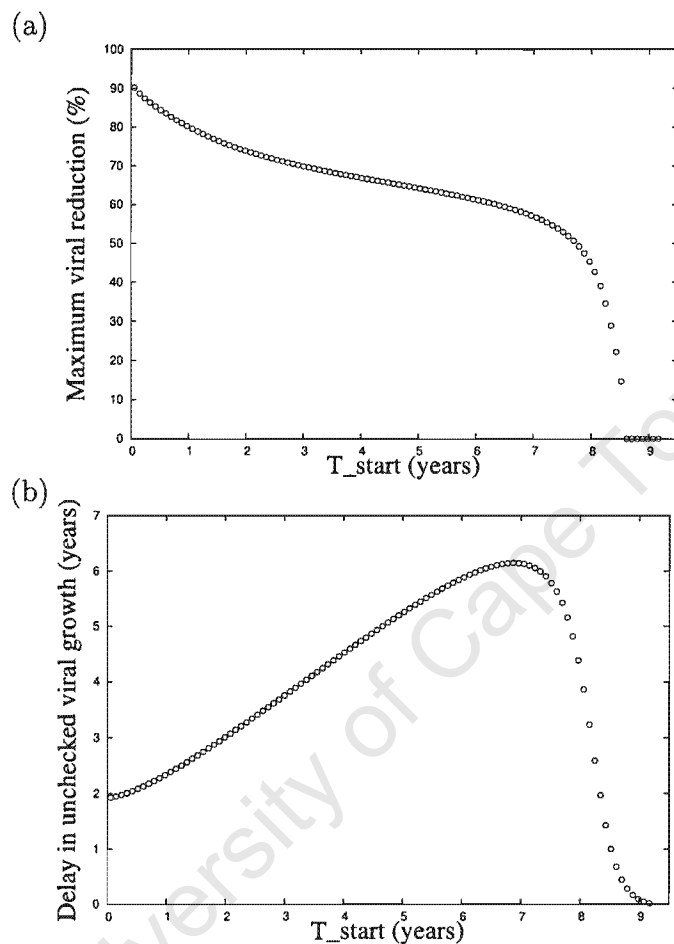


Figure 4.8: *Effect of treatment initiation time on vaccine performance assuming the disease progression case. (a) Earlier treatment initiation results in higher viral suppression due to the presence of a stronger immune response. However as the immune system progresses towards increased impairment, the vaccine eventually fails to suppress the virus. (b) For the progression case, treatment initiation is positively correlated to a delay in the onset of unchecked viral growth. Parameter values are given in Table (4.3) with $h = 0.0072$ and varying T_{start} .*

The influence of Vaccine efficiency.

Further analysis of the model showed that the vaccine efficiency also affects the performance of the vaccine. The more efficient the vaccine, the higher the viral suppression and the higher the delay in the onset of AIDS (Fig. 4.9(a) and (b)). The vaccine efficiency is correlated with the strength and the time that the vaccine remains in the body before being eliminated. For this reason, an increase in vaccine efficiency increases the level and duration of viral suppression by the vaccine. Above an efficiency threshold the vaccine can in principle eliminate the virus, however, such levels of vaccine efficiency lead to over-stimulation of the CD8 memory population. This accumulation in CD8 memory is a result of the overwhelming presence of antigen presenting DCs while at the same time having a reduced level of MHC class I bearing infected CD4 T helper cells that are required for CD8 memory differentiation into CD8 effectors. This over-stimulation of the immune system could result in the overproduction of proinflammatory cytokines such as $\text{INF-}\gamma$, which has been associated with high apoptosis, pathology and death [87]. While $\text{INF-}\gamma$ is required for maturation of DCs, its overproduction may lead to a decrease in cross presentation, as DCs lose their antigen presentation function with maturation [79]. It is therefore important to maintain a balance between achieving an improved vaccine performance and avoiding overproduction of CD8 memory cells.

4.4 The dynamics of repeated vaccination.

Since the vaccine is unlikely to eradicate the virus, it is suggested that the vaccination be repeated to maintain prolonged viral suppression. This how-

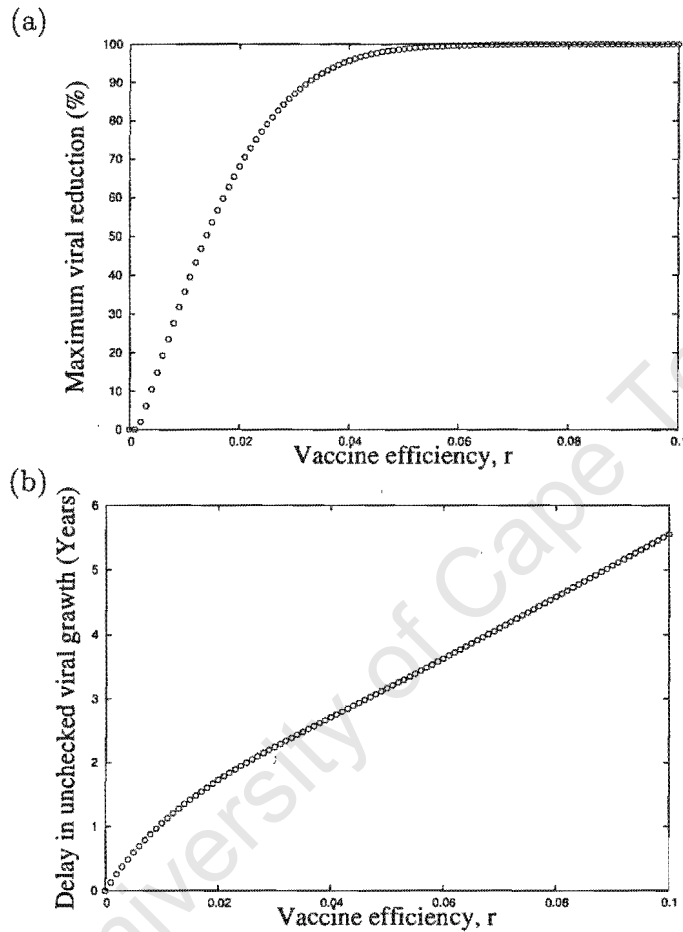


Figure 4.9: (a) The higher the vaccine efficiency, the higher the maximum viral reduction achieved during therapy. (d) A linear relation exists between the vaccine efficiency and delay in AIDS onset. The higher the vaccine efficiency, the longer the delay in onset of unchecked viral growth as the vaccine remains efficient in the body for longer. Parameter values given in Table (4.3) with $h=0.0072$ and varying r .

ever implies that the patient would have to be on lifelong therapy. In clinical trials with HAART, repeated therapy has been associated with increased side effects and the emergence of resistant strains [71, 42]. However, this might not be the case with the DC based vaccine since no major side effects were observed during the clinical trial [5]. Furthermore, the use of whole inactivated viruses reduces the possibility of the emergence of resistance in the event of repeated treatment. The only complication that may be associated with repeated vaccination would however be the risk of overstimulating the proinflammatory cytokines. This section discusses the effects of repeated vaccination as well as how to plan treatment schedules for different individuals.

Fig. (4.10), shows simulation results of a repeated treatment at 15 month intervals. Subsequent treatments would have to be initiated before the virus rebounds to high levels to achieve improved viral suppression with each repeat (Fig. 4.10(a)). However, as the vaccine continues to increase cross presentation with each cycle, direct presentation decreases due to the reduced number of infected cells. This imbalance between cross and direct presentation eventually leads to the accumulation of CD8 memory cells and the subsequent overproduction of proinflammatory cytokines (Fig. 4.10(b)).

