Chapter 2
System Biology Models and Conceptualizations Applied to Eco-Immunology

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Abstract This article outlines how ecological concepts, based on population dynamics and mathematical models, can be helpful for investigating various aspects of immunology, and in particular immune responses against infectious diseases. The review starts by describing how basic ecological concepts have been applied to investigating the *in vivo* dynamics between immune responses in viral infections *in vivo* and discusses the usefulness and limitations of such approaches. These concepts include predator-prey dynamics, competition between different clones of the same immune response, and competition between different branches of the adaptive immune system. The article continues to discuss how immune responses can be important modulators of pathogen competition *in vivo*, and this is explored with a particular example, i.e. the competition dynamics among different viral strains in the context of multiple infection of cells. Finally, these *in vivo* considerations are extended to examine how immune responses to infectious diseases can influence pathogen-host dynamics on an epidemiological levels. This is done with two examples: the effect of immunity on the apparent competition between two host species, and the effect of immunological memory on the strain composition of a pathogen population circulating in a host population. Overall, this article summarizes different aspects in which ecological concepts can be useful to understand concepts in immunology, discussing several different examples, spanning a variety of scenarios.

Keywords Coinfection · Host-pathogen dynamics · Virus competition

2.1 Introduction

The immune system is incredibly complex and involves the interactions of many different components ranging from molecules to cells to multi-cellular pathogens (Janeway et al. 2005). For a comprehensive understanding of the immune

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D. Malagoli, E. Ottaviani (eds.), *Eco-immunology*, DOI 10.1007/978-94-017-8712-3_2,

system, such interactions have to be considered on a number of different levels or scales. Interactions of the different components *in vivo* are the most common considerations. In this respect, complex intracellular interactions determine the behavior of immune cells, such as their activation status, differentiation, and their activity. On a higher level, the interactions between different immune cell types, cytokines, and pathogen populations are essential for understanding the outcome of infections and for understanding the conditions for successful pathogen control or for failure of the immune system to contain pathogens (Nowak and May 2000; Perelson 2002; Wodarz 2006). In addition to these *in vivo* considerations, however, larger scale ecological and epidemiological interactions can have important implications for our understanding of immunity as a whole, especially when investigating the immune system from an evolutionary point of view (Thomas et al. 2009).

At all of these levels, systems biology approaches have been of great value for our understanding of these complex interactions. While verbal and graphical reasoning cannot provide a full understanding of the consequences of these interactions, mathematical and computational approaches allow us to formulate a certain set of biological assumptions in terms of equations or algorithms, and to follow them to their precise logical conclusions. This can add essential information to the work derived from experimental approaches. Much of this work has strong roots in the field of ecology, in particular population dynamics. The interactions among the different molecules, cells, and pathogens can be viewed as an *in vivo* ecosystem where elements compete with each other or act in predator-prey like fashions. The review will start with a detailed discussion of this concept. This will be followed by a case study that shows how immune responses can be important for determining the outcome of interactions and for shaping the evolutionary dynamics of pathogens *in vivo*. The second part of the review will consider the immune system and immune-mediated pathogen control in a broader ecological and epidemiological setting. Rather than concentrating on the in "*in vivo* ecosystem", it will highlight how ecological interactions among hosts, as well as between host dynamics of pathogens, can shape our understanding of the role of immunity in the light of evolution.

The immune system contains several different branches, and different pathogens elicit different responses. In order to keep this review focused, the adaptive immune system will be considered in the context of viral infections. The adaptive immune system includes cells that specifically recognize pathogens and that are essential for their control, such as killer T cell responses (also called CD8 T cell or cytotoxic T lymphocytes, CTL), helper T cell responses (CD4 T helper cells), and B cell responses (leading to antibody production). The innate immune response, which provides a first line of defense and which also collaborates with the adaptive immune responses, will not be considered. Even within this restriction a vast literature exists that examines these interactions from a systems biology point of view. Hence, the review will seek a balance between explaining general concepts and providing examples that are based on my own studies.

