Modeling Evolutionary Dynamics of HIV Infection

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Abstract. We have modelled the within-patient evolutionary process during HIV infection. We have studied viral evolution at population level (competition on the same receptor) and at species level (competitions on different receptors). During the HIV infection, several mutants of the virus arise, which are able to use different chemokine receptors, in particular the CCR5 and CXCR4 coreceptors (termed R5 and X4 phenotypes, respectively). Phylogenetic inference of chemokine receptors suggests that virus mutational pathways may generate R5 variants able to interact with a wide range of chemokine receptors different from CXCR4. Using the chemokine tree topology as conceptual framework for HIV viral speciation, we present a model of viral phenotypic mutations from R5 to X4 strains which reflect HIV late infection dynamics. Our model investigates the action of Tumor Necrosis Factor in AIDS progression and makes suggestions on better design of HAART therapy.

1 Introduction

Evolutionary biology was founded by Charles Darwin on the concept that organisms share a common origin and have subsequently diverged through time. Molecular phylogenetics has provided a statistical framework for estimating historical relationships among organisms, and it has supplied the raw data to test models of evolutionary and population genetic processes. Those have found practical uses in tracing the origins of pandemias and the routes of infectious disease transmission. Our ability to obtain molecular data has increased dramatically over the last two decades and large data sets describing a wide range of evolutionary distances are used in population genetic, phylogeny and epidemiological studies. Nevertheless, phylogenetic methods based on sequence information represent often an oversimplification when we aim at capturing the short time dynamics, i.e. the early stages of the speciation process. Population genetics focuses on this topic by investigating the behavior of mutations in populations. This discipline is related to the other important idea that Darwin expressed in The Origin of Species [1], that the exquisite match between a species and its environment is explained with natural selection, a process in which individuals with

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beneficial mutations leave more offspring. Here we combine predictive quantitative theories of HIV evolution in the context of the selection pressure generated by the virus competition and the immune response. In particular phylogenies of the natural target of the HIV viruses, i.e. their cell receptors is combined with population genetics mathematical models. We show that combining the two leads to a better understanding of the complex molecular interaction underlying the macroscopically observable phenomena of HIV infection.

The smallest scale of molecular evolution generates genetic variability at population level. A special case is that of quasispecies which are clouds of very similar genotypes that appear in a population at mutation-selection balance [2]. Since the number of targets (the substrate) is limited, fitter clones tend to eliminate less fit mutants, which are subsequently regenerated by the mutation mechanism [3]. They are the combined result of mutations and recombination. Other sources of variability result from co-infection (simultaneous viral infection), superinfection (delayed secondary infection). On the contrary, selection and random drift decrease variability. The fact that deleterious or less fitted variants are not instantaneously counter selected allows for the coexistence and co-evolution of different strains of a virus within the same host. Although the conditions for the formation and survival of new strains have not always been understood, small scale evolution such as variability at population level may experience different mutation/selection balance than the genetic variability estimated from sequence analysis which represent fixed genotypes. Indeed, recent studies show that the rate of molecular evolution appears to accelerate when measured over evolutionary short timescales [4], which strongly contrast with substitution rates inferred in phylogenetic studies. Molecular virology studies appear the natural benchmark, given that viruses have usually very high mutation rates and large populations. We aim at modelling viral multi strain short and long term evolutionary dynamics during the immune response. The multi strains can be thought as viral populations. Since there is a tremendous lack of studies attempting at integrating population and phylogenetic studies, our work represents the efforts to link speciation at small and large evolutionary scale. This may result in a better understanding how to use the topology and branch lengths of existing species to predict future evolution.

In the next section we describe the relevant feature of the immune response which represents the selection pressure playing a key role in the speciation process. Then we use data from chemokine receptor sequences to estimate the rate of phenotype change in the virus and use this data to derive a selection-mutation model based on a set of differential equations. In the results we show that the models introduced are suited to model both short and long term evolutions. In particular we first show an example of speciation dynamics of viral population mediated by the immune sytem response. Then we model the phenotypic switch in co-receptor usage in HIV-1 infection and we also make some observations on the better design for HAART therapy. Finally we draw our conclusions.