An increased amount of CD8 memory cells may trigger the overstimulation of proinflammatory cytokines. These cytokines may lead to a number of side effects such as the further impairment of DCs by overmaturation, exhaustion of the pool of naive CD8 cells and development of inflammatory response syndrome (IRS) related diseases [88]. Since the patient would be on lifelong therapy, it is important to reduce that amount of CD8 cell stimulation due to repeated vaccination. The following subsection discusses how structured treatment interruptions (STIs) may be used to control the CD8 memory cells

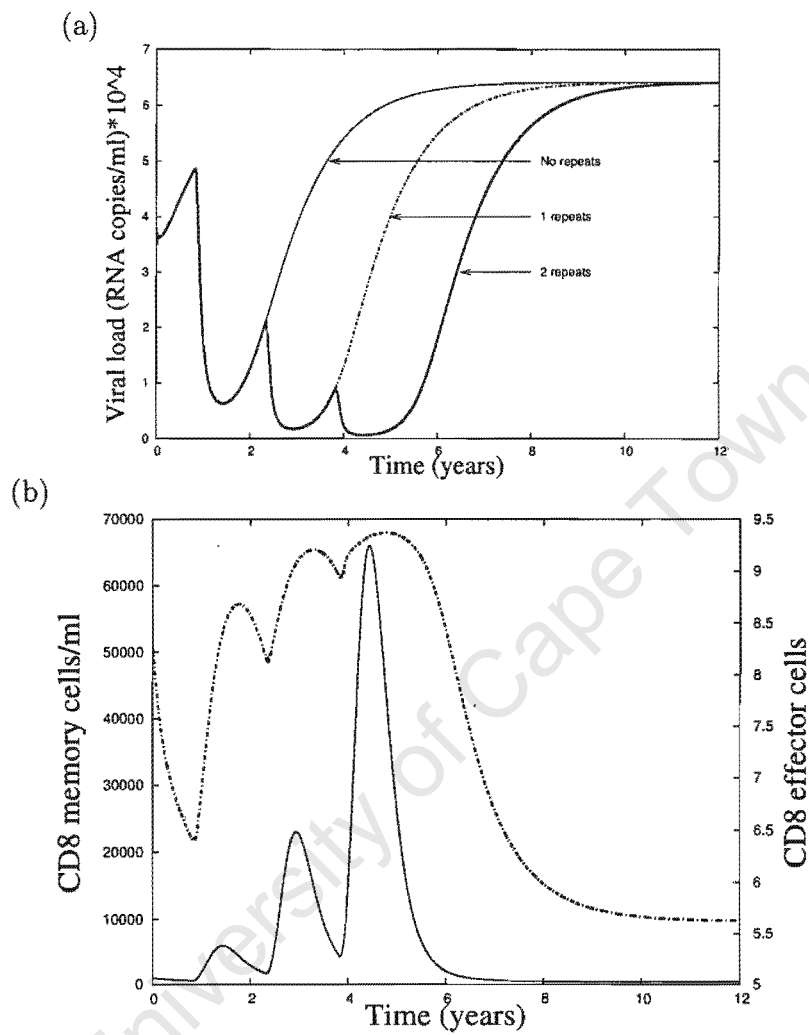


Figure 4.10: Repeated therapy at 15 months intervals. With each repeat, a lower viral load is achieved (a) while at the same time the number of CD8 memory cells continue to increase (b). In (b), dashed line represents CD8 effectors cells while the solid curve represents CD8 memory cells. Parameter value given in Table (4.3).

and viral load levels by changing the number of injections administered and the time after which a new treatment cycle starts.

4.4.1 Designing of repeated treatment schedules.

A reduction in CD8 memory cells stimulation can be achieved by either reducing the number of injections administered in a single cycle or alternatively by extending the period between treatment cycles. In addition this also helps reduce the treatment cost while a simple-to-follow schedule would be necessary to enhance patient adherence to treatment. This subsection discusses two STIs developed for different disease stages.

In the two STIs, three injections are administered at two week intervals for two cycles that are 15 months apart. This brings the viral load below a limit level of 5000 RNA copies/ml. We set this limit based on studies that showed that there was probability less than 0.08 of disease progression at such a viral load [89]. The goal is also to maintain the level of CD8 memory cells below an overstimulation mark that is defined as 50 000 cells/ml. Once a low viral load is achieved, it becomes possible to reduce the vaccine dose by reducing the number of injections in each cycle. However, a reduced dose implies an earlier viral rebound hence reducing the interval between treatment cycles can keep the viral load below the limit mark. This is based on earlier results in which it was shown that earlier treatment would improved viral suppression (fig. 4.8). Variations in the interval between cycles give different results in terms of the maximum CD8 memory cell levels and minimum viral load achieved by the STI. Table(4.4.1) summarises the outline of the two STIs discussed in this section, while fig (4.11 and 4.12) show the simulation outcome of each STI for

Cycle number	1	2	3	4...
31-STI				
Number of injections	3	3	1	1...
Interval between injections	2 weeks	2 weeks	—	—
Time lapse before next cycle	15 mths	15 mths	7.5 mths	7.5mths...
32-STI				
Number of injections	3	3	2	2...
Interval between injections	2 weeks	2 weeks	2 weeks	2 weeks
Time lapse before next cycle	15 mths	15 mths	14 mths	14mths...

Table 4.4: *A summary of the 31-STI and 32-STI repeated treatment schedules. (mths=months)*

the non progression and progression case. The STIs are referred to as the 31-STI and 32-STI depending on the number of injections administered in the first two cycles of vaccination and the number of injections given afterwards.

For the non progression case (Fig. 4.11), a 31-STI is efficient at maintaining a lower CD8 memory population compared to a 32-STI however both manage to keep the viral load below the limit mark. The choice of which STI to use in this case would depend on how well the patient's body can tolerate the different CD8 memory levels. On the other hand, for the progression case, if the treatment cycles are started early enough then the results are similar to the non progression case. However, if treatment is initiated much later, then unlike the non progression case, three injections would have to be administered in the first three cycles, instead of the first two cycles, before reducing the number of injections administered. This helps to bring the viral load down since during this stage it is much higher and the immune

response is weaker as compared to the non progression case. Even after an extra three injection cycle, the 31-STI could not able to bring the viral load below the limit mark while the 32-STI was more successful although with transient lapses of the virus(blips) above the limit mark (Fig. 4.12). Such blips have also been observed in patients undergoing therapy with HAART [90]. Furthermore, a 32-STI also leads to a better CD8 memory stimulation although not as high as in the non progression case. From the progression and non progression results it can be concluded that a 31-STI would be a better STI for patient in the chronic stage of infection whose immune system is still intact. On the other hand, for a patient in the more advanced stages of the disease or late chronic stage, a 32-STI would be a better strategy as it help improve the CD8 memory cell level.

4.5 Chapter summary and conclusion.

This chapter discussed a model describing the role of antigen presentation in HIV-1 infection. It was shown that a balance between cross and direct presentation is required for the successful establishment of CD8 memory in the model and hence prolonged viral control. The ratio of cross presentation to direct presentation is largely influenced by the ability of DCs to function as APCs as well as CD4⁺ T helper cell availability, hence the eventual breakdown of the immune system can be attributed to either DC's failure to function as antigen presenting cells or impairment of CD4⁺ T helper cells or both.

The effect of a DC-based vaccine on viral dynamics was also investigated. The hypothesis was that the vaccine may improve the APC function of DCs,

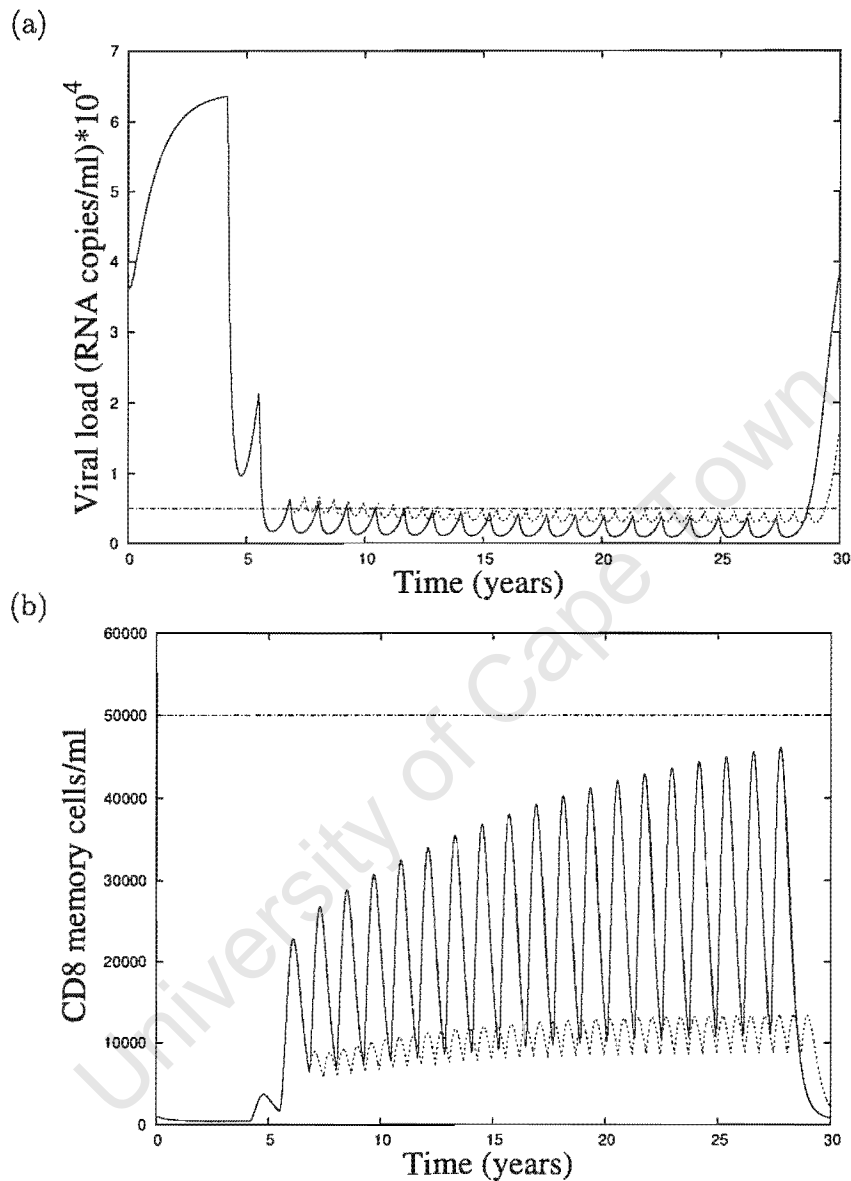


Figure 4.11: The graphs show simulation results of the 31-STI (dashed curve) and 32-STI (continuous curve) for the non progression case. (a) Shows the viral load while (b) shows the CD8 memory level both plotted relative to the limit mark for the viral load and CD8 memory respectively. This limit mark is shown by the dashed horizontal line. In both (a) and (b), a total of 42 injections are administered. Parameter values are given in Table (4.3)

