Impact of viral mutation on suppression of infection by cytotoxic T lymphocytes

Julien Arino* Neal Madras‡ Stéphanie Portet*§
Beni Sahai†

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Abstract

We develop a deterministic model describing the dynamics of infected cells wearing n epitopes recognizable by n CTL clones, in which mutation can lead to the epitopes becoming unrecognizable by their cognate CTL clone. Some mathematical and computational analyses of the resulting large scale system of $2^n + n$ equations are conducted. The model is used to examine the ability of CTL response to suppress infection in the presence of mutations and conditions are established that lead to CTL escape. We show in particular that escape happens in the case where the growth rate of the mutant with no recognizable epitopes is large enough, regardless of other conditions.

1 Introduction

Pathogens evolve by substitutions of amino acids at specific locations in DNA or RNA sequences. This process is called *mutation* and takes place during the replication phase of the pathogens. Depending on what substitution takes place and/or the location of the substitution, mutations may have different consequences [6]. They can be associated with an increase of survival or reproduction rates, or with a catastrophic cost to viral fitness that can lead to extinction of the strain [18]. Mutations can occur in epitopes, potentially leading to a decrease of the binding affinity of Cytotoxic T lymphocytes (CTL) receptors or preventing the recognition by the cognate CTL clone. Hence, specific mutations in epitopes can abrogate their CTL recognition and allow infected cells to escape CTL action (Figure 1). Because of the absence of proof-reading during virus replication, RNA viruses such as influenza and HIV exhibit higher mutation-inducing error rates are exhibited [15, 17].

*Department of Mathematics, University of Manitoba, Winnipeg, Canada.
‡Department of Mathematics and Statistics, York University, Toronto, Canada.
†Cadham Provincial Laboratory, Winnipeg, Canada.
§Corresponding author, portets@cc.umanitoba.ca.

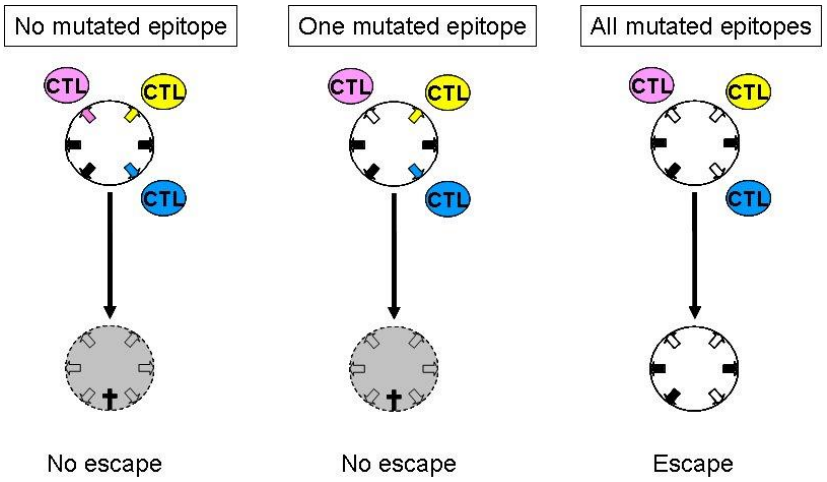

Figure 1: Mechanism of CTL escape with $v = 6$ epitopes (arrows at the cell membrane), of which $n = 3$ are recognized by CTL clones (shown here by CTLs and epitopes of the same colour); unrecognized epitopes are shown as black arrows. In the centre case, the pink epitope has mutated (indicated by a white arrow), but there are still two recognized epitopes and the cell is killed. In the case on the right, all recognizable epitopes have mutated and the infected cell escapes detection by CTLs.

For any infection in which CTLs play a role in virus elimination, CTL escape is an important mechanism to be investigated (see, e.g., [7, 16] for Hepatitis B, [14, 19] for Influenza and [11] for HIV). For instance, influenza and HIV/SIV are highly sensitive to mutations in epitopes [20]. Understanding the complex mechanisms of immune escape has major implications for drug and CTL-based vaccine design [8].

Mathematical models of viral infections including mutations abound, in particular in the context of HIV/AIDS and, to a lesser extent, influenza. Of relevance here, the model of [2] considers a population of mutants but does not track successive mutations. Similarly, the model of [1] explicitly describes host cell populations, viral density and uses a stochastic description of mutation events. Note also that from a modelling perspective, the problem is similar to that of drug resistance in cancer; see, e.g., [10].

To investigate the conditions of CTL escape, we propose a model describing the dynamics of infected cells wearing v epitopes recognized by $n \leq v$ CTL clones and the interactions between these populations. The dynamics of viruses is described implicitly in the generation of infected cells. Some mutations result in the mutation of an epitope inducing the non-recognition of this epitope by the cognate CTL clone. Backward mutations of epitopes are not considered. Contrary to the models discussed above, we track explicitly the densities of

cells infected by viruses showing specific mutations. On the other hand, our description of mutations is deterministic.

2 The mathematical model

2.1 Modelling strategy

One of the consequences of the infection of a cell by a virus is that the infected cell exhibits specific epitopes on its membrane. Assume there are v such epitopes; of these, $n \leq v$ are recognized by CTLs, i.e., there are n relevant CTL clones each recognizing a specific expressed epitope (Figure 2). An error occurring during virus replication results in the mutation of an epitope, which can induce the non-recognition of this epitope by the cognate CTL clone, leading to the latter's inability to kill infected cells expressing this epitope (Figure 1). Here, we only consider those mutations that do lead to the non-recognition of a previously recognized epitope. It is assumed that mutation in an epitope results from an error during the replication of its base pairs. Mutations can come at a cost or be beneficial for the fitness of virions; whether it is the former or the latter is described through the growth rate of the infected cell population. Backward mutations of epitopes are not considered.

For sake of simplicity, neither the virus nor host cell dynamics are explicitly described; virus replication in the infected cells, virus release as well as invasion of new host cells by the virus are implicit (Figure 3) and only the dynamics of the infected cell populations is modelled (Figure 4). Because of this, the model implicitly assumes an infinite number of target cells, which leads in some cases to unbounded solutions (see later). However, it is felt that the trade-off is acceptable because of the reduction of model complexity that results from this hypothesis.

2.2 Populations of interest

Two types of cell populations are considered: specialized killer cells (or CTLs) and virus-infected cells (Figure 2). Note that here and below, we use state variables to represent the number of cells of a certain type that are present, but also as the name of cells of that type.

- K_e is the number of copies of the specialized CTL clone recognizing an epitope of type e, with $e \in \{1, \ldots, n\}$. We also call K_e "CTLs of type e" or "K_e clones".
- For $A \subseteq \{1, \ldots, n\}$, T_A is the number of infected cells with the set A of mutated epitopes.

The population of infected cells is more specifically subdivided as follows:

- T ($A = \emptyset$) is the number of infected cells expressing all recognizable epitopes (types 1 to n). The population T is recognized by any CTL clone; it is also called the wild type-infected population, or wild type for short.

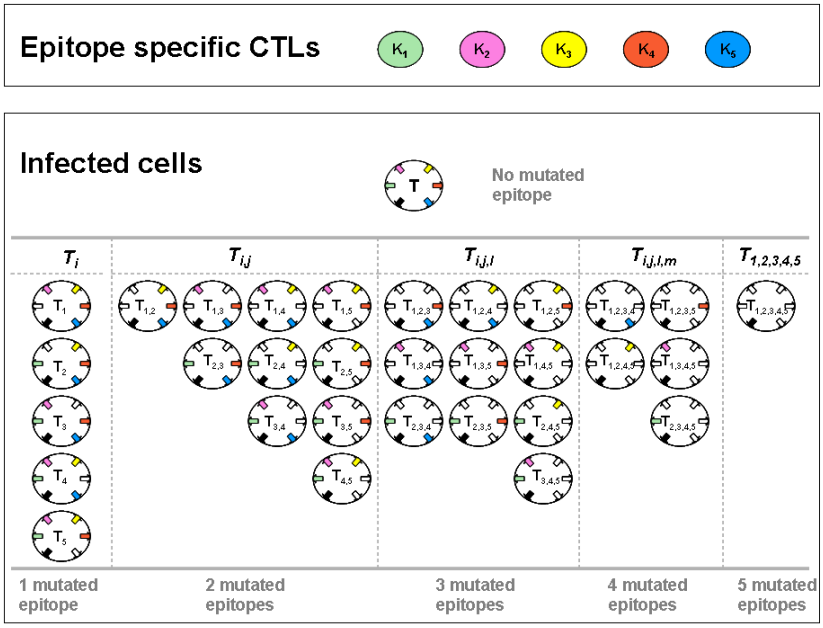

Figure 2: Populations of CTL clones, infected cells and possible mutations in the case with $v = 6$ epitopes expressed on the cell membrane and $n = 5$ recognizable epitopes. See Figure 1 for the meaning of symbols used.

- T_i is the number of infected cells in which the epitope of type i has mutated; T_i cells are recognized by all CTL clones except K_i clones.
- T_{ij} is the number of infected cells in which epitopes of types i and j have mutated; T_{ij} cells are recognized by all CTL clones except K_i and K_j. By convention, in T_{ij}, $i < j$.
- $T_{ij\ell}$ is the number of infected cells in which epitopes of type i, j and ℓ have mutated; cells of type $T_{ij\ell}$ are recognized by all CTL clones except K_i, K_j and K_ℓ. By convention, in $T_{ij\ell}$, $i < j < \ell$.