1.1 Major Features of Within-Patient HIV Evolution

Although the process of adaptive change is difficult to study directly, natural selection has been repeatedly detected in the evolution of morphological traits (such as the beak of Darwin's finches). During HIV infection, the process of adaptation requires interaction with CD4 T cell and a chemokine receptor, either CXCR4 or CCR5. During early stages of HIV-1 infection, viral isolates most often use CCR5 to enter cells and are known as R5 HIV-1. Later in the course of HIV-1 infection, viruses that use CXCR4 in addition to CCR5 (R5X4) or CXCR4 alone (X4 variants) emerge in about 50% patients (switch virus patients) [5, 6]. These strains are syncytium-inducing and are capable of infecting not only memory T lymphocytes but also naïve CD4+ T cells and thymocytes through the CXCR4 coreceptor. The switch to use of CXCR4 has been linked to an increased virulence and with progression to AIDS, probably through the formation of cell syncytia and killing of T cell precursors. X4 HIV strains are rarely, if ever, transmitted, even when the donor predominantly carries X4 virus. CXCR4 is expressed on a majority of CD4+ T cells and thymocytes, whereas only about 5 to 25% of mature T cells and 1 to 5% of thymocytes express detectable levels of CCR5 on the cell surface [7]. It is noteworthy that X4 HIV strains stimulate the production of cellular factor called Tumor Necrosis Factor (TNF), which is associated with immune hyperstimulation, a state often implicated in T-cell depletion [8]. TNF seems able to both inhibit the replication of R5 HIV strains while having no effect on X4 HIV and to down regulate the number of CCR5 co-receptors that appear on the surface of T-cells [9].

2 Bioinformatics Analysis and Mathematical Models

We assume that the phylogenetic tree describes all sorts of genetic variants, i.e. quasispecies and species. Quasispecies appear at the leaves and are seen as single specie by the distant leaves. We make the assumptions that leaves that are very close experience the same environment, i.e. they compete for the same receptor targets. Therefore, the fitness landscape within short branch length distance is shaped by competition which decrease for longer distances.

2.1 Mutational Pathway from R5 to X4

A meaningful way to estimate the mutational pathways and phenotype difference between R5 and X4 is to use phylogenetic inference on chemokine receptors families. The statistical relationships among the species can be described using a tree. Let the phylogeny to be inferred be denoted Π. A node of Π is either a currently extant leaf node, with no descendants in Π, or an it internal node, with two or more child nodes in Π. A point of Π is defined to be any point at a node or on an edge of Π. Let t_i denote the time before present that point i was extant in Π. Let π_{ij} denote the path in Π between points i and j, and $|\pi_{ij}|$ its length. Thus, where j is an ancestor of i, $|\pi_{ij}| = t_j - t_i$. More generally, for any i and j, $|\pi_{ij}| = |\pi_{ik}| + |\pi_{kj}|$, where k is the last common ancestor (LCA) of

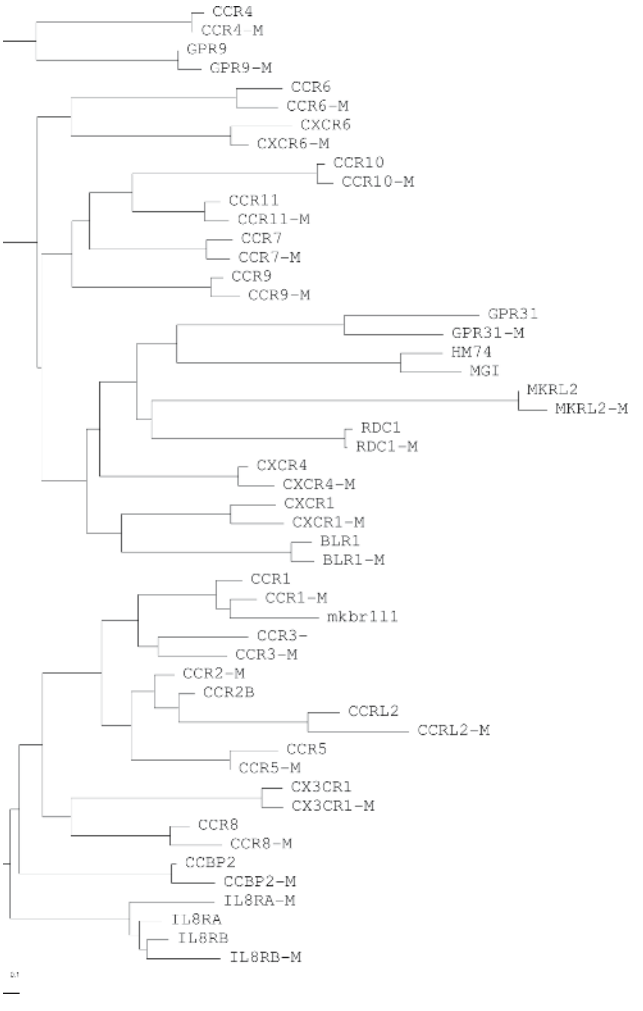

Fig. 1. The maximum likelihood phylogeny under the JTT+F+Γ model of evolution for the set of human and mouse (mouse sequences are labelled with "-M") chemokine receptors. We have considered only the external loop regions. The scale bar refers to the branch lengths, measured in expected numbers of amino acid replacements per site.