2.2 Immune-Pathogen Interactions as *in vivo* Ecological Systems

While immunology has traditionally been considered in the context of molecular and cell biology, it represents a highly complex system with many interacting populations of cells and molecules, that in turn interact with growing pathogen populations. Hence, a variety of systems biology approaches have been very useful for gaining insights into these complex interactions. A lot of this work is routed in ecological principles, particularly in the field of population biology, and mathematical approaches have played important roles in this respect. To this day, much of the theoretical work that considers the dynamics between immune cells and infectious agents is based on the concepts of predator-prey as well as competitive interactions. In order to demonstrate this point, let us consider one of the most basic models that describe the dynamics between a virus population and an anti-viral immune response (Nowak and May 2000). This model contains four variables: the populations of uninfected cells, S, infected cells, I, free virus, V, and a specific immune response, C. These quantities can either denote the total abundance in a host, or the abundance in a given volume blood or tissue. It is given by the following set of ordinary differential equations:

$$\begin{aligned} \dot{S} &= \lambda - dS - \beta SV \\ \dot{I} &= \beta SV - aI - pIC \\ \dot{V} &= kI - uV \\ \dot{C} &= \eta IC - bC \end{aligned} \tag{2.1}$$

Uninfected cells are produced with a rate λ, die with a rate d, and become infected by free virus particles with a rate β. Infected cells die with a rate a and produce new virus particles with a rate k. Free virus particles die with a rate u. An important concept in this respect is the basic reproductive ratio of the virus, R_0. This measure expresses the average number of newly infected cells produced by a single infected cell at the beginning of the infection, when almost all cells are uninfected. This measure determines whether a virus can successfully establish an infection in the host or not. If, $R_0 > 1$, then one infected cell on average gives rise to more than one newly infected cell, and the virus can successfully establish an infection. On the other hand, if $R_0 < 1$, then one infected cell on average gives rise to less than one newly infected cell, and the virus population goes extinct. On top of these basic virus dynamics, a virus-specific immune response is included in the model. In particular, this model considers a cytotoxic T lymphocyte (CTL) response, which expands in response to antigenic stimulation and kills infected cells through lysis. The CTL proliferate in response to antigenic stimulation with a rata η, and decay in the absence of stimulation with a rate b. CTL can kill infected cells with a rate p.

In this basic model, the equation for the CTL response is based on the Lotka-Volterra model for predator-prey interactions. The CTL are the predators that

proliferate in response to their prey (virus) and kill the prey. Consequently, the model has properties that are similar to those of the Lotka-Volterra class of models. If the CTL do not expand fast enough in response to antigenic stimulation, the response cannot become established and the following equilibrium is attained.

$$S^* = \frac{au}{\beta k}; I^* = \frac{\lambda\beta k - dau}{a\beta k}; V^* = \frac{\lambda\beta k - dau}{a\beta u}; C^* = 0.$$

If the CTL do expand sufficiently fast (i.e. if $\eta I^* > b$), then the population of cells grows and starts fighting the virus population. This can lead to oscillatory dynamics that will eventually dampen down towards a stable equilibrium. A typical time-series that arises from this model is shown in Fig. 2.1. The equilibrium is given by the following expressions.

$$S^* = \frac{\lambda u\eta}{\eta ud + \beta kb}; I^* = \frac{b}{\eta}; V^* = \frac{kb}{\eta u}; C^* = \frac{\eta\lambda\beta k - \eta dau - ba\beta k}{p\left(\eta ud + \beta kb\right)}$$

In this model, the equilibrium virus load is determined by the immunological parameters η and b. The faster the rate of antigen-induced CTL expansion (higher η) and the slower the death rate of CTL (lower b), the lower the number of infected cells, and thus amount of free virus. This model is deterministic in nature, that is the immune response cannot lead to the extinction of the pathogen. However, if equilibrium virus load is low or if oscillations before equilibrium is reached bring the number of infected cells to low numbers (of the order of one), this can be interpreted as immune-mediated pathogen clearance, since this would occur in a stochastic version of this model. If the immune response is not strong enough, the number of infected cells remains at significant numbers and a persistent infection is established, characterized by the equilibrium described above.