$\vdots$

- $T_{1\ldots n}$ ($A = \{1, \ldots, n\}$) is the number of infected cells expressing no recognizable epitopes, i.e., having all n epitopes mutated. None of the specialized CTLs recognize infected cells $T_{1\ldots n}$.

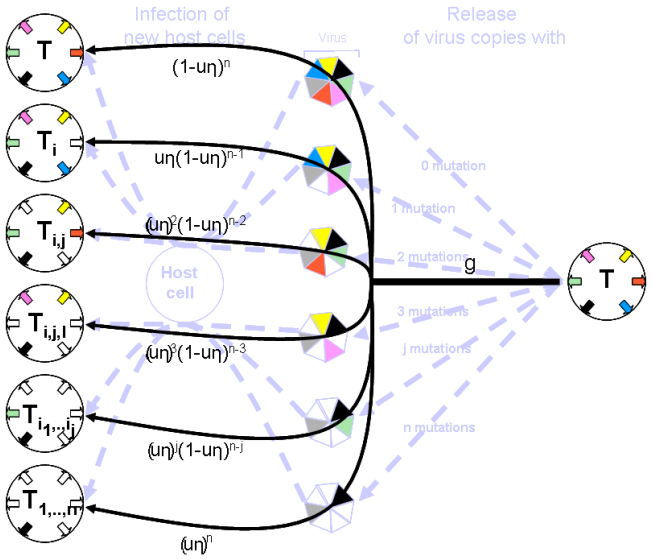

Figure 3: Dynamics of infected cell populations with $v = 6$ epitopes, of which $n = 5$ are recognized. Only the dynamics of the infected cell populations is explicitly modelled (in black). For sake of simplicity, neither the dynamics of viruses nor host cells are explicitly described; virus replication in the host cell (the infected cell), virus release, as well as invasion of new host cells by the virus are implicit (in light blue). It is assumed that during virus replication, an error can occur resulting in an epitope mutation, with u the number of base pairs constituting an epitope and η the probability of error in the copy of a base pair. The parameter g is the growth rate of infected wild type cells.

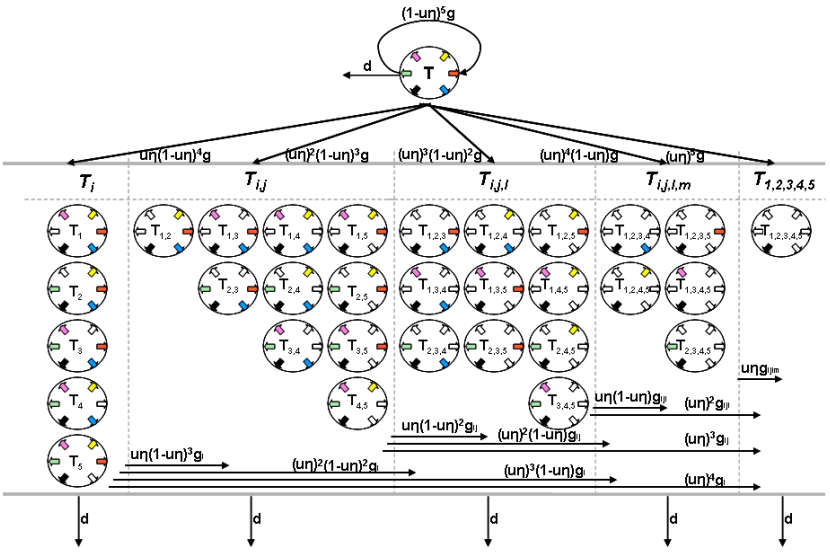

Figure 4: Dynamics of infected cells. The case shown has $v = 6$ epitopes and $n = 5$ recognizable epitopes. Parameters g_A, $A \subseteq \{1, \ldots, n\}$, are the growth rates of virus-infected cells, d is the death rate of infected cells, u is the number of base pairs constituting an epitope and η is the probability of error in base pair copy.

Finally, variable $T_{\not e}$ is the total number of infected cells expressing a non-mutated epitope of type e:

$$\begin{aligned} T_{\not e} &= T + \sum_{\substack{i=1 \\ i\neq e}}^{n} \left(T_i + \sum_{\substack{j=i \\ j\neq e,i}}^{n} \left(T_{ij} + \sum_{\substack{l=j \\ l\neq e,i,j}}^{n} \left(T_{ijl} + \sum_{\substack{m=l \\ m\neq e,i,j,l}}^{n} \cdots \right) \right) \right) \\ &= \sum_{\substack{e\notin A \\ A\subseteq\{1,\ldots,n\}}} T_A. \end{aligned} \tag{1}$$

Any infected cell counted in $T_{\not e}$ is recognized by CTLs of type e, K_e.

There is one population of infected cells, T, expressing all recognizable epitopes; there are $\binom{n}{1} = n$ populations of infected cells with 1 mutated epitope (T_i), $\binom{n}{2} = \frac{(n-1)n}{2!}$ populations of infected cells with 2 mutated epitopes (T_{ij}), etc. and finally, $\binom{n}{n} = 1$ population of infected cells with n mutated epitopes ($T_{1\ldots n}$). Hence, the model considers n CTL populations and $1 + \sum_{i=1}^{n} \binom{n}{i} = \sum_{i=0}^{n} \binom{n}{i} = 2^n$ populations of infected cells.

2.3 Model assumptions

In the absence of CTLs, the dynamics of infected cells is governed by the birth (generation) and death of infected cells (Figure 4). Death of infected cells occurs at the *per capita* rate d, implicitly combining natural death of host cells, virus-induced death and death resulting from action of the innate system (NK-induced death). Generation of new infected cells by a virus is proportional to the population of cells currently infected by this virus. The proportionality incorporates the "rate of growth" g_A, $A \subseteq \{1,\ldots,n\}$, of the infected cells T_A expressing the A mutated epitopes and the probability of mutations in epitopes. The parameter g_A, called the rate of growth of infected cells of type T_A, combines basic steps of viral infection dynamics (see, e.g., Figure 1 in [12]). Thus, g_A is a combination of level of uninfected cells, viral load at quasi-equilibrium (including the rate of virus production and clearance, which is related to virus fitness) as well as the rate of infection of host cells by virus. It is assumed that mutations considered here can affect the rate of infection by virus and/or virus fitness; both parameters are implicitly included in g_A. Hence, in the present model, the rate of growth g_A of the different types of infected cells T_A can vary in response to mutation. However, it is assumed that the rate of death of infected cells is not affected by mutation and is the same for all infected cell populations.

An epitope comprises u base pairs. The probability of error in the copy of a base pair is η. For an epitope to mutate, at least one error in its base pairs is needed. It is assumed that the number of mutated epitopes follows a binomial law with parameters n and ε; the probability $\varepsilon = u\eta$ of a mutation in an epitope is derived in Appendix A.1.

A K_e CTL clone is able to bind the non-mutated epitope of type e; interactions between K_e CTLs and infected cells expressing epitope e induce the death

of infected cells at the *per capita* rate κ_e. In turn, encounters between CTL clones of type e and infected cells expressing non-mutated epitopes of type e induce the proliferation of K_e. This infection-induced proliferation of CTLs of type e is described by a saturating function $f(\cdot)$ of the number $T_{\not e}$ of infected cells expressing the non-mutated epitope of type e:

$$f(T_{\not e}) = s\frac{T_{\not e}^m}{h_e^m + T_{\not e}^m}, \tag{2}$$

where s is the maximum rate of infection-induced proliferation of CTLs (common to all CTL clones), h_e is the number of infected cells expressing epitope e necessary to obtain the half-saturation $s/2$. The parameter h_e describes T Cell Receptor (TCR) affinity; different CTL clones may proliferate at different rates depending on TCR affinity (ability to bind epitopes). A higher affinity is described by a smaller value of h_e: fewer infected cells are needed to induce a given CTL proliferation rate. For $m = 1$, $f(\cdot)$ is a Michaelis-Menten function; for $m > 1$, it is a Hill function. Finally, in the absence of infection, CTLs are formed at the constant rate a_e and die at the *per capita* rate c_e.

2.4 Governing equations

Under the modelling assumptions listed above, the model takes the following form:

$$\frac{dT}{dt} = \left((1-\varepsilon)^n g - d\right) T - T\sum_{e=1}^{n} \kappa_e K_e, \tag{3a}$$

$$\frac{dT_i}{dt} = \left((1-\varepsilon)^{n-1} g_i - d\right) T_i + (1-\varepsilon)^{n-1}\varepsilon g T - T_i \sum_{\substack{e=1 \\ e\neq i}}^{n} \kappa_e K_e, \tag{3b}$$

where $i = 1, \ldots, n$,

$$\frac{dT_{ij}}{dt} = \left((1-\varepsilon)^{n-2} g_{ij} - d\right) T_{ij} + (1-\varepsilon)^{n-2}\left[\varepsilon(g_i T_i + g_j T_j) + \varepsilon^2 g T\right] - T_{ij} \sum_{\substack{e=1 \\ e\neq i,j}}^{n} \kappa_e K_e, \tag{3c}$$

where $i, j = 1, \ldots, n$ and $i < j$,

$$\vdots$$

$$\frac{dT_{1\ldots n}}{dt} = \left(g_{1\cdots n} - d\right) T_{1\ldots n} + \sum_{B\subsetneq\{1,\ldots,n\}} g_B \varepsilon^{n-|B|} T_B, \tag{3d}$$

$$\frac{dK_e}{dt} = a_e - c_e K_e + f(T_{\not e}) K_e, \quad e \in \{1, \cdots, n\}. \tag{3e}$$

Parameter	Interpretation
n	Number of CTL clones recognizing specific epitopes
a_e	Rate of formation of CTLs
c_e	Rate of death of CTLs
s	Maximal rate of proliferation of CTLs induced by infection
h_e	Number of infected cells expressing epitope e necessary to obtain the half-saturation $s/2$ ("affinity")
m	Type of growth dynamics of CTLs ($m = 1$: Michaelis-Menten; $m > 1$: Hill)
d	Rate of death of infected cells (natural death of cells+virus-induced death+innate immune system-induced death)
g_A	Intrinsic rate of growth of infected cells (replication of virions + transmission)
η	Probability of error in base pair copy
u	Number of base pairs per epitope
ε	Probability of mutation in epitopes ($= u\eta$)
κ_e	Per capita rate of death induced by the cognate CTL clone of type e

Table 1: Parameters used in system (3). When present, the index $e \in \{1, \dots, n\}$ refers to CTL clones recognizing epitopes of type e, while the index A refers to the set of mutated epitopes $A \subseteq \{1, \dots, n\}$ (possibly $\emptyset$).