i and j. The tree parameters are topology and branch lengths. The assessment of phylogenies using distance and likelihood frameworks depend on the choice of an evolutionary model. We have computed the maximum likelihood (ML) analysis of the CRs data set using different models of evolution: Dayhoff [10], JTT [11], WAG [12], the amino acid frequencies of the data set, ('$+F$'), and the heterogeneity of the rates of evolution, implemented using a gamma distribution ('$+\Gamma$') [13, 14]. Bootstrap and permutation tests have been used to assess the robustness of the tree topology [15]. The tree may be used to estimate the pathways of substitutions which are supposed to have phenotype changes.

2.2 Mathematical Models of Viral Dynamics Under Immune Response Pressure

A meaningful model to study the genetics of population is that of *quasispecies*. This model, first introduced by Eigen [3] in the context of molecular evolution, describes the evolution of an infinite population of haploid individuals reproducing asexually. Each individuals has a given genotype $\sigma = (\sigma_1, \ldots, \sigma_N)$, constituted by a sequence of N symbols taken from an alphabet of size k, and is subject to the selection pressure. Mutations arise as copying errors during the reproduction process. The evolution of the concentration of a sequence, $x(\sigma, t)$, is given by:

$$\frac{\mathrm{d}x(\sigma,t)}{\mathrm{d}t} = \sum_{\sigma'} p(\sigma' \to \sigma) W(\sigma') x(\sigma', t) - \phi(\sigma, t) x(\sigma, t) \tag{1}$$

where $W(\sigma)$ represents the strength of the selection, $p(\sigma' \to \sigma)$ the mutation mechanism, and ϕ is a flux keeping constant the total concentration, $\sum_\sigma x(\sigma, t)$. The model can be used to model the evolution of a single quasispecies as well as extended to study the dynamics of interaction among n different populations. In the latter case we obtain a system of n first order, non-linear, differential equations.

Models using the notion of quasispecies have been adopted to study the biological evolution of populations and recently also for the modelling of the interaction between HIV-1 and the immune system [16]. Moreover, due to its intrinsic multiscale nature - indeed the population of sequences considered can be either that of genotypes or, more generally, the one of phenotypes - the model is suited to analyze both short and long range interactions.

In a phylogenetic framework a given leaf represents the common ancestor of the individuals coevolving. If we are interested in studying the short range evolution of the viral strains competing for the same co-receptor, we concentrate on a particular leaf of the phylogenetic tree. As a paradigmatic model, we may consider that introduced by Bagnoli et al. [17]. This model describes the speciation of a quasispecies population induced by competition.

In the model different individuals compete for the shared resources of a common environment, and this effect is reflected in the term corresponding to the selection strength. In particular, the growth rate W is expressed as

$$W(\sigma, t) = \exp[H_0(\sigma) - q(\sigma, t)] \tag{2}$$

where H_0 represents the static fitness (e.g. the environment) and the term $q(\sigma, t)$ accounts for the competition. We can think $q(\sigma, t)$ to be a function of the phenotypic distance between two different sequences, mimicking the fact that the competition is stronger for individuals sharing common habits. This competitive dynamics may lead to the speciation of the population. This event results in the appearance of new branches in the phylogentic tree and, as the selection pressure is continuously acting, the branches corresponding to the fittest individuals are eventually selected (see Fig. 2.2).

Now, considering the dynamics of interaction between viruses and immune system, the competition among different viral strains is induced by the immune

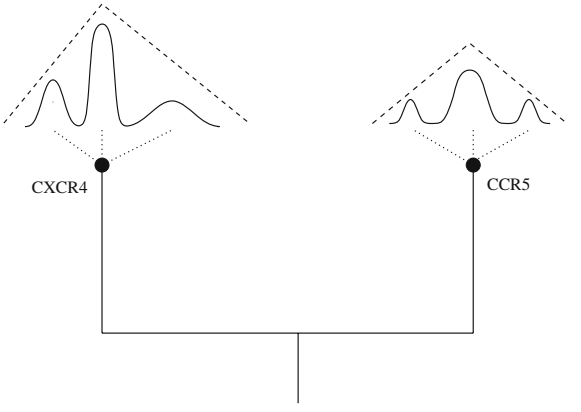

Fig. 2. Qualitative description of the short range evolution occuring on a phylogenetic tree, according to the competition model introduced by Bagnoli et al. [17]. Only two leaves (e.g. corresponding to CCR5 and CXCR4 co-receptors) are shown. In the figure we assume that only a single phenotypic character is continuosly varying and thus we assume a one-dimensional linear phenotypic space. Given the static fitness corresponding to different binding specificities (dashed line), we represent the effect of the speciation resulting from the induced competitive dynamics (solid line). The emerging new variants are then represented as dotted segments.

response. In this case a virus may escape the response by a T cell with high binding affinity, by differentiating enough. It's worth noting that this short-range dynamics alone may justify the stable multi strain infection reported in several patients (see. Sec. 3.2).