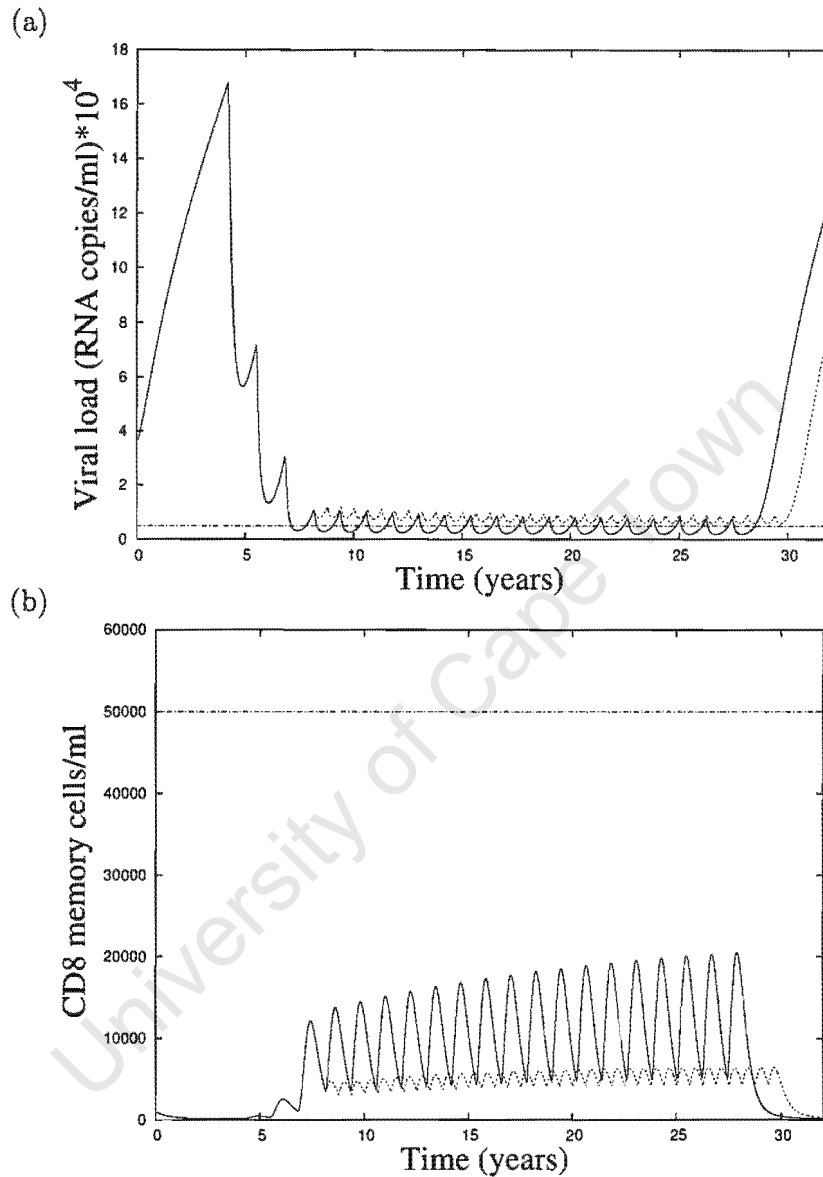


Figure 4.12: The graphs show simulation results of the 31-STI (dashed curve) and 32-STI (continuous curve) for the progression case. (a) Shows the viral load while (b) shows the CD8 memory level. In both (a) and (b), a total of 42 injections are administered. Parameter values are given in Table (4.3) with $h=0.0072$

improve CD4⁺ T helper cell availability by increasing IL-2 levels and restoration of CD8 memory cells as a result of the first two processes. From the model simulations, it was shown that soon after the vaccine is administered, an improved CD4⁺ T helper cell and CD8 memory cell levels as well as a viral reduction by up to 80% can be achieved. However the immune restoration would be temporary hence a viral rebound would occur. It can be concluded that the vaccine is likely to fail in restoring the DC APC function as well as the CD4⁺ T helper cells levels. It has been suggested that CD8 memory cells are developed as a result of priming during the primary stage of infection [33]. This may explain the failed restoration of CD8 memory cells during the chronic stage of infection.

To improve the outcome of vaccination, treatment could be given earlier when the levels of CD4⁺ T helper cell impairment is still low. Alternatively the dosage or efficacy of the vaccine may be increase however this may result in the overproduction of proinflammatory cytokine that could be detrimental to the patient. Furthermore a viral rebound is likely to occur once the effect of the vaccine vanishes in all cases. This implies that the vaccination may have to be repeated for the rest of the patient's life. In designing a treatment schedule for repeated vaccination, one has to consider cost of the vaccination, simplicity of the strategy to improve patient adherence to treatment and most important maintainance of an optimal CD8 memory cells level to avoid overproduction of cytokines. Two STIs are suggested and refered to as the 31-STI and the 32-STI (explained in table. 4.4.1). The 31-STI is efficient in maintaining low CD8 memory levels and a low viral load hence is effective during the early stages of chronic infection where repeated therapy is likely to cause overproduction of cytokines. On the other hand, the 32-STI is efficient in increasing the level of CD8 memory cells while keeping the viral load

low. This STI would therefore be effective during the late stages of chronic infection as it helps restore the immune response.

University of Cape Town

Chapter 5

Vaccination of subtype B and C infected individuals.

So far discussion has focused on the driving forces behind three mechanisms of disease progression namely progression by coreceptor switching, by impairment of the DC antigen presentation function and by depletion of $CD4^+$ T helper cells. This chapter makes use of the knowledge on these mechanisms and the findings from chapter 3 and chapter 4 to make recommendations on the possible effects of vaccination in HIV-1 subtype B and C infected individuals. In addition to the findings in chapter 3 and 4, a combined model (equation 5.1) is developed and used to investigate the effect of vaccination on coreceptor switching.

The development of this model involved the extension of the developed in chapter 3 to include antigen presentation by DCs based of the assumptions of the model in chapter 4 (equations 3.1-3.2 and 4.1-4.7). In addition this model assumes that activation of naive $CD4^+$ T helper cells is a result of

their interaction with antigen loaded DCs. This model is used to investigate the effect of vaccination on the coreceptor switching. Results of the model simulations are discussed in chapter 5.

$$\begin{aligned}
\dot{S} &= \lambda - d_s S - gSA^* \\
\dot{T} &= gSA^* - d_T T - \beta TX_4 \\
\dot{T}^* &= \beta TX_4 - d_{T^*} T^* - pT^* C \\
\dot{M} &= \mu - d_m M - \frac{\beta MR_5}{kC + 1} \\
\dot{M}^* &= \frac{\beta MR_5}{kC + 1} - d_{m^*} M^* \\
\dot{R}_5 &= k_{R_5}(1 - e_v)M^* - d_v R_5 \\
\dot{X}_4 &= k_{X_4} T^* + k_{R_5} e_v M^* - d_v X_4 \\
\dot{A} &= \phi - d_A A - hA(T^* + M^*) \\
\dot{A}^* &= hA(T^* + M^*) - d_{A^*} A^* \\
\dot{W} &= f(A^* + A_d)WS - qW(T^* + M^*) - d_w W \\
\dot{C} &= qW(T^* + M^*) - d_c C \\
\dot{A}_d &= H(t) - d_d A_d
\end{aligned} \tag{5.1}$$

where

$$H(t) = r\delta(t - T_{\text{start}} + \text{interval}(i - 1))$$

r refers to efficiency of the vaccine,

t_{start} refers to time of first injection,

interval refers to the time between any two injections

i refers to the injection number.

In chapter 3, it was found that different immune activation levels may explain

Table 5.1: Parameter values used in combined model simulations.