System (3) is a $(2^n + n)$ −dimensional system; it is considered with nonnegative initial conditions such that $T(0) > 0$, $T_A(0) = 0$ with $A \subseteq \{1, \dots, n\}$ and $A \neq \emptyset$. Parameters are listed in Table 1; all parameter values are positive.

Note that equations (3a)–(3d) can be written for $A \subseteq \{1, \dots, n\}$ as

$$\frac{dT_A}{dt} = \left((1-\varepsilon)^{n-|A|} g_A - d - \sum_{e \notin A} \kappa_e K_e \right) T_A + (1-\varepsilon)^{n-|A|} \sum_{B \subsetneq A} g_B \varepsilon^{|A|-|B|} T_B. \quad (4)$$

3 Behaviour of a few submodels

It is easy to show that initial value problems composed of system (3) with nonnegative initial conditions have uniquely defined solutions. Also, the nonnegative orthant $\mathbb{R}_+^{2^n+n}$ is positively invariant under the flow of (3). Furthermore, solutions with $T(0) > 0$ are such that $T_A(t) > 0$ for all $t > 0$ and all $A \subseteq \{1, \dots, n\}$ (see Appendix A.2).

To understand the effect of each population on the dynamics of the whole system (3), we consider here a few submodels derived from (3).

3.1 Dynamics of CTLs in the absence of infection

The first result concerns the dynamics of CTLs without stimulation by infection, i.e., (3e) for $e \in \{1, \ldots, n\}$ when $T_A \equiv 0$ for all $A \subseteq \{1, \ldots, n\}$.

Theorem 3.1 *The dynamics of the CTLs in the absence of infection is given for* $e \in \{1, \ldots, n\}$ *by*

$$\frac{dK_e}{dt} = a_e - c_e K_e. \tag{5}$$

System (5) *has the unique positive (globally) asymptotically stable equilibrium* $(a_1/c_1, \ldots, a_n/c_n)$.

The proof of Theorem 3.1 is straightforward from the uncoupled linear nature of (5). Thus, in the absence of infection (or after infection), CTL clones of type e are maintained at the constant level a_e/c_e, which is interpreted as the maintenance of memory cells.

3.2 Dynamics of infected cells in the absence of CTLs

The dynamics of infected cells in absence of CTLs is now studied. To do so, the *infection-only* subsystem is considered, consisting of equations (3a)-(3d) in which $K_e \equiv 0$, $\forall e \in \{1, \ldots, n\}$. Hereafter, equilibrium values are indicated with *.

Theorem 3.2 *The infection-free equilibrium*

$$T^* = T_i^* = T_{ij}^* = \cdots = T_{1\ldots n}^* = 0$$

of the infection-only subsystem (3a)-(3d) *is (globally) asymptotically stable if the following condition is satisfied:*

$$\forall A \subseteq \{1, \ldots, n\}, \quad (1-\varepsilon)^{n-|A|} g_A < d. \tag{6}$$

The proof of Theorem 3.2 is given in Appendix A.3. Condition (6) in Theorem 3.2 leads to the eradication of the infection in absence of CTL intervention. For the infection-free equilibrium of the infection-only subsystem (3a)-(3d) to be stable, cells with no mutated epitope should have a growth rate $(1-\varepsilon)^n g < d$; those with 1 mutated epitope should have $(1-\varepsilon)^{n-1} g_i < d$. Continuing, infected cells with j mutated epitopes should have a growth rate satisfying $(1-\varepsilon)^{n-j} g_{i_1 \cdots i_j} < d$ and, ultimately, cells with all mutated epitopes should grow at a rate $g_{1\ldots n} < d$.

Condition (6) plays a role that is similar to the reproductive fitness of the virus, which is proportional to the number of offspring it can produce during the lifetime of the cell it infects [3]. To state Theorem 3.2 in terms of reproductive fitness, the infection-free equilibrium is (globally) asymptotically stable if

$$\max_{A \subseteq \{1,\ldots,n\}} \frac{(1-\varepsilon)^{n-|A|}}{d} < 1.$$

3.3 Dynamics in the absence of mutations

Next, the interactions between infected cells and CTLs are considered in the absence of mutations. System (3) with no mutation simplifies to the following system:

$$\frac{dT}{dt} = (g-d)\,T - T\sum_{e=1}^{n}\kappa_e K_e, \tag{7a}$$

$$\frac{dK_e}{dt} = a_e - c_e K_e + \frac{sT^m}{h_e^m + T^m}K_e, \quad e \in \{1,\dots,n\}. \tag{7b}$$

The next result (proved in Appendix A.4) defines the condition for the eradication of infection by CTL clones in the absence of mutations.

Theorem 3.3 *System* (7) *has an infection-free equilibrium* $(0, a_1/c_1, \dots, a_n/c_n)$ *that is locally asymptotically stable if*

$$g < d + \sum_{e=1}^{n}\frac{\kappa_e a_e}{c_e} \tag{8}$$

and unstable if the reverse inequality holds.

Thus, in the absence of mutations, infection occurs whenever the reverse inequality to (8) holds.

4 Complete model

4.1 Preliminary considerations

The following result (proved in Appendix A.5) provides information on the existence of equilibria of the complete system (3) with mutation.

Theorem 4.1 *i) A necessary condition for existence of an interior infection equilibrium (with all types of infected cells present) for system* (3) *is*

$$(1-\varepsilon)^n g > d. \tag{9}$$

ii) If $T_A^* = 0$ *for some* $A \subseteq \{1,\dots,n\}$*, then* $T_B^* = 0$ *for all* $B \subseteq A$*.*

iii) Suppose that $T(0) > 0$*. A sufficient condition for solutions of system* (3) *to become unbounded is*

$$g_{1\dots n} > d. \tag{10}$$

In *ii)*, we say that if an infected cell equilibrium component is equal to zero, then equilibrium components of all possible infected cell ancestors are also equal to zero. The notion of infected cell ancestors is illustrated for $n = 2$ in Figure 5 in which all possible equilibria of the system are listed.

Condition (10) in *iii)* provides a sufficient condition for CTL escape, since in this case, at least the infected cell population $T_{1\dots n}$ persists.

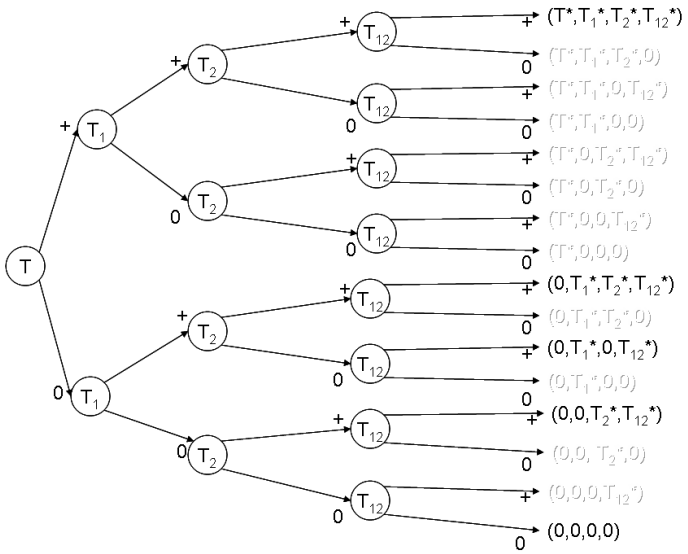

Figure 5: Infection components of possible equilibria with $n = 2$ epitopes. A "+" near an arrow head indicates that the variable at the origin of the arrow is a positive component of the equilibrium, "0" indicates that the equilibrium value for that variable is zero. In black, the possible equilibria; in gray, equilibria ruled out using *ii)* in Theorem 4.1.

4.2 Case of $n = 1$ epitope

The complete model is first considered with one recognizable epitope, i.e., system (3) is studied with $n = 1$; the state variables are T, T_1 and K_1. The following result, proved in Appendix A.6, lists all possible equilibria of (3) and their local asymptotic stability in this case.

Theorem 4.2 *The infection-free equilibrium,*

$$\left(0, 0, \frac{a_1}{c_1}\right),$$

always exists and is locally asymptotically stable if the following conditions are satisfied:

- $(1-\varepsilon)g < d + \dfrac{a_1\kappa_1}{c_1}$, *[control of wild type]*
- $g_1 < d$. *[control of mutant]*

The (unique) infection equilibrium,

$$E_{IE} = \left(T^*, \frac{\varepsilon g}{d - g_1}T^*, \frac{(1-\varepsilon)g - d}{\kappa_1}\right),$$

where $T^* = h_1\left(\frac{a_1\kappa_1 - c_1((1-\varepsilon)g-d)}{(c_1-s)((1-\varepsilon)g-d)-a_1\kappa_1}\right)^{1/m}$, *exists whenever*

$$g_1 < d \tag{11a}$$

and additionally, if $c_1 < s$, *there holds that*

$$d + \frac{a_1\kappa_1}{c_1} < (1-\varepsilon)g, \tag{11b}$$

while if $c_1 > s$, *there holds that*

$$d + \frac{a_1\kappa_1}{c_1} < (1-\varepsilon)g < d + \frac{a_1\kappa_1}{c_1 - s}. \tag{11c}$$

When it exists, the infection equilibrium is locally asymptotically stable.