While this basic framework is based on predator-prey dynamics, it has to be noted that it is a very simplified representation of how immune responses, and CTL responses in particular, work. Many models have been developed that are based on this simple framework, but include many other experimentally established findings in the assumptions. Before the host is infected with a given pathogen, specific CTL are present in a "naïve" state, that is they are resting and found only at low numbers. Upon infection, the pathogen activates these naïve CTL, and the activated CTL undergo clonal expansion that eventually leads to the differentiation into effector cells, which are the cells that actually fight the infection. As the infection is suppressed and/or cleared, the effector cells either die or differentiate into memory CTL that can persist for long periods of time in the absence of antigenic stimulation. Models have been developed that explicitly take into account details of this differentiation pathway (Antia et al. 1998; Antia et al. 2005). Further, while the basic model assumes that CTL expansion is always proportional to the amount of antigen present, experiments have shown that in the initial expansion phase, an antigen-independent phase of "programmed proliferation" is observed, which has also been incorporated into models of CTL dynamics (Antia et al. 2003; Wodarz et al. 2000a; Wodarz and Thomsen 2005). To summarize, many developments of the basic model of CTL

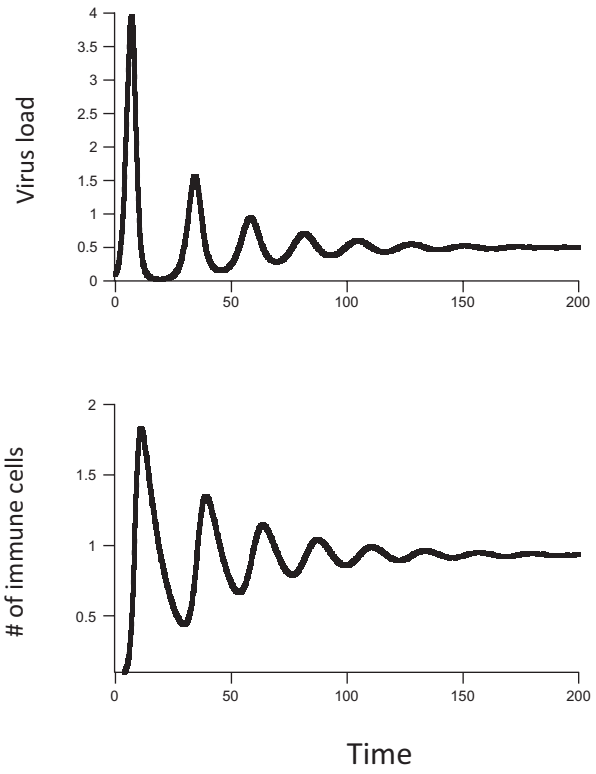

Fig. 2.1 Basic simulation of the dynamics between immune responses and an infection, based on model (1). Virus growth is followed by immune cell expansion, and immune cell-mediated activity reduces virus load. Subsequent damped oscillations bring the system towards an equilibrium. The level of virus load at this equilibrium shows how well the infection is controlled. If virus load lies below a threshold, this indicates virus clearance in practical terms. After expansion, the population of immune cells remains at an elevated memory level. The exact dynamics with which the equilibrium is approached can vary depending on the model and does not have much biological meaning. In this graph, there are prolonged oscillations before equilibrium is reached. Even if this is predicted by simple mathematical models, however, other factors not taken into account in the model, such as space, can reduce these oscillations and stabilize the dynamics

dynamics can be found in the literature, which however, are all rooted in and related to ecological population dynamics models.