2.3 Long Range Competition and R5 to X4 Switching

Here we introduce a mathematical model to study the long range competition, mediated by the immune system response, occurring between different HIV-1 phenotypes around different leaves of the phylogenetic tree. Indeed, the viral quasispecies not only compete for using the same co-receptor (short range competition), but also for establishing a preferential chemokine signalling pathway (long-range competition). In someone who is newly infected by HIV, several variants of the virus, called R5, are often the only kind of virus that can be found. In about half of the people who develop advanced HIV disease, the virus begins to use another co-receptor called CXCR4 (X4 viral phenotype). This model supports the hypothesis that it may not be exhaustion of homeostatic responses, but rather thymic homeostatic inability along with gradual wasting of T cell supplies through hyper activation of the immune system that lead to CD4 depletion in HIV-1 infection.

We are interested in the switching in coreceptor usage and thus, by considering CD8+ cells to be at their equilibrium concentration and disregarding the effects of B cell, we concentrate on CD4 dynamics. We map the different leaves of the phylogenetic tree on a linear phenotypic space, composed by the viral

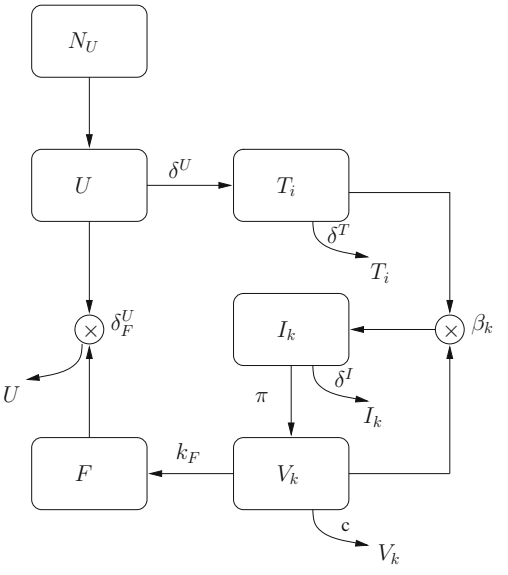

Fig. 3. Schematic description of the model for the switching from R5 to X4 viral phenotype. Naive T-cells, U, are generated at constant rate N_U and removed at rate δ^U. They give birth to differentiated, uninfected T-cells, T. These in turn are removed at constant rate δ^T and become infected as they interact with the virus. Infected T-cells, I, die at rate δ^I and contribute to the budding of viral particles, V, that are cleared out at rate c. As soon as the X4 phenotype arise, the production of the TNF starts, proportional to the X4 concentration and contribute to the clearance of naïve T-cells, via the δ_F^U parameter.

phenotypes competing for different co-receptor usage. At the beginning of the infection the only viral population present is that of R5 strains. Later on, as the infection evolves, we focus on the appearance of X4 viruses and on their subsequent interaction with R5 strains.

The model is the following:

$$\frac{\mathrm{d}U}{\mathrm{d}t} = N_U - \delta^U U - \delta_F^U U F \tag{3}$$

$$\frac{\mathrm{d}T_i}{\mathrm{d}t} = \delta^U U - \left(\sum_k \beta_k V_k\right) T_i - \delta^T T_i \tag{4}$$

$$\frac{\mathrm{d}I_k}{\mathrm{d}t} = \left(\sum_{k'} \mu_{kk'} \beta_{k'} V_{k'}\right) \left(\sum_i T_i\right) - \delta^I I \tag{5}$$

$$\frac{\mathrm{d}V_k}{\mathrm{d}t} = \pi I_k - c V_k \tag{6}$$

$$\frac{\mathrm{d}F}{\mathrm{d}t} = k_F \sum_{k \in X4} V_k \tag{7}$$

In the equations above, the variables modelled are the pool of immature CD4+ T cells, U, the different strains of uninfected and infected T cells (T and I, respectively), HIV virus, V, and the concentration of TNF, F. A schematic view of the model is depicted in Fig.3. The value of the parameters introduced are summarized in Table 2.3.

In particular, Equation (3) describes the constant production of immature T cells by the thymus N_U and their turning into mature T cells at rate δ^U. If X4 viruses are present, upon the interaction with TNF, immature T-cells are cleared at fixed rate δ_F^U.