Note that (11a) relates to Theorem 4.1: $d < (1-\varepsilon)g$ is the necessary condition in *i)* and $g_1 < d$ is the contrapositive of the sufficient condition in *iii)* when $n = 1$. Also, if $c_1 < s$, then the condition for existence (and local asymptotic stability) of the infection equilibrium is that the infection-free equilibrium loses stability because the control of the wild type fails. For $c_1 > s$ but very close to s, (11c) is roughly equivalent to (11b). Then, as the difference between c_1 and s increases, condition (11c) becomes harder and harder to satisfy.

Note now that the dynamics of T_1 does not influence that of the other variables; see System (21) in the proof (Appendix A.6). So one can consider the

subsystem consisting of the equations for T and K_1, (21a) and (21c), respectively. From the proof, it is clear that in that subsystem, if the condition for control of the wild type in Theorem 4.2 holds, then the infection free equilibrium $(T^*, K_1^*) = (0, a_1/c_1)$ is locally asymptotically stable. Conditions for the existence of the infection equilibrium $(T^*, ((1-\varepsilon)g - d)/\kappa_1)$, with T^* as in Theorem 4.2, remain as in the theorem, (11b) if $c_1 < s$ and (11c) if $c_1 > s$. Thus, if the condition for the control of the mutant is broken, i.e., if $g_1 > d$, then T_1 becomes unbounded; see the equation for the dynamics of T_1, (21b), in the proof. However, while local asymptotic stability of the equilibria for the three variables T, T_1 and K_1 is lost because of the unboundedness of T_1, that of the subsystem with only the wild type and CTL populations is governed by the condition on control of the wild type: if $(1-\varepsilon)g < d+\kappa_1 a_1/c_1$, the infection-free equilibrium is locally asymptotically stable, while if $(1-\varepsilon)g > d+\kappa_1 a_1/c_1$ (and $(1-\varepsilon)g$ not too large in the case where $c_1 > s$), the infection equilibrium is locally asymptotically stable.

The unbounded situation is unrealistic and is a consequence of the absence of target cell populations in the model, which, in effect, is equivalent to assuming that the target cell population is infinite. However, in practice, the situation where $T_1 \to \infty$, which is written $T_1^* = \infty$, corresponds to one of escape and a particularly interesting case is that where $(T^*, K_1^*, T_1^*) = (0, +, \infty)$, with the wild type absent but the mutant present because of the selection pressure exerted by CTL killing.

Numerically, it is observed that in the case where conditions (11b) or (11c) fail to hold, there are instances where T becomes unbounded. However, we have been so far unable to ascertain mathematically the dynamics in this case.

Special case of growth-neutral mutations. Suppose that mutations have neither beneficial nor detrimental effects on the growth rate of infected cells, i.e., $g = g_1$. Then all conditions of Theorem 4.2 reduce to $g < d$. In this case, there is a unique equilibrium, the infection-free equilibrium, which is locally asymptotically stable when $g < d$; the infection equilibrium does not exist. When $g > d$, there is escape, solutions become unbounded (case *iii)* in Theorem 4.1).

4.3 Case of $n = 2$ epitopes

System (3) is now considered with $n = 2$ epitopes. The following theorem establishes the existence of equilibria and is proved in Appendix A.7.

Theorem 4.3 *Consider system* (3) *with* $n = 2$. *There exist*

- *an infection-free equilibrium* $\left(0, 0, 0, 0, \frac{a_1}{c_1}, \frac{a_2}{c_2}\right)$, *which always exists;*
- *a boundary infection equilibrium of type 1,* $(0, T_1^*, T_2^*, T_{12}^*, K_1^*, K_2^*)$, *with*

$$T_1^* = h_2 \left(\frac{a_2\kappa_2 - c_2\left((1-\varepsilon)g_1 - d\right)}{(c_2 - s)\left((1-\varepsilon)g_1 - d\right) - a_2\kappa_2} \right)^{1/m},$$

$$T_2^* = h_1 \left(\frac{a_1\kappa_1 - c_1\left((1-\varepsilon)g_2 - d\right)}{(c_1 - s)\left((1-\varepsilon)g_2 - d\right) - a_1\kappa_1} \right)^{1/m},$$

$$T_{12}^* = \frac{\varepsilon}{d - g_{12}} \left(g_1 T_1^* + g_2 T_2^* \right),$$

$$K_1^* = \frac{(1-\varepsilon)g_2 - d}{\kappa_1}, \quad K_2^* = \frac{(1-\varepsilon)g_1 - d}{\kappa_2};$$

- *a boundary infection equilibrium of type 2,* $(0, 0, T_2^*, T_{12}^*, K_1^*, K_2^*)$*, with*

$$T_1^* = 0, \quad T_2^* = h_1 \left(\frac{a_1\kappa_1 - c_1\left((1-\varepsilon)g_2 - d\right)}{-a_1\kappa_1 + (c_1 - s)\left((1-\varepsilon)g_2 - d\right)} \right)^{1/m},$$

$$T_{12}^* = \frac{\varepsilon}{d - g_{12}} g_2 T_2^*, \quad K_1^* = \frac{(1-\varepsilon)g_2 - d}{\kappa_1}, \quad K_2^* = \frac{a_2}{c_2};$$

- *a boundary infection equilibrium of type 3,* $(0, T_1^*, 0, T_{12}^*, K_1^*, K_2^*)$*, with*

$$T_1^* = h_2 \left(\frac{a_2\kappa_2 - c_2\left((1-\varepsilon)g_1 - d\right)}{-a_2\kappa_2 + (c_2 - s)\left((1-\varepsilon)g_1 - d\right)} \right)^{1/m}, \; T_2^* = 0,$$

$$T_{12}^* = \frac{\varepsilon}{d - g_{12}} g_1 T_1^*, \quad K_1^* = \frac{a_1}{c_1}, \quad K_2^* = \frac{(1-\varepsilon)g_1 - d}{\kappa_2};$$

Boundary infection-equilibria of type k*, with* $k \in \{1, 2, 3\}$*, exist under the following conditions:*

- $g_{12} < d$*,*
- *For* $T_i^* \neq 0$ *with* $i \in \{1, 2\}$*,*
  - *if* $c_j > s$ $(j \neq i)$*,* $d + \frac{a_j\kappa_j}{c_j} < (1-\varepsilon)g_i < d + \frac{a_j\kappa_j}{c_j - s}$*,*
  - *if* $c_j < s$ $(j \neq i)$*,* $d + \frac{a_j\kappa_j}{c_j} < (1-\varepsilon)g_i$*.*

For illustration, possible equilibria of system (3) with $n = 2$ epitopes are listed in Figure 5. Note that an interior infection equilibrium of the form $(T^*, T_1^*, T_2^*, T_{12}^*, K_1^*, K_2^*)$ could exist if the necessary condition $(1-\varepsilon)^2 g > d > g_{12}$ were to hold. However, we have not been able to prove that such an equilibrium does indeed exist.

The next result (proved in Appendix A.8) concerns the infection-free equilibrium.

Theorem 4.4 *The infection-free equilibrium* $(0, 0, 0, 0, \frac{a_1}{c_1}, \frac{a_2}{c_2})$ *is locally asymptotically stable if*

- *Wild type condition:* $(1-\varepsilon)^2 g < d + \kappa_1 \frac{a_1}{c_1} + \kappa_2 \frac{a_2}{c_2}$*,*
- *Mutant condition 1:* $(1-\varepsilon)g_1 < d + \kappa_2 \frac{a_2}{c_2}$*,*

- *Mutant condition 2:* $(1-\varepsilon)g_2 < d + \kappa_1 \frac{a_1}{c_1}$,
- *Mutant condition 3:* $g_{12} < d$.

Note that conditions for the existence of the boundary equilibrium of type 1 are the contrapositives of "Mutant condition 1" and "Mutant condition 2" in Theorem 4.4. The following result (with proof in Appendix A.9) gives the conditions for the local asymptotic stability of the boundary infection-equilibria of type 1 to 3.

Theorem 4.5 *When it exists, the boundary infection equilibrium of type 1,* $(0, T_1^*, T_2^*, T_{12}^*, K_1^*, K_2^*)$*, is locally asymptotically stable if*

$$(1-\varepsilon)^2 g + d < (1-\varepsilon)(g_1 + g_2).$$

When it exists, the boundary infection equilibrium of type 2, $(0, 0, T_2^*, T_{12}^*, K_1^*, K_2^*)$*, is locally asymptotically stable if*

$$(1-\varepsilon)^2 g < (1-\varepsilon)g_2 + \kappa_2 \frac{a_2}{c_2}.$$

When it exists, the boundary infection equilibrium of type 3, $(0, T_1^*, 0, T_{12}^*, K_1^*, K_2^*)$*, is locally asymptotically stable if*

$$(1-\varepsilon)^2 g < (1-\varepsilon)g_1 + \kappa_1 \frac{a_1}{c_1}.$$

At the boundary-infection equilibria, wild type-infected cells are eradicated by the immune response; however, infected cells with mutated epitopes escape from CTL control. The local asymptotic stability of boundary equilibria of types 1 to 3 thus presents a new situation where escape occurs, namely, one where the wild type is eradicated while some mutants persist. Thus, Theorem 4.5 states additional conditions for escape in the case of $n = 2$ epitopes (recall that it suffices that $g_{12} > d$ for the infected cell population with all mutated epitotes to become unbounded, another situation with escape).