Parameter	value	Parameter	value
λ	3 day ⁻¹ ml ⁻¹	d_{T^*}	0.24 day ⁻¹
ϕ	0.125 day ⁻¹ ml ⁻¹	d_v	2.4 day ⁻¹
β_1	0.00024 day ⁻¹ ml ⁻¹	d_A	0.008 day ⁻¹
μ	1.5 day ⁻¹ ml ⁻¹	d_{A^*}	0.06 day ⁻¹
f	0.00063 day ⁻¹	d_c	0.3 day ⁻¹
q	0.1 day ⁻¹	d_w	0.01 day ⁻¹
d_s	0.001	d_d	0.0085 day ⁻¹
h	0.02 day ⁻¹	e_v	5.28*10 ⁻⁵
g	1	k_{X_4}	90 day ⁻¹
p	0.9	k_{R_5}	62.5 day ⁻¹
r	0.01	d_T	0.01 day ⁻¹

the variations in parameter values resulting in variations in the choice of coreceptors used for viral entry into target cells. In subtype B a coreceptor switch from dominance of the R5 strain to the X4 strain is more frequent and is associated with the onset of AIDS [42, 13, 46, 48]. This association is generally due the more virulent nature of the X4 strain [46, 10]. However in subtype C, although the R5 strain is less virulent compared to the X4 strain, it has been suggested that cytokines produced as a result of immune activation lead to a faster disease progression by increasing susceptibility of cells to HIV infection, stimulating viral replication and altering cell mediated immunity [64, 67, 63]. This explains how subtype C infected individuals progress to AIDS with no switching to the more virulent X4 strain.

In the absence of a coreceptor switch, as is the case with HIV-1 subtype C, the impairment of the APC function of DCs as well as a decline of the CD4⁺

T help population may be a mechanism for disease progression as shown in chapter 4. Due to the importance of DCs in the response of the immune system, a dendritic cell based vaccine would be a good candidate for treatment of patients. In chapter 4 it was also shown that vaccination would help boost the CD8 memory cells as well as CD4⁺ T helper cells while reducing the viral load. However since vaccination contributes to immune activation, what then is the effect of vaccination with a DC based vaccine on coreceptor choice and hence disease progression in HIV-1 subtype B individuals?

5.1 Effect of vaccination on coreceptor switching.

To investigate the effect of vaccination of coreceptor switching, the models from chapter 3 and 4 were combined and simulations were carried out. In the results below, two cases are defined depending on the vaccination initiation time relative to the coreceptor switch time.

Case 1: Vaccination before a coreceptor switch.

In this case if vaccination is initiated before a coreceptor switch has occurred, it results in a delay in the switch time (Fig. 5.1 b). This can be attributed to increased immune activation as a result of boosting of CD8 cells by the vaccine. An explanation of how immune activation result in such a change in switch time was explained in chapter 3.

Case 2: Vaccination after a coreceptor switch.

In this case if vaccination is initiated after a coreceptor switch has already

occurred, a switch back to R5 dominance occurs. The boosting of CD8 cells results in an increase in the number of CD8 effectors and hence a negative selection pressure on both the X4 and R5 viral strains. However the R5 strain recovers faster than the X4 strain hence out competing it. As the effect of the vaccine vanishes and the immune response weakens, the X4 strain re-emerges and eventually a second switch from R5 to X4 dominance occurs (Fig. 5.1 c).

5.2 Conclusions and recommendations.

Since individuals in subtype C regions are associated with a higher cytokine expression levels, vaccination may be detrimental to the individual if its results in overproduction of cytokines such as $\text{INF}-\gamma$. This can however be avoided through a number of ways. (1) A lower dosage of the vaccine can be given to such individuals to reduce the possibility of overproduction of cytokines. However a lower dose implies less efficiency in viral load reduction. (2) An anti-parasitic drug could be given before administering the vaccine to reduce the immune activation. Alternatively, vaccination together with immunomodulatory drugs such as linomide may be helpful. Linomide has the ability to block proinflammatory cytokines, prevent apoptosis of CD8 and CD4 cells, as well as increasing the production of nitric oxide which has antiviral and antimicrobial properties [91]. This however would imply additional costs for treatment. (3) To avoid reducing the dose of the vaccine or using additional drugs, a structured treatment schedule can be used to maintain a low viral load while at the same time preventing the overproduction of cytokines by minimising CD8 memory production. This could be done with a strategy

such as the 31-STI that was discussed in chapter 4.

On the other hand, in 50% of HIV-1 subtype B infected individuals, complications associated with increased cytokine levels may not be a huge concern. For these individuals, the major concern lies in the high probability of coreceptor switching which is an indicator of accelerated disease progression associated with accelerated CD4⁺ T cell depletion [54, 3]. In this case the initiation time of vaccination would be an important factor as was shown in chapter 4. Although it is not clear whether the collapse of the immune system is the cause or a result of the coreceptor switch, occurrence of the switch can be an additional indicator of the stage of disease progression. Since the treatment strategy to use for repeated vaccination depends of the stage of disease progression when vaccination is started, the coreceptor switch time can be used to determine which strategy to use. Therefore, if a coreceptor switch has occurred the immune response is likely to be weaker hence the 32-STI would be a better strategy to use as it will efficiently reboot the immune response while suppressing the virus. Unfortunately this classification of the stage of disease progression can not be used before the switch has occurred since only 50% of the HIV-1 subtype B infected individuals will exhibit a coreceptor switch.

For those HIV-1 subtype B infected individuals that will not experience a switch, the mechanism of progression to AIDS would thus be similar to HIV-1 subtype C infected individuals. In this case the stage of disease progression would have to be determined by the the strength of the immune response, viral load and level of CD4⁺ T cells as is the case with HIV-1 subtype C infected individuals. It can thus be concluded that a dendritic cell based vaccine developed in HIV-1 subtype B region can thus be used on both

individuals in an HIV-1 subtype B and subtype C region as long as one takes into account the level of immune activation in the individual at the time of vaccination to avoid severe side effect.

5.3 Limitations and future work.

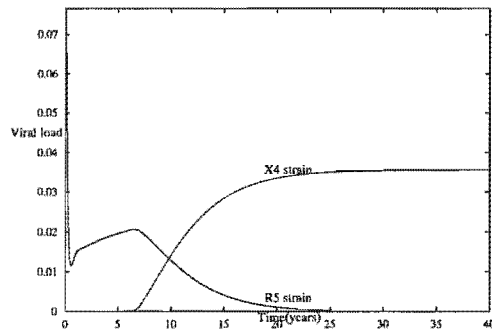
- Although the model developed in chapter 3 was based on previous models and experimental work, there is still need to validate the model with experimental data. Ideally, it would be useful to obtain individual data of the amount R5 and X4 strains over a period of time. In addition a measure of the efficiency of the lytic and non-lytic immune responses in these individuals would also be of great value to test the theory.
- In the model in chapter 3, the evolution of coreceptors is considered to be a deterministic process. However since the conditions within different individuals vary, it would be interesting to investigate whether the results of the model differ if the process is stochastic.
- One of the limitations in the models in chapter 3 and 4 are that they do not account for the effect of the humoral response on HIV-1 infection. HIV-1 neutralising antibodies have been suggested to play a crucial role in viral dynamics [34] hence it may be important to investigate how the interaction between cellular and humoral responses can explain these dynamics.
- For future work a full analysis of the combined model in chapter 5 would be useful to understand the behaviour of the model when certain parameter values are changed.

- Although different treatment strategies have been investigated in different scenarios, for future work it would be interesting to investigate the outcomes of therapy if the patient skips at least one cycle of the STI. This would be helpful considering that patient adherence to treatment is not always 100%.

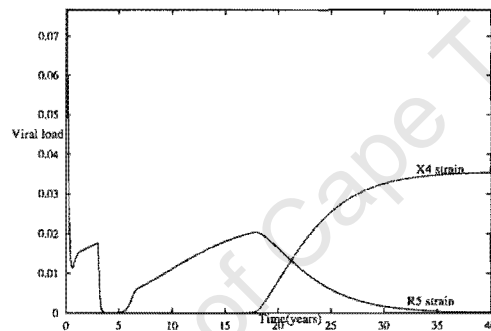
5.4 Papers submitted for publication.

- (1.) Coreceptor switching in HIV-1 subtype B and subtype C. Accepted in The Bulletin of Mathematical Biology.
- (2.) The long-term outcomes of a therapeutic dendritic cell-based HIV-1 vaccine. Submitted to PLoS Medicine

(a)



(b)



(c)

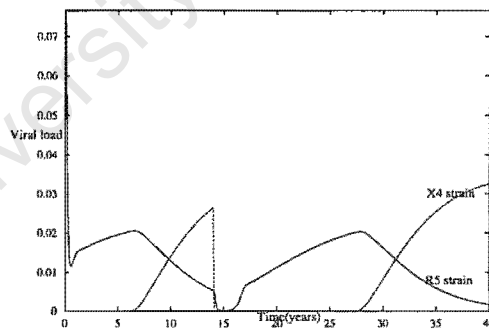


Figure 5.1: Outcomes of the combined model. (a) A coreceptor switch without vaccination (coreceptor switch at year 10). (b) Delayed coreceptor switch when vaccinated before the switch (vaccination at year 3). (c) Repeated coreceptor switching when vaccinated after initial switch (vaccination at year 14). parameter in Table (5.1).