Equation (4) describes how uninfected mature T cells of strain i are produced at fixed rate δ^U by the pool of immature T cells. Those cells, upon the interaction with any strain of the virus, V_k, become infected at rate $\beta_k = \beta \quad \forall k$. The infectiousness parameter, β, is not constant over time, but depends on the interplay between R5 and X4 viruses. In particular, due to the presence of TNF, the infectivity of R5 strains is reduced ($\beta_{R5}(t) = \beta - k_{R5}F(t)$), while the one of X4 viruses increases, with constant of proportionality k_{X4} ($\beta_{X4}(t) = \beta + k_{X4}\ F(t)$), mimicking the cell syncytium effect induced by the TNF molecule.

Table 1. Model for the R5 to X4 phenotypic switch: a summary of the additional parameters introduced. The value of the other parameters are medical literature referred, see also [18].

Parameter	Symbol	Value	Units of Meas.
Production of immature T cells	N_U	100	cell/μl t^{-1}
Death rate of immature T cells	δ^U	0.1	t^{-1}
Death rate of immature T cells upon the interaction with TNF	δ_F^U	10^{-5}	μl/cell t^{-1}
Decreasing infectivity of R5 phenotype due to TNF	k_{R5}	10^{-7}	(μl/cell)^2 t^{-1}
Increasing infectivity of R5 phenotype due to TNF	k_{X4}	10^{-7}	(μl/cell)^2 t^{-1}
Increasing death rate of immature T cells due to TNF	δ_{X4}^I	0.0005	μl/cell t^{-1}
Rate of production of TNF	k_F	0.0001	t^{-1}

Equation (5) describes the infection of mature T-cells. Infected T-cells of strain k arise upon the interaction of a virus of strain k with any of the mature T-cell strains. The infected cells, in turn, are cleared out at a rate δ^I. When TNF is released, this value increases linearly with constant δ_{X4}^I, $\delta^I(t) = \delta^I + \delta_{X4}^I\ F(t)$.

Equation (6) describes the budding capacity i.e. the mean number of virions produced in the unit of time by each infected T cell. We have used a value close to that reported in medical literature by [19].

Finally, in Equation (7), we model the dynamics of accumulation of TNF by assuming the increase in TNF level to be proportional, via the constant k_F, to the total concentration of X4 viruses present.

3 Results

3.1 Phenotype Change Patterns of R5 and X4 Strains

RNA viruses have been reported to have substitution rates of the order of $1{\cdot}10^{-3}$ substitution per site per replication [20]. Since a large fraction of amino acid

substitutions are neutral or quasi-neutral to structural changes, they do not change dramatically the fitness of the virus [21]. Nevertheless, sometimes even a single mutation can change the fitness in a substantial way. In our model we take into consideration only non-synonymous mutations and, therefore, we explored values slight higher value than that, (i.e. 10^{-4} and 10^{-5}). These values can be compared with the phenotype changes required to bind to CCR5 or CXCR4 receptors. In other words, research into HIV dynamics has much to gain from investigating the evolution of chemokine co-receptor usage. Although CCR5 and CXCR4 are the major coreceptors used by HIV-1 a number of chemokine receptors display coreceptor activities in vitro. Also several other chemokine receptors, possibly not present on the T cell membrane, may act as targets. To date, a number of human receptors, specific for these chemokine subfamilies, have been described, though many receptors are still unassigned. Several viruses, for example Epstein-Barr, Cytomegalovirus, and Herpes Samiri, contain functional homologous to human CRs, an indication that such viruses may use these receptors to subvert the effects of host chemokines [22]. Cells different from CD4+ and CD8+ T cells, such as macrophages, express lower levels of CCR5 and CXCR4 on the cell surface [23–25], and low levels of these receptors expressed on macaque macrophages can restrict infection of some non-M-tropic R5 HIV-1 and X4 simian immunodeficiency virus (SIV) strains [26, 27].

Fundamental to the evolutionary approach is the representation of the evolution of sequences along lineages of evolutionary trees, as these trees describe the complex patterns of dependence amongst sequences that are caused by their common ancestry [12, 28, 29]. The ML tree, obtained using the JTT+F+Γ model of evolution, is shown in Figure 1. The topology clearly shows that the CCR family is not homogeneous: CCR6, CCR7, CCR9 and CCR10 are separated from the other CCRs; in particular, CCR10 clusters with CXCRs; CXCR4 and CXCR6 do not cluster with the CXCRs. The tree shows that there are many mutational steps between CCR5 and CXCR4. The phylogeny suggests that the mutations that allow the virus env to cover a wide phenotypic distance from R5 to X4, may also lead to visit other receptors. Since the external loops of CRs contain the binding specificities and have higher rates of evolution than internal loops and transmembrane segments [30], the tree Fig. 1 shows a relative longer mutational pathway between CCR5 and CXCR4 with respect to pathway linking CCR5 to other receptors.