Special case of growth-neutral mutations. Suppose that mutations have neither beneficial nor detrimental effects on the growth rate of infected cells, i.e., $\forall A \subseteq \{1, 2\}$, $g_A = g$. Then the necessary condition for the existence of an interior infection equilibrium is $(1-\varepsilon)^2 g > d > g$, which never holds. Similarly, conditions in Theorem 4.3 for existence of boundary infection-equilibria (of types 1, 2 and 3) are not satisfied. There are no infection equilibria. In this case, there is a unique equilibrium, the infection-free equilibrium, which is locally asymptotically stable when $g < d$ (all conditions of Theorem 4.4 reduce to the unique condition $g < d$). Otherwise, if $g > d$, all infected cell populations are unbounded. The condition of eradication of infection does not depend on CTLs (similarly to Theorem 3.2) and only depends on the growth rate of the wild type.

4.4 General case of n epitopes

The following result is proved in Appendix A.10 and generalizes Theorems 4.2 and 4.4 to the case of n epitopes.

Theorem 4.6 *The infection-free equilibrium,*

$$\left(\mathbf{0}_{2^n}, \frac{a_1}{c_1}, \ldots, \frac{a_n}{c_n}\right),$$

is locally asymptotically stable if the following condition is satisfied:

$$\forall A \subseteq \{1, 2, \ldots, n\}, \quad (1-\varepsilon)^{n-|A|} g_A < d + \sum_{e \notin A} \kappa_e \frac{a_e}{c_e}. \tag{12}$$

Condition (12) can be interpreted in terms of the maximal rate of growth of infected cells of different types. More precisely, for the of infection-free equilibrium to be locally asymptotically stable, the growth rate of cells with no mutated epitopes (wild type) must satisfy

$$(1-\varepsilon)^n g < d + \sum_{e=1}^{n} \kappa_e \frac{a_e}{c_e}.$$

Those with 1 mutated epitope must satisfy

$$(1-\varepsilon)^{n-1} g_i < d + \sum_{\substack{e=1 \\ e \neq i}}^{n} \kappa_e \frac{a_e}{c_e}.$$

Continuing, cells with j mutated epitopes should satisfy

$$(1-\varepsilon)^{n-j} g_{i_1 \cdots i_j} < d + \sum_{\substack{e=1 \\ e \neq i_1, \ldots, i_j}}^{n} \kappa_e \frac{a_e}{c_e}.$$

Finally, cells with all epitopes mutated need a growth rate $g_{1\cdots n} < d$.

Special case of growth-neutral mutations. Similarly to the case of $n = 1$ and $n = 2$, if mutations do not lead to any change in the rate of growth of infected cells, $g = g_A$ for $\forall A \subseteq \{1, \ldots, n\}$, the condition for infection control reduces to $d > g$.

5 Numerical considerations

The simulations presented here are carried out with $n = 5$ epitopes composed of $u = 30$ base pairs. The growth rate is randomly chosen such that $g_A \in (0, 10]$ (day^{-1}) and $g_{1\ldots n} = d/5$ day^{-1}, where the death rate of infected cells is $d = 0.9$ day^{-1}. The probability η of error in base pair copy is taken between

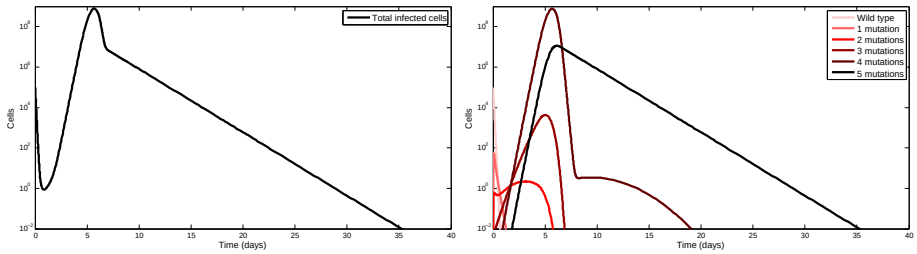

Figure 6: No escape scenario. Parameter values satisfy the no-escape conditions of Theorem 4.6 for $n = 5$. Left: total number of infected cells. Right: Number of infected cells of different types.

10^{-8} and 10^{-3} [4, 5]. For CTLs, rates of growth (resp. death) are randomly chosen such that $a_e \in [1, 10]$ (cell·day^{-1}) (resp. $c_e \in [10^{-1}, 10^{-3}]$ day^{-1}). For CTL proliferation induced by infected cells, Michaelis Menten functions are used (m=1), the maximal rate of proliferation is $s = 1$ day^{-1}, the "affinity" is randomly chosen such that $h_e \in [10^4, 10^6]$ (cell). The per capita rates of death of infected cells induced by CTLs of type e are randomly chosen such that $\kappa_e \in [10^1, 10^3]$ (cell^{-1}·day^{-1}).

In the numerical simulations, it is assumed that "eradication" happens when the total number of infected cells becomes less than 10^{-6}.

5.1 No escape scenario

In Figure 6, the time evolution of the total number of infected cells (left) and of the different infected cells (right) is shown, in conditions where Theorem 4.6 rules out escape. While infection is eradicated in 35 days, after about 8 days the only remaining cells are those expressing 4 and 5 mutated epitopes. The effect of mutations under the conditions with no escape are then investigated (Figure 7): the probability of error in base pair copy is made to vary from 10^{-6} to 9×10^{-3} (so the probability of mutation in epitopes $\varepsilon \in [30 \times 10^{-6}, 30 \times 9 \times 10^{-3}]$), other parameter values are taken as in Figure 6. Even in the no escape scenario, mutations increase the time to infection eradication and the "virus load" (Figure 7). When the probability of mutation ε is less than $30 \times 8 \times 10^{-6}$, eradication of infection takes place within the first day. Otherwise, eradication of infection takes between 40 to 50 days. The higher the probability of mutation, the faster the disappearance of wild type virus-infected cells; in all cases, the wild type is extinct within the first day. Furthermore, the higher the probability of mutation, the faster the appearance of cells bearing 5 mutations.

5.2 Escape scenario

In Figure 8, the time evolution of the total number of infected cells (top) and of the different infected cells (bottom left) is shown in conditions where Theorem 4.6 fails. Even if the number of infected cells (total or bearing specific

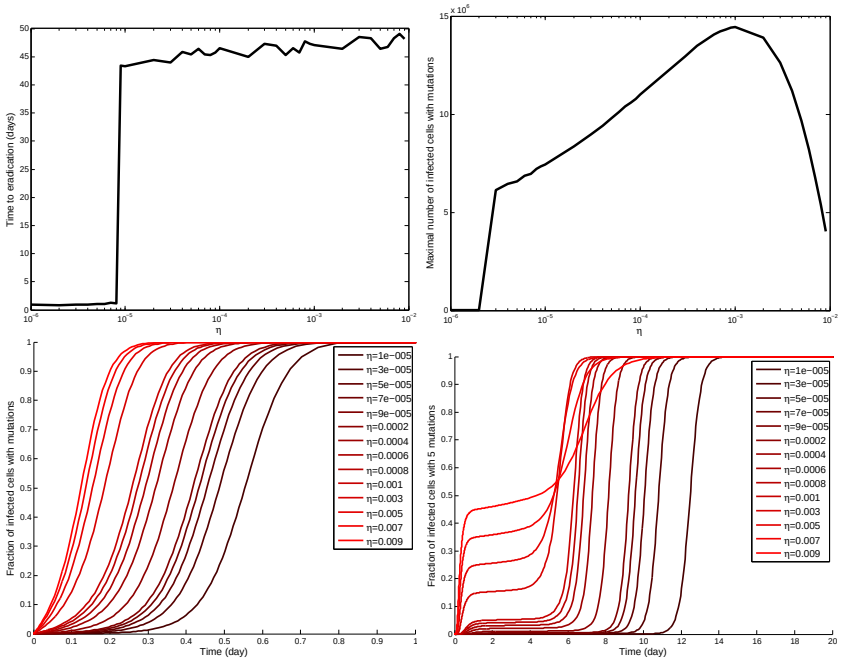

Figure 7: Effect of the probability η of error in base pair copy, giving a probability of mutation $\varepsilon = 30 \times \eta$, under the conditions of no escape of Theorem 4.6. "Eradication" happens when the total number of infected cells becomes less than 10^{-6}. Top left: Time to eradication. Top right: Maximum number of infected cells with mutations. Bottom left: Time evolution of the fraction of mutants. Bottom right: Fraction of cells with 5 mutations amongst the total population of infected cells with mutations.