Bibliography

- [1] Avert. World HIV and AIDS statistics. [http:// www.avert.org/ world-stats.htm](http://www.avert.org/world-stats.htm), 2005.
- [2] Monosi. B, T. Oliveira, C. Seebregts, and S. Cassol. HIV-1 prevalence in Africa. <http://www.bioafrica.net/index.html>, 2003.
- [3] Regoes. R. R and S. Bonhoeffer. The HIV coreceptor switch: a population dynamical perspective. *Trends Microbiol*, 13(6):269–277, 2005.
- [4] Panaman. B. Animal experimentation. [http:// www.wolftrust.org.uk/aec-a-entries-animalexp.html](http://www.wolftrust.org.uk/aec-a-entries-animalexp.html), 2003.
- [5] Lu. W, L. C. Arraes, W. T. Ferreira, and J. M. Andrieu. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nature*, 10:1359–1365, 2004.
- [6] Pastores. C, A. Ramos, and D. E. Mosier. Intrinsic obstacles to human immunodeficiency virus type 1 coreceptor switching. *J. Virol.*, 78:7565–7574, 2004.
- [7] Regoes. R. R and S. Bonhoeffer. HIV coreceptor usage and drug treatment. *J. Theor. Biol.*, 217:443–457, 2002.

- [8] Cilliers. T, J. Nhlapo, M. Coetzer, D. Orlovic, T. Ketas, W. C. Olson, J. P. Moore, A. Trkola, and L. Morris. The CCR5 and CXCR4 coreceptors are both used by human immunodeficiency virus type 1 primary isolates from subtype C. *J. Virol.*, 77:4449–4456, 2003.
- [9] Dimitrov. D. S, X. Xiao, D. Chabot, and C. C. Broder. HIV coreceptors. *J. Membr. Biol.*, 166:75–90, 1998.
- [10] Doms. R. W and J. P. Moore. HIV 1 coreceptor use: A molecular window into viral tropism. In *HIV Database Review Articles*. HIV sequence database, 1997. <http://www.hiv.lanl.gov/content/hiv-db/REVIEWS/reviews.html>.
- [11] Witten G. Q and A. S. Perelson. Modeling the cellular-level interaction between the immune system and HIV. *S. A. Jour. Science*, 2004.
- [12] Perelson. A. S and P. W. Nelson. Mathematical analysis of HIV-1 dynamics in vivo. *SIAM*, 41:3–44, 1999.
- [13] Callaway. D. S, R. M. Ribeiro, and M. A. Nowak. Virus phenotype switching and disease progression in HIV-1 infection. *Proc. RSoc. Lond. B Biol. Sci.*, 266:2523–2530, 1999.
- [14] Ho. D. D, A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz. Rapid turnover of plasma virion and CD4 lymphocytes in HIV-1 infection. *Nature*, 373:123–126, 1995.
- [15] Perelson. A. S, D. C. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho. HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, viral generation time. *Science*, 271:1582–1586, 1996.

- [16] Douek. D. C, L. J. Picker, and R. A. Koup. T cell dynamics in HIV 1 infection. *Annu. Rev. Immunol.*, 21:265–304, 2003.
- [17] Koot. M., A. B. Van't Wout, N. A. Kootstra, R. E. de Goede, M. Tersmette, and H. Schuitemaker. Relation between changes in cellular load .evolution of phenotype and the clonal composition of virus populations in the course HIV 1 infection. *J. Infect. Dis.*, 173:349–354, 1996.
- [18] Connor. R. I, K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau. Change in coreceptor use coreceptor use correlates with disease progression in HIV 1 infected individuals. *J. Exp. Med.*, 185:621–628, 1997.
- [19] Lusso. P. HIV and chemokine: implications for pathogenesis, therapy and vaccine. INSTITUT PASTEUR, october 2003.
- [20] Collman. R. G. HIV 1 env chemokine receptor interactions in primary human macrophages: entry and beyond. RESEARCH INITIATIVE Treatment Action, 2003.
- [21] Wodarz. D, A. L. Lloyd, V. A. A. Jansen, and M. A. Nowak. Dynamics of macrophage and T cell infection by HIV. *J. Theor. Biol.*, 196:101–113, 1999.
- [22] Berger. E. A. HIV-1 entry and tropism: the chemokine receptor connection. *AIDS*, 11:s3–s16, 1997.
- [23] Schuitemaker. H. Macrophage tropic HIV 1 variants: initiators of infection and AIDS pathogenesis? *Journal of Leukocyte Biology.*, 56:218–224, 1994.

- [24] Connor. R. I, H. Mohri, Y. Cao, and D. D. Ho. Increased viral burden and cytopathicity correlate temporally with CD4+ T lymphocyte decline and clinical progression in human immunodeficiency virus type 1 infected individuals. *J. Virol.*, 67:1772–1777, 1993.
- [25] Collman. R, J. W. Balliet, S. A. Gregory, H. Friedman, D. L. Kolson, N. Nathanson, and A. Srinivasan. An infectious molecular clone of an unusual macrophage tropic and highly cytopathic strain of human immunodeficiency virus type 1. *J. Virol.*, 66:7517–7521, 1992.
- [26] WHO. HIV vaccine trial results are an important step forward in developing an effective vaccine, say WHO and UNAIDS. <http://www.who.int/mediacentre/news/releases/2003/pr19/en/>, February 2003.
- [27] Nowak. M. A and C. R. Bangham. Population dynamics of immune responses to persistent viruses. *Science*, 272(5258):74–79, 1996.
- [28] Jin. X, D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. J. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.*, 189(6):991–998, 1999.
- [29] De Boer. R. J and A. S. Perelson. Target cell limited and immune control models of HIV infection: a comparison. *J Theor Biol*, 190(3):201–214, 1998.
- [30] Wodarz. D, K. M. Page, R. A. Arnaout, A. R. Thomsen., J. D. Lifson, and M. A. Nowak. A new theory of cytotoxic T-lymphocyte memory

implications for HIV treatment. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 355:329–43, 2000.

- [31] Kesmir. C and R. J. De Boer. Clonal exhaustion as a result of immune deviation. *Bull Math Biol.*, 65:359–374, 2003.
- [32] Wodarz. D, P. Klenerman, and M. A. Nowak. Dynamics of cytotoxic T lymphocyte exhaustion. *Proc. R. Soc. Lond. B. Biol. Sci.*, 265:191–203, 1998.
- [33] Bevan. M. J. Helping the CD8 + T cell response. *Nat. Rev. Immunol.*, 4:595–602, 2004.
- [34] Wodarz. D, R. M. May, and M. A. Nowak. The role of antigen-independent persistence of memory cytotoxic T lymphocytes. *Int. Immunol.*, 12:467–77, 2000.
- [35] Janssen. E. M, E. E. Lemmens, T. Wolfe, U. R. S. Christen, M. G. Von Herrath, and S. P. Schoenberger. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature*, 421:852 – 856, 2003.
- [36] Kundig. M, Bachmann. M. F, Hoffmann. U. W Oehen. S, Simard. J. J. L, Kalberer. C. P, Pircher. H, Ohashi. P. S, Hengartner. H, and Zinkernagel. R. M. On the role of antigen in maintaining cytotoxic T-cell memory. *Proc. Natl. Acad. Sci. U. S. A.*, 93(18):9716–9723, 1996.
- [37] Wodarz. D. Helper dependent versus helper independent CTL responses in HIV infection: Implications for drug therapy and resistance. *J. Theor. Biol.*, 213:447–459, 2001.

- [38] D Wodarz and D. C. Krakauer. Defining CTL induced pathology: implications for HIV. *Virology.*, 274:94–104, 2000.
- [39] Thomsen. R. A, A. Nansen, S. O. Andreasen, D. Wodarz., and J. P. Christensen.. Host factors influencing viral persistence. *Phil. Trans. Roy. Soc. Lond. B*, 355:1031–1041, 2000.
- [40] Wodarz. D, J. P. Christensen, and R. A. Thomsen. The importance of lytic and non-lytic immune responses in viral infections. *Trends. Immunol*, 23(4):194–200, 2002.
- [41] Connor. R. I and D. D. Ho. Human immunodeficiency virus type 1 variants with increased replicative capacity develop during the asymptomatic stage before disease progression. *J. Virol*, 68:4400–4408, 1994.
- [42] Farber. J. M and E. A. Berger. HIV's response to a CCR5 inhibitor: I'd rather tighten than switch! *PNAS.*, 99(4):1749–1751, 2002.
- [43] Wodarz. D and M. A. Nowak. The effect of different immune responses on the evolution of virulent CXCR4 trophic HIV. *Proc R Soc Lond B Biol Sci.*, 265:2149–2158, 1998.
- [44] Abebe. A, D. Demissie, J. Goudsmit, M. Brouwer, C. L. Kuiken, G. Polakakis, H. Schuitemaker, A. L. Fontanet, and T. F. Rinke de Wit. HIV-1 subtype C syncytium and non syncytium inducing phenotypes and coreceptor usage among Ethiopian patients with AIDS. *AIDS*, 13:1305–1311, 1999.
- [45] Cecilia. D, S. S. Kulkarni, S. P. Tripathy, R. R. Gangakhedkar, R. S. Paranjape, and D. A. Gadkari. Absence of coreceptor switch with disease progression in human immunodeficiency virus infections in India. *Virology*, 271:253–258, 2000.