These models have been applied to examine various biological questions. They have been fit to experimental data in order to measure important kinetic parameters of T cell responses (Perelson 2002; De Boer et al. 2001, 2003a, b, c; Bonhoeffer et al. 2000; Ribeiro et al. 2002a, b; Asquith et al. 2002; Ganusov et al. 2005). Various aspects of immune responses, such as the ability of a response to clear a pathogen (Nowak and Bangham 1996; Wodarz and Nowak 2000a; Chao et al. 2004), the potential for immune responses to cause pathology (Wodarz et al. 2002; Wodarz and Krakauer 2000), correlates of protection against re-infection (Antia et al. 1998, 2005; Borghans et al. 1999a; Wodarz et al. 2000b), and the dynamical interactions

between different branches of the immune system as well as between different immune cell clones (Nowak and May 2000; Borghans et al. 1999b; De Boer and Perelson 1994; Segel and Bar-Or 1999; Perelson et al. 1976; Bergmann et al. 1999, 2001; Yates et al. 2000; Scherer and Bonhoeffer 2005; Funk et al. 2005) have been investigated with mathematical models. Many other applications exist, which cannot all be reviewed here due to lack of space. Instead, the article will outline further aspects of CTL dynamics that are strongly related to ecological dynamics, in particular competition dynamics.

In the simple model described above, the immune cells were considered as a single population which responds to the pathogen and fights it. In reality, however, several species of immune cells can recognize the same pathogen and respond. They recognize different parts of the same pathogen. Not only do different branches of the immune system, such as antibodies and T cells, respond simultaneously. Even within one branch of the immune system, such as the killer T cells, different species or clones of cells recognize different parts of the same pathogen and respond. Thus, a diversity of immune responses develops. As explained above, the interactions between immune responses and pathogens can be considered a predator-prey system. In this analogy, there are different species of predators living off the same prey population. This means that the predators are in competition for their common food resource. The predator species which is most efficient at capturing the prey can reduce the food resource to levels which are too low for other predator species to survive. This can result in competitive exclusion where only one predator species remains. Similar considerations apply to the immune system (De Boer and Perelson 1994; Wodarz and Nowak 2000b; Nowak 1996; Nowak et al. 1995). If one immune cell clone is more efficient at responding to a virus, it can reduce virus load to levels which are too low for the other immune cell clones to become stimulated. This can result in the dominance of one immune cell clone and the exclusion of the others. This concept is called immunodominance. An immune response with a dominant immune cell clone is also called a narrow response. Another possible outcome of competition dynamics in ecology is coexistence. This can come about if there is a degree of niche separation between the species. Similarly, the coexistence of multiple immune cell clones can be observed. This is called a broad response, and different reasons can contribute to this outcome. One of the reasons might be the evolution of pathogens *in vivo* towards escape from immune responses (Nowak 1996; Nowak et al. 1995). Viruses can mutate the proteins which are recognized by the immune system. The mutant will initially grow unopposed. It is very likely, however, that another immune cell clone exists within the body which can recognize the mutant. This immune cell clone will become stimulated and expand. Now, two responses are present: the original response which controls the wild-type virus, and the new response which controls the mutant. Further mutants can arise, and further immune cell clones can become stimulated in order to fight the mutants. In this way, a broad immune response is found with many immune cell clones coexisting. Such non-equilibrium dynamics might be observed in some HIV infected patients (Nowak 1996; Nowak et al. 1995). Another factor which might determine whether a broad or a narrow immune response is observed could be the level of pathogen control in persistent infections. Mathematical studies (Wodarz and Nowak 2000b) suggest that efficient control of a persistent

infection correlates with a broad response, while less efficient control correlates with a narrow response. On a simplified level, the reason is that the competition between the immune cell clones is reduced in the presence of efficient control while this is not the case if the virus can replicate at higher levels. Efficient control can be brought about by a long life-span of CTL in the absence of antigen (low value of parameter *b*), which reduces the level of competition and can lead to a prolonged coexistence of several CTL clones, before extinction of the weaker clones is observed after a relatively long period of time. Yet, other studies have shown that the interplay between CTL responses and other branches of the immune system, such as helper cell responses, can lead to complex dynamics with multiple steady sates, in which the coexistence of different CTL clones can occur (Korthals Altes et al. 2003).