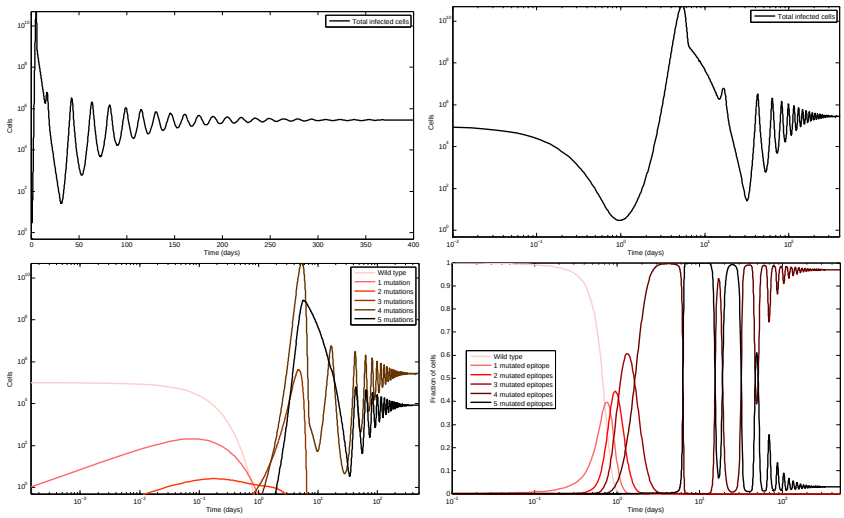

Figure 8: Escape scenario. Parameter values fail conditions of no escape of Theorem 4.6 for $n = 5$. Top row: temporal evolution of the total number of infected cells (note the log time scale on top right, used to emphasize initial transients). Bottom row: temporal evolution (in log scale) of the number (left) and distribution (right) of cells infected with the various mutants.

epitopes) goes down dramatically, infection persists; only cells expressing 4 and 5 mutated epitopes persist. Note that the time evolution of the total number of infected cells in this scenario is reminiscent of qualitative observations of the viral load made in the case of HIV: a high peak of the viral load is followed by drastic drop down and finally an re-increase of the viral load.

In Figure 9, the effect of the probability of mutation is illustrated. The lower the mutation probability, the lower the trough in the number of infected cells and equilibrium values of infected cells. Mutations increase the viral load.

6 Discussion

Cytotoxic T lymphocytes constitute an important component of the antiviral immune response. By recognizing virus-encoded epitopes clasped in the groove of MHC molecules on the cell surface, CTLs target and kill infected cells and thereby play an essential role in suppressing viral infections. Specific mutations in these epitopes can abrogate their CTL recognition and allow an infected cell to escape CTL action. A number of viral pathogens, especially those with RNA genome, exhibit high rates of mutation and thus rapidly produce immune-escape or drug-resistant mutants that have been attributed as barriers to antiviral vaccine or drug design, respectively. However, infection with some of the rapidly mutating viruses can be effectively controlled by CTLs in vivo. This suggests

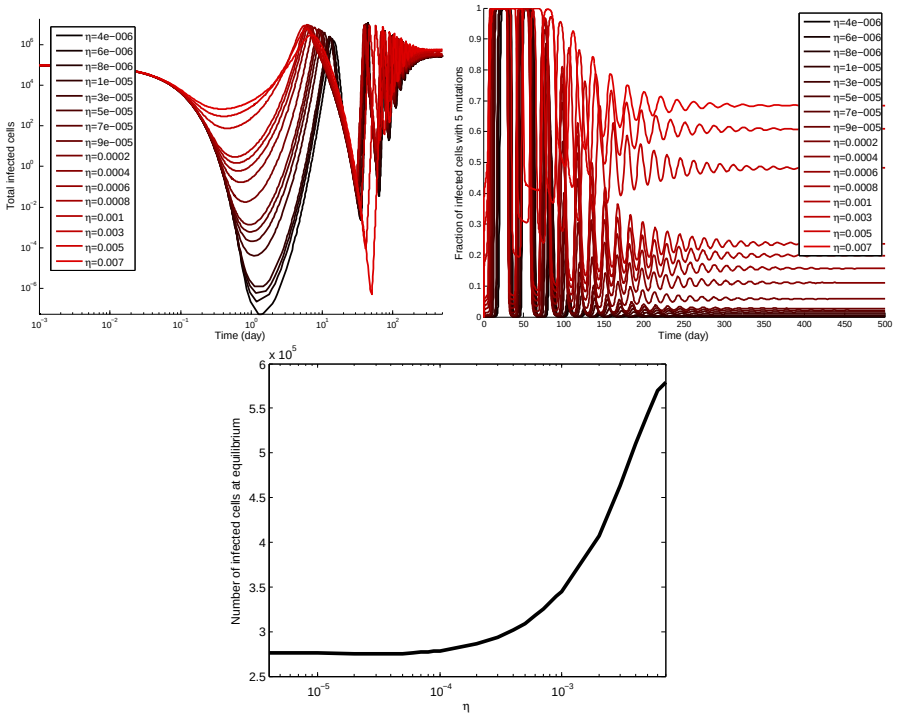

Figure 9: Effect of the probability η of error during base pair copy, under conditions of escape. Conditions of Theorem 4.6 are not satisfied. Top left: total number of infected cells (log time axis used to emphasize initial transients). Top right: Fraction of the total number of infected cells that are infected with viruses having 5 mutated epitopes. Bottom: Number of infected cells at the equilibrium as a function of η.

that a true relationship between viral mutations and the ability of oligoclonal CTLs to suppress an infection is not fully understood. Owing to its importance in pathogenesis, vaccine or drug design and viral control, we developed a deterministic model consisting of oligoclonal CTLs and infected cells bearing one or more types of CTL-escape epitopes to define conditions necessary for CTL-escape and persistence of infection. The model is used to examine the ability of CTL response of varying oligoclonality to suppress infection in the presence of mutations. For the sake of simplicity, the dynamics of target cell populations and their infection by pathogens are not considered here.

In this work, CTL escape can happen in two situations: either there is an equilibrium with a positive number of infected cells (of any type) or one at least of the infected cell populations goes unbounded. Unboundness is an unrealistic situation which results from ignoring the dynamics of target cells. The conditions for absence of CTL escape are fully determined and given in the general case of n recognizable epitopes in Theorem 4.6. Different situations with CTL escape exist; each possible escape scenario necessitates the negation of at least one of the conditions necessary to prevent escape. Moreover, when the infection persists in a situation with no unbounded solutions, the TCR affinities h_e determine the level of infection; see the expressions for the infection equilibria in Theorems 4.2 and 4.3. Furthermore, even in a situation without CTL escape, the presence of mutations slows down the eradication of infection: the larger the probability of mutation, the slower the eradication. In a situation with CTL escape, mutations increase the level of infection: the larger the probability of mutation, the larger the level of infection.

Because of the structure of the model, it is not possible (case *ii* in Theorem 4.1) to observe a situation where the presence of mutations would lead the wild type cells to escape elimination by CTLs because the latter are mobilized to successfully eliminate all cells infected by mutated viruses. However, considering the case of $n = 2$ epitopes, we find boundary equilibria that can be locally asymptotically stable and at which the infection persists for some mutated cells and is eradicated by CTLs for others (Theorems 4.3 and 4.5). This suggests that from the virus perspective, there could exist a cooperative mechanism for escape: mutations, by "diluting" the effect of the cognate CTL clone, lower the efficiency of the latter and therefore give the opportunity to a higher fitness strain to establish itself. Further work will be needed in order to establish if such behaviour is observable in the more general case of n epitopes and whether the number of recognizable epitopes is important.

Comparing the situation where there are no mutations (Theorem 3.3) and the growth-neutral cases of Theorems 4.2, 4.4 and 4.6, it is possible to characterize the effect of mutations on the conditions required for infection eradication. When mutations are neither beneficial nor detrimental, infection is eradicated if $g < d$ (Theorems 4.2, 4.4 and 4.6), i.e., the kill rate of cognate CTL clones becomes irrelevant and the persistence of infection is governed solely by the population dynamics of infected cells. Furthermore, the occurrence of mutations induces a stronger condition for the control of the infection than in the case of no mutations (Theorem 3.3), $d + \sum_{e=1}^{n} \kappa_e a_e / c_e > d > g$. Thus, in pres-

ence of mutations, eradication of the infection is more difficult and only depends on the growth rate of the wild type.

The model introduced here is quite complex and of high dimensionality and more work is needed in order to better understand its dynamics. Also, in some cases, the cell populations go to very low numbers before returning to higher values; see, for instance, Figure 9. This implies that the problem should also be investigated using a stochastic approach, since escape could be a consequence of the reappearance of infected cells following a phase where the infection has almost been eradicated; see, e.g., [13].

Acknowledgements

This work was initiated by Beni Sahai and originally carried out by Beni, NM and SP. Because of Beni's untimely passing, this work was left without its "biological head". JA, NM and SP would like to emphasize that biological errors are their doing, not Beni's. This work is dedicated to Beni's memory. We thank an anonymous referee whose comments helped improve the manuscript.

A Appendix – Details and proofs

A.1 Probability of mutation

An epitope is composed of u base pairs. Let η be the probability of error in the copy of a base pair (substitution); we assume that the copy of base pairs are independent events. We make the assumption that the approximation $(1-\eta)^u \approx 1-u\eta$ can be made. This implies that $(1-\eta)^{un} \approx (1-u\eta)^n$. In order to simplify notation, we let $u\eta = \varepsilon$.