- [46] L. Morris, T. Cilliers, H. Bredell, M. Phoswa, and D. Martin. CCR5 is the major coreceptor used by HIV-1 subtype C isolates from patients with active tuberculosis. *AIDS. Res. Human Retrovir*, 17:697–701, 2001.
- [47] Johnston. E. R, L. S. Zijenah, S. Mutetwa, R. Kantor, C. Kittinunvorakoon, and D. A. Katzenstein. High frequency of syncytium inducing and CXCR4 tropic viruses among human immunodeficiency virus type 1 subtype C infected patients receiving antiretroviral treatment. *J. Virol.*, 77:7682–7688, 2003.
- [48] Pollakis. G, A. Abebe, A. Kliphuis, M. I. Chalaby, M. Bakker, Y. Mengistu, M. Brouwer, J. Goudsmit, H. Schuitemaker, and W. A. Paxton. Phenotypic and genotypic comparisons of CCR5 and CXCR4 tropic human immunodeficiency virus type 1 biological clones isolated from subtype C infected individuals. *J. Virol.*, 78:2841–2852., 2004.
- [49] Ball. S. C, A. Abraha, K. R. Collins, A. J Marozsan, H. Baird, M. E. Quinones-Mateu, A. Penn-Nicholson, M. Murray, N. Richard, M. Lobritz, P. A. Zimmerman, T. Kawamura, A. Blauvelt, and E. J. Arts. Comparing the ex vivo fitness of CCR5 tropic human immunodeficiency virus type 1 isolates of subtypes B and C. *J. Virol.*, 77:1021–1038, 2003.
- [50] Este. J. A, C. Cabrera, J. Blanco, A. Gutierrez, G. Bridger, G. Henson, B. Clotet, D. Schols, and E. De Clercq. Shift of clinical human immunodeficiency virus type 1 isolates from X4 to R5 and prevention of emergence of the syncytium-inducing phenotype by blockade of CXCR4. *J Virol*, 73(7):5577–5585, 1999.
- [51] Trkola. A, S. E. Kuhmann, J. M. Strizki, E. Maxwell, T. Ketas., T. Mor-

- gan, P. Pugach, S. Xu., L. Wojcik, J. Tagat, A. Palani, S. Shapiro, J. Clader, S. McCombie. S, G. R. Reyes, B. M. Baroudy, and J. P. Moore. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. *Proc. Natl. Acad. Sci. USA*, 99(1):395-400, 2002.
- [52] Trkola. A, T. J. Ketas, K. A. Nagashima, T. Cilliers L. Zhao., L. Morris, J. P. Moore, P. J. Maddon, and W. C. Olson. Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. *J. Virol*, 75(2):579-588, 2001.
- [53] Moore. J. P, S. G. Kitchen, P. Pugach, and J. A. Zack. The CCR5 and CXCR4 coreceptors central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses.*, 20:111-126, 2004.
- [54] Harouse. J. M, C. Buckner, A. Gettie, R. Fuller, R. Bohm, J. Blanchard, and C. Cheng-Mayer. CD8+ T cell mediated CXC chemokine receptor 4 simian/human immunodeficiency virus suppression in dually infected rhesus macaques. *Proc. Natl. Acad. Sci. U S A.*, 100:10977-10982, 2003.
- [55] Stevenson. M. Portals of entry: uncovering HIV nuclear transport pathways. *Trends. Cell. Biol.*, 6:9-15, 1996.
- [56] Schuitemaker. H, M. Koot, N. A. Kootstra, W. A. Dercksen, J. M. Lange, J. K. Schattenkerk, F. Miedema, and M. Tersmette. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection progression of disease is associated with a shift from monocytotropic to T cell tropic virus population. *J Virol.*, 66:1354-1360, 1992.

- [57] Van't Wout. A. B, N. A. Kootstra, G. A. Mulder, N. Albrecht-van., H. J. Scherpbier., J. Veenstra., K. Boer, R. K. Coutinho, F. Miedema, and H. Schuitemaker. Macrophage tropic variants initiate human immunodeficiency virus type 1 infection after sexual parenteral and vertical transmission. *J. Clin. Invest.*, 94:2060–2067, 1994.
- [58] Zinkernagel. R. M, M. F. Bachmann, T. M. Kundig, S. Oehen, H. Pirchet, and H. Hengartner. On immunological memory. *A. Rev. Immunol.*, 14:333–367, 1996.
- [59] Moskophidis. D, F. Lechner, H. Pircher, and R. M. Zinkernagel. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature.*, 362:758–761, 1993.
- [60] Starfield. M. A and A. L. Bleloch. *Building models for conservation and wildlife management*. The Burgess Press, second edition, 1991.
- [61] Plackett. R. L and J. P. Burman. The design of optimum multifactorial experiments. *Biometrika.*, 33:305–332, 1946.
- [62] D. L. Beres and D.M. Hawkins. Plackett-Burman technique for sensitivity analysis of many parametered models. *Ecological modelling.*, 141:171–183, 2001.
- [63] Clerici. M, S. Butto, M. Lukwiya, M. Saresella, S. Declich, D. Trabattioni, C. Pastori, S. Piconi, C. Fracasso, M. Fabiani, P. Ferrante, G. Rizzardini, L. Lopalco, and et al. Immune activation in Africa is environmentally driven and is associated with upregulation of CCR5. *AIDS.*, 14:2083–2092, 2000.
- [64] Clerici. M, S. Declich, and G. Rizzardini. African enigma: Key player

- in human immunodeficiency virus pathogenesis in developing countries?
Clinical and Diagnostic Laboratory Immunology., 8:864–866, 2001.
- [65] Perelson A. S, P. Essunger, Y. Cao, M. Vesanen, A. Hurley, K. Saksela, M. Markowitz, and D. D. Ho. Decay characteristics of HIV 1 infected compartments during combination therapy. *Nature.*, 387:188–191, 1997.
- [66] Fraziano. M, G. Cappelli, M. Santucci, F. Mariani, M. Amicosante, M. Casarini, S. Giosue, A. Bisetti, and V. Colizzi. Expression of CCR5 is increased in human monocyte derived macrophages and alveolar macrophages in the course of in vivo and in vitro Mycobacterium tuberculosis infection. *AIDS Res. Hum. Retrovir.*, 15:869, 1999.
- [67] Rizzardini. G, D. Trabattoni, M. Saresella, S. Piconi, M. Lukwiya, S. Declich, P. Ferrante, and M. Clerici. Immune activation in HIV infected African individuals. *AIDS.*, 12:2387–2396, 1998.
- [68] Finnegan. A, K. A. Roebuck, B. E. Nakai, D. S. Gu, M. F. Rabbi, S. Song, and A. L. Landay. IL-10 cooperates with TNF-alpha to activate HIV 1 from latently and acutely infected cells of monocyte/macrophage lineage. *J. Immunol.*, 156:841–851, 1996.
- [69] Ramalingam. S, R. Kannangai, T. S. Vijayakumar, S. Subramanian, O. C. Abraham. O. C, Rupali. P, Jesudason. M. V, and Sridharan. G. Increased number of CCR5+ CD4 T cells among south Indian adults probably associated with the low frequency of X4 phenotype of HIV-1 in India. *Indian. J. Med. Res.*, (116):90–95, 2002.
- [70] Abbas. A. K, D. M. Lichtman, and J. S. Pober. *Cellular and Molecular Immunology*, chapter 6. W.B. Saunders Company, 1991.