Just as different cell clones from a single branch of the immune system can compete against each other, so can different branches of the immune system (Arnaout and Nowak 2000; Wodarz 2003b). The two main branches which directly fight pathogens are antibody responses and killer T cell responses (CTL). Both populations expand when they are stimulated by a pathogen, and both responses work to remove the pathogen from the body. Antibodies can attack any pathogen which is present in the extracellular environment of the body. Killer T cells attack pathogens which are inside host cells (intracellular pathogens). These are mostly viruses. Viruses have both intra- and extra-cellular stages. Thus, the competition between antibody and killer T cell responses is mostly relevant in the context of viral infections. Mathematical modeling has investigated the basic competition dynamics between antibody and killer T cell responses (Arnaout and Nowak 2000; Wodarz 2003b). One of the outcomes is competitive exclusion. If antibodies are more efficient at fighting a virus, they can reduce virus load to levels which are too low to stimulate the killer T cells. Similarly, if killer T cells are more efficient at fighting the virus, they can reduce virus load to levels which are too low to stimulate the antibodies. In addition to these exclusion outcomes, there is also a coexistence outcome. Both antibodies and killer T cells persist and fight the infection. This is possible because they recognize different stages of the viral life cycle. The killer T cells are stimulated by intracellular virus, while antibody responses are stimulated by extracellular virus. For example, even if the killer T cells are very efficient at reducing the number of infected cells to low numbers, the population of free (extracellular) virus particles can still be sufficiently large to stimulate the antibody response. Such competition between killer T cells and antibodies might be observed in Hepatitis C virus (HCV) infection. A relatively small fraction of patients who become infected with HCV clear the virus from the blood and no further disease is observed. The rest of the patients develop persistent infection. This is initially asymptomatic, and the asymptomatic phase lasts for a long time. After about 10–20 years, liver pathology (hepatitis) is observed in a fraction of virus carriers. During the initial stages of infection (acute phase), both killer T cells and antibody responses expand. The role of killer T cells and antibodies for the resolution of HCV infection is debated in the literature (Farci et al. 2000; Klenerman et al. 2000; Cox et al. 2005a, b). Studies of the early phase of the infection showed that both humans or chimpanzees who cleared the virus from blood developed strong and sustained killer T cell responses (Thimme et al. 2001; Lechner et al. 2000a; Cooper et al. 1999; Lechner et al. 2000b, c;

Chang et al. 2001). In chimpanzee studies, killer cell-mediated clearance is associated with the absence of strong antibody responses (Cooper et al. 1999), and this supports the notion of competition between the two branches of the immune system. Humans and chimpanzees who developed persistent infection were characterized by an initial killer T cell response which was not sustained at high levels beyond the early phase of infection (Thimme et al. 2001; Lechner et al. 2000a; Cooper et al. 1999; Lechner et al. 2000b, c). Persistent infection has, however, been observed to be associated with vigorous antibody responses (Farci et al. 2000; Major et al. 1999), again pointing to the occurrence of competition. Thus, it was argued that a strong killer T cell response is crucial for the resolution of infection. It has been hypothesized that the progression of HCV infection from the asymptomatic phase to the pathogenic phase could be explained by the competition between antibody and killer T cell responses (Wodarz 2003b). As described above, persistent HCV infection is characterized by an ongoing antibody response, and by the absence of a significant killer T cell response. It is thought that HCV might be non-cytopathic. That is, the virus does not kill the cells it infects (the liver cells). Liver cell death would then require the activity of killer T cells. Since those are absent during the earlier stages of the infection, pathology is not observed. According to the ecological arguments presented here, the killer T cells are absent because they have been excluded by the antibody response. During the course of infection, HCV accumulates mutations and can evolve to escape the antibody responses. As the virus escapes antibody responses, the degree of immunological control is reduced and virus load rises. This provides increased levels of stimulation for the killer T cells. The more the virus evolves away from the antibodies (i.e. the higher the degree of viral diversity), the higher the level of stimulation of the killer T cell responses. Once the number of escape mutants has crossed some threshold, there is enough stimulation for the killer T cell population to expand. Now, the ongoing killer T cell response continuously removes infected liver cells, but fails to clear the infection. As a high percentage of liver cells tends to be infected by HCV, this can cause significant degrees of pathology (also called immunopathology (Thomsen et al. 2000; Chang et al. 1997; Zinkernagel and Hengartner 1994)). Therefore, virus evolution of antibody escape can shift the competition dynamics from a parameter region where the outcome is competitive exclusion of the killer T cells to another parameter region, where we observe coexistence between antibodies and killer T cells. This in turn may cause disease.