For an epitope to mutate, at least one error in its base pairs is needed. Considering n epitopes, the probability that the copy happens without an error, i.e., the probability of no mutation, is

$$\begin{aligned} \mathbb{P}[\text{no error}] &= (1-\eta)^{un} \\ &\approx (1-\varepsilon)^n \end{aligned} \tag{13}$$

and the probability of at least one mutation is

$$\begin{aligned} \mathbb{P}[\text{at least an error}] &= 1 - \mathbb{P}[\text{no error}] \\ &= 1-(1-\eta)^{un} \\ &\approx 1-(1-\varepsilon)^n. \end{aligned} \tag{14}$$

The probability of exactly 1 mutated epitope among n epitopes is

$$\begin{aligned} \mathbb{P}[\text{error in exactly 1 epitope}] &= \tbinom{n}{1}(1-(1-\eta)^u)(1-\eta)^{u(n-1)} \\ &\approx \tbinom{n}{1}\varepsilon(1-\varepsilon)^{(n-1)}. \end{aligned} \tag{15}$$

and the probability of a mutation in epitope j only is:

$$\begin{aligned}\mathbb{P}[\text{error in epitope } j \text{ only}] &= (1-(1-\eta)^u)(1-\eta)^{u(n-1)} \\ &\approx \varepsilon(1-\varepsilon)^{(n-1)}. \end{aligned} \tag{16}$$

The probability of exactly j mutated epitopes among n epitopes present on cells is

$$\begin{aligned}\mathbb{P}[\text{error in exactly } j \text{ epitopes}] &= \tbinom{n}{j}(1-(1-\eta)^u)^j(1-\eta)^{u(n-j)} \\ &\approx \tbinom{n}{j}\varepsilon^j(1-\varepsilon)^{(n-j)}. \end{aligned} \tag{17}$$

The probability of mutation in the epitope j and the epitope i only is:

$$\begin{aligned}\mathbb{P}[\text{error in the epitopes } j \text{ and } i \text{ only}] &= (1-(1-\eta)^u)^2(1-\eta)^{u(n-2)} \\ &\approx \varepsilon^2(1-\varepsilon)^{(n-2)}. \end{aligned} \tag{18}$$

Finally, the probability of exactly v mutated epitopes among the $n-j$ previously non-mutated epitopes is $\binom{n-j}{v}(1-(1-\eta)^u)^v(1-\eta)^{u(n-j-v)}$, i.e.,

$$\begin{aligned}&\mathbb{P}[\text{error in exactly } v \text{ of the } n-j \text{ previously non-mutated epitopes}] \\ &\qquad\qquad \approx \tbinom{n-j}{v}\varepsilon^v(1-\varepsilon)^{(n-j-v)}. \end{aligned} \tag{19}$$

A.2 Positive invariance under the flow

Proposition A.1 *Suppose that initial conditions of* (3) *are nonnegative and such that* $T_A(0) > 0$ *for all* $A \subseteq \{1,\dots,n\}$. *Then* $T_A(t) > 0$ *for all* $t \geq 0$ *and* $A \subseteq \{1,\dots,n\}$.

Proof Assume that $T_A(0) > 0$ for all $A \subseteq \{1,\dots,n\}$. We proceed by induction on A.

First, consider (3a) and write it as $T' = f(t,T)$, where the time dependence indicates the role of the K_e's. As $f \in C^\infty$, solutions to this scalar equation exist and are unique for all t. Now note that $f(t,0) = 0$ for all t, implying that $T(t) = 0$ for all t is (an equilibrium) solution to the equation. Existence of a solution such that $T(0) > 0$ and $T(t_1) = 0$ for some $t_1 > 0$ would then violate uniqueness of solutions. Thus, $T(t) > 0$ for all $t > 0$ if $T(0) > 0$.

Now consider (3b). Assume that there exists $t_1 > 0$ such that for some $i \in \{1,\dots,n\}$, $T_i(t_1) = 0$ and that t_1 is the first such time. Then

$$\frac{dT_i}{dt}(t_1) = (1-\varepsilon)^{n-1}\varepsilon g T(t_1) > 0,$$

since $T(t) > 0$ for all $t \geq 0$. However, since $T_i(0) > 0$ and t_1 is the first time such that $T_i(t_1) = 0$, it follows that T_i is decreasing left of t_1 and nonincreasing at t_1, a contradiction. Therefore $T_i(t) > 0$ for all $t \geq 0$. And the same reasoning holds for all $i \in \{1,\dots,n\}$.

Continuing the argument for $T_{ij},\dots,T_{i\dots n}$ gives the result. ∎

From the proof, we see that the following more general result actually holds.

Corollary A.2 *Suppose that initial conditions are nonnegative and such that* $T(0) > 0$. *Then* $T_A(t) > 0$ *for all* $t > 0$ *and* $A \subseteq \{1, \ldots, n\}$.

A.3 Proof of Theorem 3.2

The *infection-only* subsystem (3a)-(3d) is a linear system

$$\frac{d}{dt}\mathbf{T} = \mathbf{A}\mathbf{T}, \tag{20}$$

where $\mathbf{T}$ is the vector of all infected cell populations,

$$\mathbf{T} = [T, T_1, \ldots, T_n, T_{12}, \ldots, T_{1\ldots n}]^T.$$

The coefficient matrix $\mathbf{A}$ is a $2^n \times 2^n-$lower triangular matrix. System (20) has a unique equilibrium, the infection-free equilibrium

$$T^* = T_i^* = T_{ij}^* = \cdots = T_{1\ldots n}^* = 0,$$

if and only if $\mathbf{A}$ is invertible, that is, if and only if

$$\det(\mathbf{A}) = ((1-\varepsilon)^n g - d)\left((1-\varepsilon)^{n-1} g_1 - d\right) \cdots (g_{1\cdots n} - d) \neq 0.$$

Therefore, the conditions of existence of the infection-free equilibrium are

$$\begin{aligned} (1-\varepsilon)^n g &\neq d \\ (1-\varepsilon)^{n-1} g_i &\neq d \\ &\vdots \\ g_{1\cdots n} &\neq d. \end{aligned}$$

The infection-free equilibrium is (globally) asymptotically stable if $\Re(\lambda_i) < 0$ for $i \in \{1, \ldots, 2^n\}$, with λ_i the eigenvalues of $\mathbf{A}$. As $\mathbf{A}$ is a triangular matrix, the λ_i are the diagonal entries of $\mathbf{A}$, or also factors of $\det(\mathbf{A})$. Therefore, the conditions to have $\Re(\lambda_i) < 0$ for $i \in \{1, \ldots, 2^n\}$ give (6).

A.4 Proof of Theorem 3.3

The infection-free equilibrium satisfies $T^* = 0$. From (7b),

$$K_e^* = \frac{a_e}{c_e}, \quad \forall e \in \{1, \ldots, n\}.$$

The Jacobian matrix of system (7) at the infection-free equilibrium takes the form

$$J = \mathsf{diag}\left(g - d - \sum_{e=1}^{n} \kappa_e K_e, -c_1, -c_2, \ldots, -c_n\right).$$

Thus, the infection-free equilibrium is locally asymptotically stable if (8) holds.

A.5 Proof of Theorem 4.1

Suppose that (3) is at an equilibrium. Then, from (3a), we find that $T^* > 0$ requires $(1-\varepsilon)^n g/d > 1$. This proves assertion *i)*.

To prove *ii)*, suppose again that system (3) is at an equilibrium and use (4). Assume that $T_A^* = 0$ for some $A \subseteq \{1, \ldots, n\}$. It follows that

$$(1-\varepsilon)^{n-|A|} \sum_{B \subsetneq A} g_B \varepsilon^{|A|-|B|} T_B^* = 0,$$

for all proper subsets B of A. As all parameters are positive, this implies that for all $B \subsetneq A$, $T_B^* = 0$.

Finally, to prove assertion *iii)* consider (3d) and assume that $g_{1\ldots n} - d > 0$. As $T(0) > 0$, it follows from Corollary A.2 that $T_{1\ldots n} > 0$ for all $t > 0$. Since $g_{1\ldots n} - d > 0$, it follows that $dT_{1\ldots n}/dt > 0$ and therefore, $T_{1\ldots n}$ grows unbounded.

A.6 Proof of Theorem 4.2

System (3) with $n = 1$ takes the form

$$\frac{dT}{dt} = ((1-\varepsilon)g - d)T - \kappa_1 K_1 T, \tag{21a}$$

$$\frac{dT_1}{dt} = (g_1 - d)T_1 + \varepsilon g T, \tag{21b}$$

$$\frac{dK_1}{dt} = a_1 - c_1 K_1 + \frac{sT^m}{h_1^m + T^m} K_1. \tag{21c}$$

The infection-free equilibrium is the solution with $T^* \equiv 0$ and $T_1^* \equiv 0$, leading to $K_1^* = a_1/c_1$ using (21c). The Jacobian matrix of system (21) at an arbitrary point is

$$J = \begin{bmatrix} (1-\varepsilon)g - d - \kappa_1 K_1 & 0 & -\kappa_1 T \\ \varepsilon g & g_1 - d & 0 \\ \dfrac{smh_1^m T^{m-1} K_1}{(h_1^m + T^m)^2} & 0 & \dfrac{sT^m}{h_1^m + T^m} - c_1 \end{bmatrix}. \tag{22}$$

Evaluating (22) at the infection-free equilibrium $(0, 0, \frac{a_1}{c_1})$ gives a lower-triangular matrix with diagonal entries $(1-\varepsilon)g - d - \frac{a_1\kappa_1}{c_1}$, $g_1 - d$ and $-c_1$. The conditions for the local asymptotic stability of the infection-free equilibrium follow.