- [71] Culshaw. R. V, S. Ruan, and R. J. Spiteri. Optimal HIV treatment by maximising immune response. *J. Math. Biol.*, 48:545 – 562, 2004.
- [72] Marzo. A. L, V. Vezys, K. D. Klonowski, S. J. Lee., G. Muralimohan, M. Moore, F. D. Tough, and L. Lefrancois. Fully functional memory CD8 T cells in the absence of CD4 T cells. *J. Immunol*, 173:969–975, 2004.
- [73] Shedlock. D. J, J. K. Whitmire, J. Tan, A. S. MacDonald., R. Ahmed, and H. Shen. Role of CD4 T cell help and costimulation in CD8 T cell responses during *Listeria monocytogenes* infection. *J. Immunol*, 170:2053–2063, 2003.
- [74] Lakkis. F. G and M. H. Sayegh. Memory T cells: A hurdle to immunologic tolerance. *J. Am. Soc. Nephrol.*, 14:2402–2410, 2003.
- [75] Ribeiro. R. M, H. Mohri, D. D. Ho., and A. S. Perelson. In vivo dynamics of T cell activation, proliferation, and death in HIV-1 infection: Why are CD4+ but not CD8+ T cells depleted? *Proc. Natl. Acad. Sci. U. S. A*, 99:15572–15577, 2002.
- [76] Altes. H. K, D. Wodarz, and V. A. A. Jansen. The dual role of CD4 T helper cells in the infection dynamics of hiv and their importance for vaccination. *Jour. Theor. Bio*, 14:633–646, 2002.
- [77] Kawamura. T, H. Gatanaga, D. L. Borris, M. Connors, H. Mitsuya, and A. Blauvelt. Decreased stimulation of CD4+ T cell proliferation and IL-2 production by highly enriched populations of HIV-infected dendritic cells. *J. Immunol.*, 170:4260–4266, 2003.
- [78] Smed-Sorensen. A, L. Lore, L. Walther-Jallow, J. Andersson, and A. L. Spetz. HIV-1 infected dendritic cells up-regulate cell surface markers

but fail to produce IL-12 p70 in response to CD40 ligand stimulation. *Blood*, 104:2810–2817, 2004.

- [79] Lu. W and J. M. Andrieu. In vitro human immunodeficiency virus eradication by autologous CD8 + T cells expanded with inactivated-virus-pulsed dendritic cells. *J. Virol.*, 75:8949–8956, 2001.
- [80] Steinman. R. M. DC-SIGN: a guide to some mysteries of dendritic cells. *Cell.*, 100:491–494, 2000.
- [81] Geijtenbeek. T. B, D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, and Y. van Kooyk. DC-SIGN, a dendritic cell specific HIV-1 binding protein that enhances trans-infection of T cells. *Cell*, 100:587–597, 2000.
- [82] Ramirez. M. C and L. J. Sigal. Macrophages and dendritic cells use the cytosolic pathway to rapidly cross-present antigen from live, vaccinia-infected cells. *J Immunol.*, 169:6733–6742, 2002.
- [83] Wodarz. D and V. A. A. Jansen. A dynamical perspective of CTL cross-priming and regulation: implications for cancer immunology. *Immunol. Lett.*, 86:213–227, 2003.
- [84] Lu. W, X. Wu, L. Lu, W. Guo, and J. M. Andrieu. Therapeutic dendritic-cell vaccine for simian AIDS. *Nature*, 9:27–32, 2003.
- [85] Horig. H, F. A. Medina, W. A. Conkright, and H. L. Kaufman. Strategies for cancer therapy using carcinoembryonic antigen vaccines. *Exp. Rev. Mol. Med.*, 2000. <http://www.expertreviews.org/0000168Xh.htm>.

- [86] Chun. T. W and A. S. Fauci. Latent reservoirs of HIV: Obstacles to the eradication of virus. *Proc. Natl. Acad. Sci. USA*, 96:10958–10961, 1999.
- [87] Gavrilescu. L. C and E. Y. Denkers. IFN-gamma overproduction and high level apoptosis are associated with high but not low virulence toxoplasma gondii infection. *J. Immunol.*, 167(2):902–909, 2001.
- [88] Leonard. B. E. The immune system, depression and the action of antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry.*, 25:767–804, 2001.
- [89] Mellors. J. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *science*, 272:1167–1170, 1996.
- [90] Bajaria. S. H, Webb. G, and Kirschner. D. E. Predicting differential responses to structured treatment interruption during HAART. *Bull. Math. Biol.*, 66:1093–1118, 2004.
- [91] del Real. G, M. Llorente, L. Bosca, S. Hortelano, A. Serrano, P. Lucas, J. L. Toran L. Gomez, C. Redondo, and C. Martinez. Suppression of HIV-1 infection in linomide-treated SCID-hu-PBL mice. *AIDS*, 12(8):865–872, 1998.

Appendix A

Results tables

A.1 PBSA single parameter results.

Table A.1: PBSA results for the individual parameter effects, with an increase of 10% in all parameters. The sign of the change in switch time shows the direction of change, therefore, a positive result implies an increase in switch time while a negative result implies a decrease in switch time.

Parameter	Change in switch time (days)
k_{X_4}	-903.0
p	677.0
d_m	-651.0
μ	426.5
k	-424.0
k_{R_5}	415.0

Table A.1 – (continued)

Parameter	Change in switch time (days)
d_m^*	-379.5
λ	-285.5
d_c	-273.0
r	-271.5
g	-236.5
d_T	186.5
d_w	-160.5
e_v	145.5
f	110.5
dv	-102.0
d_T^*	94.5
d_s	78.0
β	-22.5

A.2 PBSA paired interaction results.

Table A.2: PBSA results from two way interactions of different parameters, with an increase of 10% for all cases. (Only interaction that resulted in a change in switch time of ≥ 1 year are shown as they are the one considered to be significant).

Parameter	Change in switch time (days)
$k_{X_4} \times d_m$	-1371
$k_{X_4} \times d_c$	-1319
$k_{X_4} \times r$	-1285
$k_{X_4} \times g$	-1281
$k_{X_4} \times d_v$	-1279
$k_{X_4} \times k$	-1263
$k \times d_m$	-1263
$k_{R_5} \times p$	1251
$k_{X_4} \times d_{m^*}$	-1211
$e_v \times p$	1194
$k_{X_4} \times d_{T^*}$	-1158
$p \times \mu$	1157
$d_{m^*} \times r$	-1114
$d_m \times \lambda$	-1018
$d_m \times g$	-991
$k_{R_5} \times \mu$	984
$k_{X_4} \times d_w$	-964
$k_{X_4} \times \lambda$	-952
$d_{T^*} \times p$	949
$d_m \times d_s$	-942

Table A.2 – (continued)

Parameter	change in switch time (days)
$d_c \times d_m$	-891
$\beta \times d_m$	-880
$d_{m^*} \times k$	-858
$p \times d_T$	848
$d_{m^*} \times d_m$	-847
$\beta \times \mu$	835
$d_w \times \lambda$	-820
$k \times g$	-794
$k_{X_4} \times d_s$	-785
$d_v \times p$	761
$r \times d_m$	-760
$d_{m^*} \times d_c$	-741
$k_{X_4} \times d_T$	-721
$\lambda \times g$	-716
$e_v \times k_{X_4}$	-713
$\mu \times d_T$	709
$p \times d_s$	702
$d_v \times d_m$	-682
$k \times d_w$	-681
$k_{X_4} \times \mu$	-652
$d_w \times d_m$	-650
$\beta \times k$	-642
$k \times r$	-642
$k \times \lambda$	-642
$k_{R_5} \times d_{T^*}$	636

Table A.2 – (continued)

Parameter	change in switch time (days)
$k_{R_5} \times d_T$	632
$\beta \times k_{X_4}$	-628
$k \times d_s$	-625
$f \times \mu$	623
$\mu \times d_s$	599
$d_v \times d_{m^*}$	-595
$d_{m^*} \times \lambda$	-591
$k_{R_5} \times d_m$	-569
$f \times \lambda$	-557
$k_{X_4} \times f$	-553
$d_v \times d_c$	-539
$r \times d_c$	-534
$d_{T^*} \times d_m$	-533
$p \times f$	532
$k_{R_5} \times d_c$	529
$e_v \times d_m$	-522
$\beta \times p$	516
$d_c \times \lambda$	-512
$p \times \tau$	498
$e_v \times \mu$	496
$d_{m^*} \times d_w$	-474
$p \times d_c$	468
$\beta \times \lambda$	-458
$d_w \times g$	-455
$d_c \times g$	-452

Table A.2 – (continued)

Parameter	change in switch time (days)
$e_v \times f$	450
$r \times \lambda$	-445
$d_{m^*} \times p$	444
$f \times d_T$	440
$r \times d_w$	-435
$d_s \times g$	-431
$f \times d_m$	-428
$e_v \times k_{R_5}$	424
$d_m \times d_T$	-417
$d_{T^*} \times d_c$	-412
$d_w \times d_c$	-412
$k_{R_5} \times d_s$	402
$d_{m^*} \times g$	-392
$d_v \times \lambda$	-374
$d_w \times d_T$	370
$d_{m^*} \times d_T$	-367