3.2 Modeling Co-evolutive Dynamics and Speciation

Focusing on short term evolution we investigate how the co-evolutionary and competitive dynamics of viral strains, mediated by the immune response, may lead to the formation of new viral strains. In particular, if the recognition ability of viral antigens by T cells is non-uniform over different viral variants and the immune system does not discriminate among highly similar phenotypes, a competition is induced. In Fig. 4 we consider a phenotypic space composed by 25 different variants of the virus, and make a first inoculum at phenotype 15 (Fig. 4a), followed by a second delayed inoculum at phenotype 5 at time $t = 1$. The

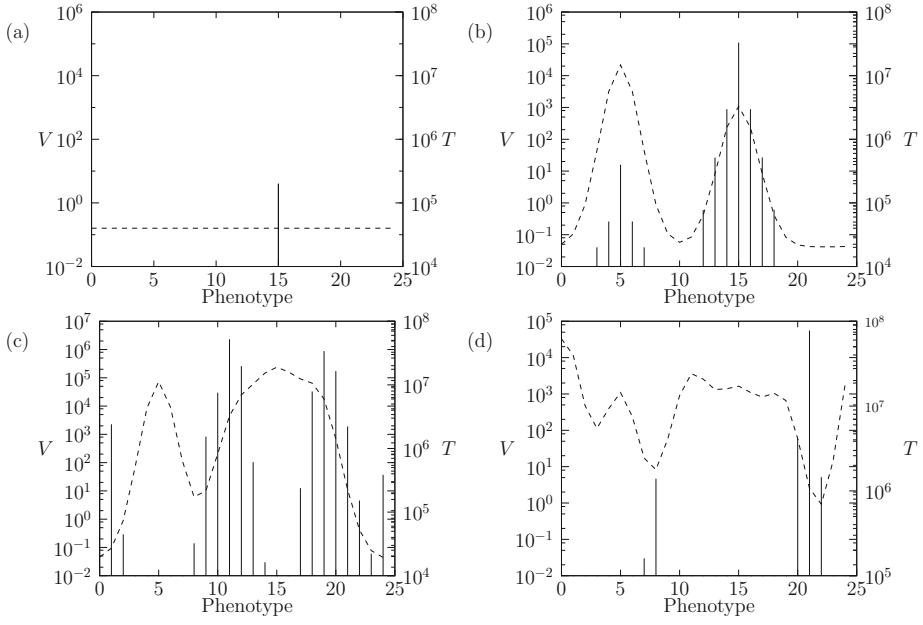

Fig. 4. Snapshots of competitive dynamics between different viral strains (vertical stems) and T lymphocites (dashed line) at four different times: $t = 0$ (a), $t = 4.5$ (b), $t = 5.25$ (c) and $t = 5.75$ (d). Virus strain 15 is present at time $t = 0$, while strain 5 is inoculated at time $t = 1$. Mutation rate $\mu = 10^{-4}$.

different interaction strength between T cells and viral phenotypes favors those viral phenotypes targeted by the weakest response. The result of the induced competition is the separation of the quasispecies centered around phenotype 15 into two clusters (quasi-speciation), Fig. 4c. It's worth noting that, due to the adaptive response by the immune system, a complex, time evolving co-evolution is established between viral populations and immune response (Figs. 4b-d).

3.3 R5 to X4 Switch and HAART Therapy

From the results derived in Sec. 3.1, it is now possible to get a better insight in the observed phenotypic switch in co-receptor usage by HIV-1 virus, by studying the coevolutive dynamics leading to X4 strain appearance by successive mutations of the ancestor R5 strain. In particular we may calculate the modelled time of switching in co-receptor usage. This time depends both on the mutation rate μ and on the phenotypic distance between R5 and X4 strains, d_P. By comparing the modelled value with the mean time inferred by the phylogenetic tree, we may tune the those model parameters to give the correct time of appearance of the X4 phenotype.