2.3 Immune Responses as Modulators of Virus Competition

The previous section mainly considered the dynamics of immune responses and ecological parallels in these dynamics. This section takes a more integrated approach and examines how immune responses can alter ecological interactions among pathogens and therefore play an important selective force in the *in vivo* evolution of pathogens. This will be done by considering a specific case study in the

context of HIV infection. If two virus strains share the same target cell population and are opposed by the same immune responses, standard models predict that the virus with the larger basic reproductive ratio excludes its competitor. Recent work, however, indicates, that viral competition dynamics can be significantly altered if models assume that multiple copies of the viral genome can infect the same cell (multiple infection or coinfection), and that immune responses can play an instrumental role in shaping the outcome of the dynamics.

Most work on virus/HIV dynamics was performed under the assumption that each cell is infected by a single copy of HIV. It is observed in vitro that upon infection, the virus induces down modulation of its receptor on the surface of the infected cell, rendering the cell resistant to further infection events. It has, however, become clear that multiple copies of HIV can infect the same cell, a process we call coinfection (Chen et al. 2005; Dang et al. 2004; Jung et al. 2002; Levy et al. 2004; Mattapallil et al. 2005; Gelderblom et al. 2008). While receptor down-modulation does occur (Lama 2003; Levesque et al. 2004), it only happens about 24 h post infection, leaving a time window that allows further copies of HIV to infect the cell (Lama 2003; Nethe et al. 2005). Further biological details about the process of coinfection are reviewed in (Wodarz and Levy 2011). Coinfection can have important consequences for the evolutionary dynamics of the virus *in vivo* (Gelderblom et al. 2008; Bonhoeffer et al. 2004; Fraser 2005; Vijay et al. 2008; Althaus and Bonhoeffer 2005; Kouyos et al. 2006, 2007; Iwabu et al. 2008; Dixit and Perelson 2004; Rouzine and Coffin 2005), for example through recombination between different genotypes that are packaged within the same virus particle.

However, coinfection can also have important consequences for the basic competition dynamics between different virus strains. This can be demonstrated in the context of the following model that is given by a set of ordinary differential equations (Wodarz and Levy 1999).

$$\begin{aligned}
\dot{x} &= \lambda - dx - \beta_1' x\left(y_1 + y_{12}\right) - \beta_2' x\left(y_2 + y_{12}\right) \\
\dot{y}_1 &= \beta_1' x\left(y_1 + y_{12}\right) - a_1 y_1 - \beta_2' y_1\left(y_2 + y_{12}\right) - p y_1 z \\
\dot{y}_2 &= \beta_2' x\left(y_2 + y_{12}\right) - a_2 y_2 - \beta_1' y_2\left(y_1 + y_{12}\right) - p y_2 z \\
\dot{y}_{12} &= \beta_1' y_2\left(y_1 + y_{12}\right) + \beta_2' y_1\left(y_2 + y_{12}\right) - a_2 y_{12} - p y_{12} z \\
\dot{z} &= cz\left(y_1 + y_2 + y_{12}\right) - bz
\end{aligned} \tag{2.2}$$

The variables y_1 and y_2 denote the populations of cells infected only by virus 1 and virus 2, respectively. The variable y_{12} denotes cells which are coinfected by both viruses. The variable z denotes a specific immune response which expands upon antigenic stimulation (such as B cell and T cells). We assume that virus 2 is more cytopathic than virus 1 (i.e. $a_2 > a_1$). Virus 1 replicates with a rate β'_1, and virus 2 replicates with a rate β'_2. It is assumed that the viral cytopathicity and replication rate are related according to $\beta'_1 = fa_1/(g + a_1)$ and $\beta'_2 = fa_2/(g + a_2)$. Virus 1 is produced from cells y_1 and y_{12}, and virus 2 is produced from cells y_2 and y_{12}. Cells infected with virus 1 die with a rate a_1, and cells infected with virus 2 die with a rate a_2. Cells infected with both viruses show dominance and die with a rate a_2 because