Appendix B

Computer programs

B.1 PBSA for the coreceptor model

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
%This is an octave code for the Plackett-Burman sensitivity      %  
%analysis technique.                                           %  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
%percentage change in parameters  
change=10;  
%design size  
designsize=20;  
%total number of parameters to be changed  
noofparameters=19;  
%number of scenarios for single parameter effects  
noofscenarios=2*designsize;  
output=fopen("pbsaresults.dat","w+");
```



```

%placement of parameter values into matrix form
parameter(1,1)=2.4*10^-4;
parameter(1,2)=0.22*parameter(1,1)^2;
parameter(1,3)=62.4;
parameter(1,4)=90;
parameter(1,5)=2.4;
parameter(1,6)=0.03;
parameter(1,7)=0.24;
parameter(1,8)=0.78;
parameter(1,9)=50;
parameter(1,10)=0.005;
parameter(1,11)=0.01;
parameter(1,12)=0.5;
parameter(1,13)=0.1;
parameter(1,14)=1.5;
parameter(1,15)=0.005;
parameter(1,16)=5;
parameter(1,17)=0.001;
parameter(1,18)=0.01;
parameter(1,19)=0.01;
%upper and lower levels of parameter values based of the
%percentage change
upper=parameter(1,:)*(change/100 + 1);
lower=parameter(1,:)*(1-change/100);
%the design matrix M
M=[1,1,-1,-1,1,1,1,1,-1,1,-1,1,-1,-1,-1,-1,1,1,-1];
for j=1:designsize-2;

```

```

    for i=1:designsize-2;
        M(j+1,1)=M(j,designsize-1);
        M(j+1,i+1)=M(j,i);
    end
end
M(designsize,1:designsize-1)=-1;
M(designsize+1:2*designsize,1:designsize-1)=
-M(1:designsize,1:designsize-1);
%parameter values for different scenerios
global scenario
for i=1:2*designsize;
    for j=1:noofparameters;
        if (M(i,j)==1) scenario(i,j)=upper(1,j);
        else scenario(i,j)=lower(1,j);
        endif
    end
end
end
%running of different scenarios
global i;
for i=1:noofscenarios;
    function xdot = f(x, t);
    global i scenario;
    %reassignment of parameter values according to scenario
    bta=scenario(i,1);
    ep=scenario(i,2);
    kr=scenario(i,3);
    kx=scenario(i,4);

```

```

dv=scenario(i,5);
dm=scenario(i,6);
dt=scenario(i,7);
p=scenario(i,8);
k=scenario(i,9);
r=scenario(i,10);
dw=scenario(i,11);
f=scenario(i,12);
dc=scenario(i,13);
mu=scenario(i,14);
dM=scenario(i,15);
lambda=scenario(i,16);
ds=scenario(i,17);
g=scenario(i,18);
dT=scenario(i,19);
%model equations
xdot=zeros(9,1);
xdot(1) = lambda - ds * x(1) - (g * x(1) * (x(3)+ x(5)));
xdot(2) = (g * x(1) * (x(3)+ x(5))) - dT * x(2) - bta * x(2) *
x(7);
xdot(3) = (bta * x(2) * x(7)) - (p * x(3) * x(9)) - dt * x(3);
xdot(4) = mu - dM * x(4) - (bta * x(4) * x(6))/(k * x(9) + 1);
xdot(5) = (bta * x(4) * x(6))/(k * x(9) +1) - dm * x(5);
xdot(6) = kr * (1 - ep) * x(5) - dv * x(6);
xdot(7) = kr * ep * x(5) + kx * x(3) - dv * x(7);
xdot(8) = r * (x(3) + x(5)) * x(8) * x(2) - f * (x(3) + x(5)) *
x(8) - dw * x(8);

```

```

xdot(9) = f * (x(3) + x(5)) * x(8) - dc * x(9);
endfunction

%solving of model equations
%length of each simulation
days=5400;

%initial conditions
x0 = [1000; 500; 0; 1000; 0; 10^-3; 0*10^-3; 100; 0];
t = linspace (0, days, days);

%ODE solver options
lsode_options("integration method","stiff");
lsode_options("relative tolerance",1e-8);
[y,ISTATE, MSG]= lsode(["f"], x0, t);

%identification and recording of switch time
temp(i,1)=days;
for j=2:days;
    if (y(j-1,6)>y(j-1,7))&&(y(j,6)<y(j,7)) temp(i,1)=t(1,j);
    endif
endfor
switchtime(i,1) =temp(i,1);
endfor
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%calculating individual effects of parameters
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
for k=1:noofparameters;
    for i=1:noofscenarios;
        Cn(i,k)=switchtime(i,1)*M(i,k);
    endfor

```

```

    Pr(k)=k;
endfor
effect=sum(Cn,1)/designsize;
%rounding off and sorting of effects
effect1=[effect;Pr];
signs=sign(effect);
effect2=round(abs(effect1));
[s, i]=sort(-effect2(1, :));
effect3=effect2(:,i);
%printing of results including a key for parameters
fprintf(output,"Key: 1=beta; 2=ep; 3=kr; 4=kx; 5=dv;
6=dm; 7=dt; 8=p; 9=k; 10=r; 11=dw; \n 12=f; 13=dc 14=lambda;
15=dM; 16=mu; 17=ds; 18=g; 19=dT \n \n");
fprintf(output,"Parameter   Change in switch time  \n");
for i=1:noofparameters;
    fprintf(output,"%i \t %7f \t ",effect3(2,i),
            signs(1,effect3(2,i))*effect3(1,i)/2);
    fprintf(output,"\n");
end
fprintf(output,"\n");
fprintf(output,"\n");
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%calculation of pairwise interaction effects
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%number of scenarios or pairs
noofpairs=bincoeff(noofparameters,2);
%signs matrix for calculation of paired effects

```

```

for i=1:noofparameters-1;
    for j=i+1:noofparameters;
        k=((i-1)*noofparameters)-(sum(0:i-1))+j-i;
        IM(1:noofscenarios,k)=M(1:noofscenarios,i).*
        M(1:noofscenarios,j);
        order(1,(k))=i;
        order(2,(k))=j;
%assignment of interaction scores according to matrix signs
        for t=1:noofscenarios;
            tk(t,k)=switchtime(t)*IM(t,k);
        endfor
    endfor
endfor
%calculation switch time change due to paired interaction and
%sorting
paireffect=sum(tk,1)/designsize;
for i=1:noofpairs;
    total(i)=(paireffect(1,i)+effect(1,order(1,i))+
    effect(1,order(2,i)))/2;
endfor
tsigns=sign(total);
ptotal1=[total;[1:k];order(1,:);order(2,:)];
ptotal=round(abs(ptotal1));
[s, i] = sort (-ptotal(1,:,:,:));
ptotal2=ptotal(:,i);
%printing of results
fprintf(output,"Parameter    Change in switch time  \n");

```

```

for i=1:noofpairs;
    fprintf(output,"%i X %i \t %7f",ptotal2(3,i),ptotal2(4,i),
    tsigns(1,ptotal2(2,i))*ptotal2(1,i));
    fprintf(output,"\n");
endfor
fprintf(output,"\n");
fprintf(output,"\n");
fclose (output);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

B.2 DC vaccine single therapy programme

```

#####
#This is an XPPAUTO programme for the dendritic cell based      #
#vaccine model with a single vaccination cycle.                 #
#####
#Model equations
th'=s-dt*th-bta*th*v-bta2*(1-x)*at*th
tht'=bta*th*v+bta2*(1-x)*at*th-dit*tht-p*c*tht
v'=k*dit*tht-dv*v
a'=-mu-da*a-h*a*tht
at'=h*a*tht-dat*at
w'=f*th*(at+vac)*w-q*tht*w-dw*w
c'=q*tht*w-dc*c
#impulse function for the vaccine
vac'=impulse(t)-daa*vac

```

```

impulse(t)=dose*heav((tstart+(ntimes-1)*(interval-1)+ntimes*
duration)-t)*heav(t-tstart)*heav(mod(t-(tstart+duration),
(interval-1)+duration)-(interval-1))
#Initial conditions
init th=750 tht=0.007 v=3.65 w=950 c=8.25 a=15 at=0.02 vac=0
#Parameter values
par dose=0.03 tstart=300 duration=1 interval=14 ntimes=3
par dt=0.01 s=8.6 mu=0.125 da=0.008 daa=0.0085
par dit=0.42 dv=3.1 k=3800 x=0.9 bta2=0.05 bta=0.00105
par dc=0.085 dw=0.009 p=50 f=0.00063 q=0.1 h=0.01 dat=0.06
#setting up xppaut options
@ meth=stiff total=4320 trans=120 bound=1000000000000
@ toler=1e-8 bell=0 maxstor=10000000 DT=1
#new buttons
@ but=run:ig but=view:x but=exit:fq
#setting up AUTO options
@ NTST=10000 NMAX=500000 NPR=100000 DSMIN=0.0001 DSMAX=0.01
done
#####

```


University of Cape Town