In Fig. 5 we observe the results of the stimulated production of TNF. Indeed, this regulate the interactions between immune response and the virus and

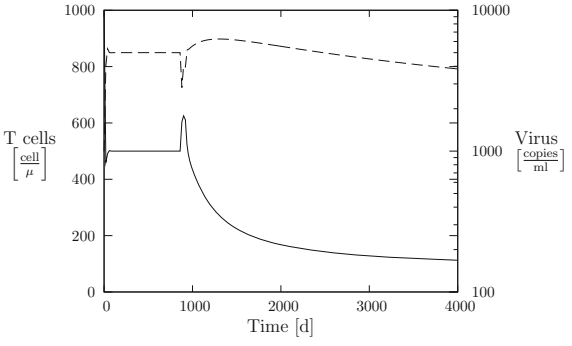

Fig. 5. Time evolution of the concentrations of uninfected T-cells (straight line) and viruses (dashed line), during R5 to X4 switch, occurring at time $t \approx 900$. The time of appearance of the X4 strains depends on the mutation rate and on the phenotypic distance between R5 and X4 viruses. After the appearance of the X4 phenotype a continuous slow decline in CD4+ T-cells level leads to AIDS phase (CD4 counts below 200cells/ml).

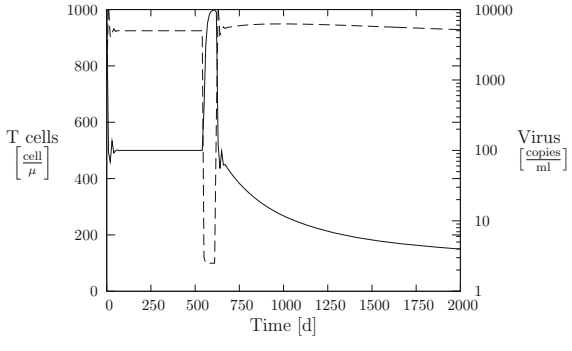

Fig. 6. The efficacy of HAART therapy may be disrupted by a sudden interruption in drugs treatment. If time has passed for mutations to populate the R5 strains closer to the X4 phenotypes, an earlier appearance of X4 strains may occur. Uninfected T-cells (straight line) and viruses (dashed line). Parameters as in Fig. 5.

between the different strains of HIV virus. The results of these interactions are a decline in T-cells level, leading to the AIDS phase of the disease, and the decline in levels of viruses using the R5 coreceptor. In the figure the temporal evolution of the infection is shown, with the appearance of the X4 strain, and the successive decline in T-cells abundances.

By using this model it is also possible to predict some scenarios in HAART treatment (see Fig. 6). This therapy is usually able to decrease the concentration of the virus in the blood and delay the X4 appearance. We have found time dynamics similar to those reported in [31]; see also [32, 33]. Note that our

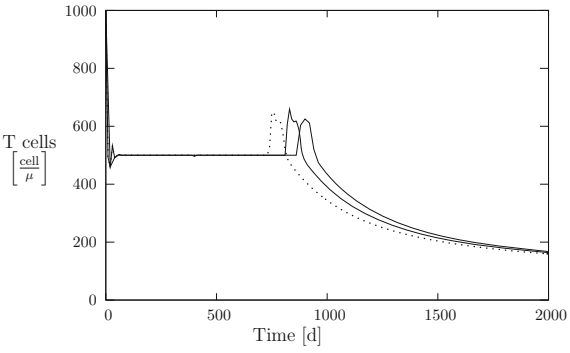

Fig. 7. CD4+ T-cells concentration during HIV-1 super-infection by a R5 viral strain. Evolution without superinfection, straight line; superinfection occurring at time t=100 and 400, dotted and dashed line, respectively. For a superinfection event occurring after the R5 to X4 switching the dynamics is qualitatively the same as for a single infection, (straight line). If the second delayed infection occurs before the R5 to X4 switching, the time of appearance of X4 viruses may be shorter, when the super-infecting strain is closer to the X4 phenotypes, (dotted and dashed line). Parameters as in Fig. 5.

model considers only the HIV virions which are in the blood. The clearance of virions hidden in cells or other tissues are known to be very slow [34, 35]. Now we investigate what may happen in the case of a sudden interruption in the use of the drugs. In Fig. 6 we observe how the X4 strain may appear sooner, if the different R5 strains experience the same selection pressure. In fact during the treatment the concentration of the different strains of R5 viruses is kept to a very low level while T-cell abundances increase. As the therapy is interrupted, all the strains give rise to a renewed infection. Now also the strains closer to the X4 co-receptor using viruses are populated, and a mutation leading to an X4 strain occurs sooner.

We have finally studied the case of a superinfection dynamics. In Fig. 7 we show T-cells evolution for different times of the superinfection event.

We may observe that if the superinfection occurs after the appearance of the X4, the new R5 strain does not have any effect on T-cells behavior. On the other hand is worth noting that if the new R5 inoculum take place before the X4 appearance, this may speed up the switching to the X4 phenotype if the new strain is mutationally close to the X4.