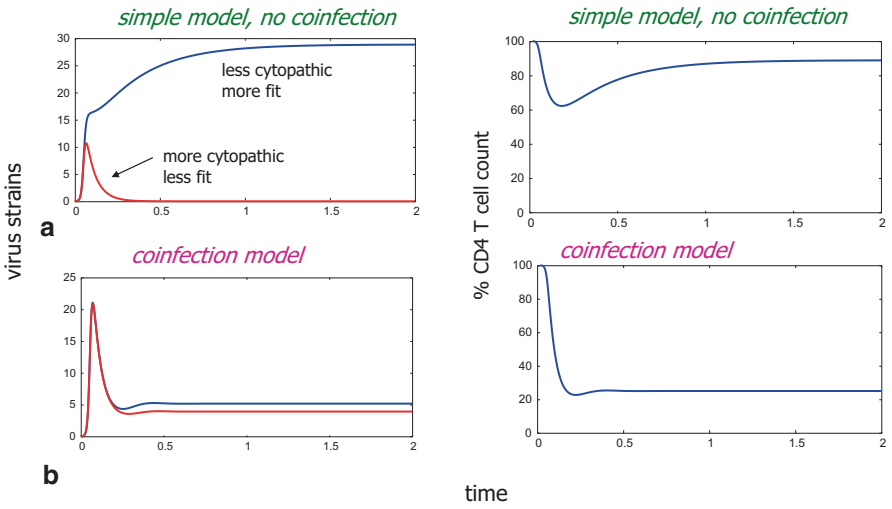

Fig. 2.2 Simulations showing the effect of HIV coinfection on the outcome of competition between a less cytopathic fitter strain and a more cytopathic strain that is less fit, based on model (2). **a** In the absence of coinfection, the fitter strain outcompetes the less fit strain. Because the fitter strain is weakly cytopathic, the CD4 T cell count is not predicted to dip significantly. **b** In the presence of coinfection, coexistence of the two virus strains is observed. Because the more cytopathic strain persists, the CD4 T cell count plunges to low levels

virus 2 is assumed to be more cytopathic than virus 1. Immune responses expand upon exposure to all types of infected cells. The simplest expression to describe this is given by $cz(y_1+y_2+y_{12})$, where c is the immune responsiveness (Nowak and May 2000). Further, immune cells die with a rate b. In the simplest form, the model assumes that immune responses kill infected cells with a rate p.

Assume that the basic reproductive ratio of each virus in isolation is greater than one. That is, in isolation, each virus would be able to sustain an infection. From now on, we assume that the cytopathicity of virus strain 1 is such that the basic reproductive ratio of the virus is at its maximum, i.e. this virus strain is characterized by maximum fitness ($a_1 = a_{fit}$). We further assume that virus strain 2 has a higher degree of cytopathicity ($a_2 > a_{fit}$) which enables it to induce more pronounced target cell depletion. As a result of these assumptions, the more pathogenic virus strain 2 is always less fit than the less pathogenic virus strain 1. We ask under which conditions the more cytopathic virus strain 2 can persist in the virus population. The less cytopathic virus strain 1 always persists. The more cytopathic virus strain 2 persists if $\frac{\beta'_2(x_1+y_1)}{a_2} > 1$, where $x_1 = a_1/\beta'_1$ and $y_1 = \lambda/a_1 - d/\beta'_1$. In other words, the less cytopathic strain can exclude the more cytopathic one, but not vice versa. If the above condition is fulfilled, a more cytopathic strain can coexist with a less cytopathic strain, even though it has a lower replicative fitness and would be driven extinct in the absence of coinfection (Fig. 2.2). The reason for the coexistence is as follows. Because the coinfected cells die with the rate determined by the more cytopathic virus,

http://www.springer.com/978-94-017-8711-6

Eco-immunology
Evolutive Aspects and Future Perspectives
Malagoli, D.; Ottaviani, E. (Eds.)
2014, XII, 179 p. 15 illus., 9 illus. in color., Hardcover
ISBN: 978-94-017-8711-6