4 Discussion

Phylogenetic inference of chemokine receptors shows that there are several mutational patterns linking CCR5 to several receptors that have the same branch length of that from CCR5 to CXCR4. There is a massive abundance of signalling disruptions in the immune systems during AIDS progression, particularly after the transition R5 to X4. These disruptions may be due to variants of the virus

which bind other chemokine receptors. This hypothesis also suggests that R5-late strains in not-X4 AIDS, which are known to be different from R5 early strains, may have accumulated mutations enabling them to interact with other chemokine receptors. Therefore, our model suggests the sooner the HAART the better, because the presence of a large number of R5 will increase the mutational spectra in R5 strains (late R5) and the probability of getting closer to the binding specificities of other chemokine receptors. Contrary to our phylogenetic statistical analysis, our mathematical model describes short term evolutionary dynamics through competitions among viruses at each tips of the tree. Following Kimura, we can subdivide mutations into advantageous, neutral or deleterious where the deleterious can be further subdivided into the proportions that are very slightly deleterious, and deleterious. Deleterious mutations are not expected to become fixed in large populations, but nevertheless can persist in the population for long periods of time. The average time before loss correlates with deleteriousness. Thus, as observation times diminish, we should observe a greater proportion of slightly deleterious mutations that have yet to be lost, with the most deleterious observed only in the short-term pedigree studies. For some reasons, the evolutionary continuum between variation at population genetics level and the long-term evolution has not been adequately studied. Although it is a continuum, the techniques required may change as the timescale decreases. For example, some concepts from long-term evolution (binary evolutionary trees with sequences studied only at the tips) have been extended into populations where trees are no longer binary, and ancestral sequences (at internal nodes) are still present in the population. There are hints that a formal multi-scale study is necessary.

The interest in HIV strain is motivated by concern about developing strain specific drugs. Quasispecies are likely the key for understanding the emerging infectious diseases and has implications for transmission, public health counselling, treatment and vaccine development. Moreover, the observed co-evolutionary dynamics of virus and immune response opens the way to the challenging possibility of the introduction or modulation of a viral strain to be used in therapy against an already present aggressive strain, as described by Schnell and colleagues [36]. The authors showed that the introduction of an engineered virus can achieve HIV load reduction of 92% and recovery of host cells to 17% of their normal levels (see also the mathematical model in Ref. [37]).

Different drug treatments can alter the spectrum of strains. Will R5 blocking drugs cause HIV to start using X4? And will that be worse than letting the R5-using virus stay around along at its own, slower, but no less dangerous activity?

Recent works show that TNF is a prognostic marker for the progression of HIV disease [8, 38]. We focused on both the inability of the thymus to efficiently compensate for even a relatively small loss of T cells precursors and on the role of TNF in regulating the interactions between the different strains of HIV virus. The second model we have introduced shows that keeping low the concentration of TNF, both the depletion of T-cells precursors repertoire and the R5 overcome by X4 strains slow down.

The model makes possible to investigate intermittency or switching dominance of strains and the arising of new dominant strains during different phases of therapy; how superinfection will evolve in case of replacement of drug-resistant virus with a drug-sensitive virus and acquisition of highly divergent viruses of different strains; to investigate whether antiviral treatment may increase susceptibility to superinfection by decreasing antigen load.

Let us extend the viral framework for a general understanding of the molecular evolutionary process along a tree under natural selection. If we focus on a quasispecies fitness landscape, the fitness' main component is probably related to the entrance of the virus in the cell, i.e. the interaction with the receptor. Other components are the budding characteristics and numerosity and the spectrum of mutants (hopeful monsters) generated. Therefore the height of the fitness curve mainly reflects the binding energy, while the windows of strain existence in the x axis reflects how many changes may still result in a sufficient binding. Our work may reveal relevant to phylogenetic studies on divergence date estimation which suffer from the difficulties of estimating the correct rate of molecular evolution for different branches. It is relatively straightforward to test if the data conform to a molecular clock . If the assumption of rate constancy does not hold across a tree and therefore the clock is rejected, however, the current methodology is lacking robustness in assessing the amount of relaxation from a clock hypothesis. Our approach in modeling the evolution of virus species is to investigate the different degree of competition among strains. Strains which are in the same fitness landscape have correlated rates of evolution. This agrees very well with the current use of local clock models which allow the molecular rate to vary throughout the tree, but with closely related species sharing similar rates. This approach is justified with the assumption that molecular rates are heritable because they are related to physiological, biochemical, and life-history characteristics of the species in question. That's precisely the idea of our local fitness landscape. Although the inference of rates is confounded by uncertainties in calibration points, by tree topology, and by asymmetric tree shapes, our present studies should be considered a theoretical framework for understanding how different smooth fitness landscapes which can be imagined at the leaves and nodes of a phylogenetic tree are linked by the topology and branch lengths reflecting a multiscale stepwise process of adaptation under natural selection.

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