Now consider the infection equilibrium. Setting $\frac{dT_1^*}{dt} = 0$ in (21b), it follows that

$$T_1^* = \frac{\varepsilon g}{d - g_1} T^*, \tag{23}$$

which implies that there must hold that $g_1 < d$ for $T_1^* > 0$. From (21a),

$$K_1^* = \frac{(1-\varepsilon)g - d}{\kappa_1}, \tag{24}$$

from which it follows that there must hold that $d < (1-\varepsilon)g$. Putting these two conditions together yields the constraint

$$g_1 < d < (1-\varepsilon)g. \tag{25}$$

Now, setting $\frac{dK_1^*}{dt} = 0$ in (21c) and using (24), we thus seek $T^* > 0$ solution of

$$-\left(a_1 - c_1 K_1^* + s K_1^*\right) {T^*}^m = h_1^m (a_1 - c_1 K_1^*). \tag{26}$$

Let $M(T^*)$ and $Z(T^*)$ be the left and right hand sides of (26), respectively. $M(T^*)$ is a monomial passing through the origin and the graph of $Z(T^*)$ is a horizontal line. As we are concerned here with solutions $T^* > 0$ to (26), we consider the restriction of $Z(T^*)$ and $M(T^*)$ to quadrants I and IV. There are three cases.

1. If $a_1 - c_1 K_1^* > 0$, $Z(T^*)$ lies in quadrant I and $-(a_1 - c_1 K_1^* + s K_1^*) < 0$, so $M(T^*)$ is a decreasing function and lies in quadrant IV. There is no intersection.

2. If $a_1 - c_1 K_1^* < 0$, $Z(T^*)$ lies in quadrant IV. Then,

   (2.a) if $-(a_1 - c_1 K_1^* + s K_1^*) > 0$, $M(T^*)$ is a increasing function and lies in quadrant I and there is no intersection;

   (2.b) if $-\left(a_1 - c_1 K_1^* + s K_1^*\right) < 0$, $M(T^*)$ is a decreasing function and lies in quadrant IV and there is a unique intersection.

Consequently, conditions leading to a (unique) intersection are

$$\begin{cases} \frac{a_1\kappa_1}{c_1} < (1-\varepsilon)g - d < \frac{a_1\kappa_1}{c_1 - s}, & \text{for } c_1 > s, \\ \frac{a_1\kappa_1}{c_1} < (1-\varepsilon)g - d, & \text{for } c_1 < s. \end{cases} \tag{27}$$

Combining (25) and (27), one finds the conditions (11) for the existence of a (unique) infection equilibrium in the theorem.

From (23), (24) and (26), the infection equilibrium is $E_{IE} = (T^*, \frac{\varepsilon g}{d - g_1} T^*, K^*)$, with

$$T^* = h_1 \left(\frac{a_1\kappa_1 - c_1\left((1-\varepsilon)g - d\right)}{(c_1 - s)\left((1-\varepsilon)g - d\right) - a_1\kappa_1} \right)^{1/m}.$$

The Jacobian (22) evaluated at the infection equilibrium takes the form

$$J_{E_{IE}} = \begin{bmatrix} 0 & 0 & -\kappa_1 T^* \\ \varepsilon g & g_1 - d & 0 \\ \dfrac{s m h_1^m {T^*}^{m-1} K_1^*}{(h_1^m + {T^*}^m)^2} & 0 & -c_1 + \dfrac{s {T^*}^m}{h_1^m + {T^*}^m} \end{bmatrix}, \tag{28}$$

which has characteristic polynomial

$$P(\lambda) = (g_1 - d - \lambda)\left(\lambda^2 + \left(c_1 - \frac{s {T^*}^m}{h_1^m + {T^*}^m} \right) \lambda + \frac{s m \kappa_1 h_1^m {T^*}^m K_1^*}{(h_1^m + {T^*}^m)^2} \right).$$

It follows that, in order for $\Re(\lambda_i) < 0$, $\forall i \in \{1, 2, 3\}$, the following conditions must hold:

- $g_1 - d < 0$. This is true when the infection equilibrium exists.
- From the Routh-Hurwitz criterion, $c_1 - \frac{s{T^*}^m}{h_1^m + {T^*}^m} > 0$ and $\frac{sm\kappa_1 h_1^m {T^*}^m K_1^*}{(h_1^m + {T^*}^m)^2} > 0$. The latter is always true. The former leads to

$$\frac{-a_1\kappa_1 + c_1((1-\varepsilon)g - d)}{(1-\varepsilon)g - d} < c_1 \quad \Leftrightarrow \quad -a_1\kappa_1 < 0,$$

  which is always true.

Therefore, when the infection equilibrium E_{IE} exists, it is locally asymptotically stable.

A.7 Proof of Theorem 4.3

System (3) with $n = 2$ takes the form

$$\frac{dT}{dt} = ((1-\varepsilon)^2 g - d)T - (\kappa_1 K_1 + \kappa_2 K_2)T, \tag{29a}$$
$$\frac{dT_1}{dt} = ((1-\varepsilon)g_1 - d)T_1 + (1-\varepsilon)\varepsilon g T - \kappa_2 K_2 T_1, \tag{29b}$$
$$\frac{dT_2}{dt} = ((1-\varepsilon)g_2 - d)T_2 + (1-\varepsilon)\varepsilon g T - \kappa_1 K_1 T_2, \tag{29c}$$
$$\frac{dT_{12}}{dt} = (g_{12} - d)T_{12} + \varepsilon g_1 T_1 + \varepsilon g_2 T_2 + \varepsilon^2 g T, \tag{29d}$$
$$\frac{dK_1}{dt} = a_1 - c_1 K_1 + \frac{s(T+T_2)^m}{h_1^m + (T+T_2)^m} K_1, \tag{29e}$$
$$\frac{dK_2}{dt} = a_2 - c_2 K_2 + \frac{s(T+T_1)^m}{h_2^m + (T+T_1)^m} K_2. \tag{29f}$$

The infection-free equilibrium corresponds to the equilibrium solution with $T_A^* \equiv 0$ for all $A \subseteq \{1,2\}$, which, from (29e) and (29f), gives $K_1^* = a_1/c_1$ and $K_2^* = a_2/c_2$.

To find boundary equilibrium points, we assume $T^* = 0$ and seek equilibria with positive components for T_A populations with $A \subseteq \{1,2\}$, $A \neq \emptyset$. First, note that $T_{12}^* = 0$ needs to be excluded (case *ii)* in Theorem 4.1) as it would result in all $T_A^* = 0$ for $A \subseteq \{1,2\}$. So, we consider the system

$$((1-\varepsilon)g_1 - d - \kappa_2 K_2^*)T_1^* = 0, \tag{30a}$$
$$((1-\varepsilon)g_2 - d - \kappa_1 K_1^*)T_2^* = 0, \tag{30b}$$
$$\varepsilon g_1 T_1^* + \varepsilon g_2 T_2^* + (g_{12} - d)T_{12}^* = 0, \tag{30c}$$
$$a_1 + \left(\frac{s{T_2^*}^m}{h_1^m + {T_2^*}^m} - c_1\right) K_1^* = 0, \tag{30d}$$
$$a_2 + \left(\frac{s{T_1^*}^m}{h_2^m + {T_1^*}^m} - c_2\right) K_2^* = 0. \tag{30e}$$

Note that if $T_1^* = T_2^* = 0$, then from (30c), $T_{12}^* = 0$ if $g_{12} \neq d$, so we exclude this case as well. From (30a) and (30b), $T_i^* = 0$ or $K_j^* = ((1-\varepsilon)g_i - d)/\kappa_j$, for $i, j = 1, 2$, $i \neq j$.

Consider the boundary equilibrium of type 3, i.e., $T_1^* > 0$, $T_2^* = 0$. From (30a), $K_2^* = ((1-\varepsilon)g_1 - d)/\kappa_2$, from which it follows that for the equilibrium to exist, there must hold that $(1-\varepsilon)g_1 > d$. From (30c), $T_{12}^* = \varepsilon g_1 T_1^*/(g_{12} - d)$. It follows that for T_{12}^* to be positive, there must hold that $g_{12} > d$. Then, using (30d) with $T_2^* = 0$ gives $K_1^* = a_1/c_1$ and (30e) gives

$$T_1^* = h_2 \left(\frac{a_2\kappa_2 - c_2((1-\varepsilon)g_1 - d)}{(c_j - s)((1-\varepsilon)g_1 - d) - a_2\kappa_2} \right)^{1/m}.$$

To investigate the biological relevance of the latter, notice that there are two cases. If $c_2 > s$, then $T_1^* > 0$ if

$$d + \frac{a_2\kappa_2}{c_2} < (1-\varepsilon)g_1 < d + \frac{a_2\kappa_2}{c_2 - s},$$

whereas if $c_2 < s$, then $T_1^* > 0$ requires

$$d + \frac{a_2\kappa_2}{c_2} < (1-\varepsilon)g_1.$$

The case of the boundary equilibrium of type 2 with $T_1^* = 0$ and $T_2^* > 0$ is similar, with the role of 1 and 2 reversed.

Now we consider the case where $T_1^* > 0$ and $T_2^* > 0$, i.e., the type 1 boundary equilibrium. From (30a) and (30b), $K_j^* = ((1-\varepsilon)g_i - d)/\kappa_j$, for $i, j = 1, 2$, $i \neq j$. These equilibria are relevant if $(1-\varepsilon)g_i > d$, $i = 1, 2$. Substituting these equilibria into (30d) and (30e) gives the values of T_i^* in the result. The same condition must hold as in the case of type 2 and type 3 equilibria for T_1^* and T_2^* to be positive, i.e., for $i, j = 1, 2$, $i \neq j$, if $c_i > s$, then $T_j^* > 0$ if

$$d + \frac{a_i\kappa_i}{c_i} < (1-\varepsilon)g_j < d + \frac{a_i\kappa_i}{c_i - s},$$

whereas if $c_i < s$, then $T_j^* > 0$ requires

$$d + \frac{a_j\kappa_j}{c_j} < (1-\varepsilon)g_i.$$

In turn, substituting the values of T_1^* and T_2^* into (30c) gives the value of T_{12}^*, which exists under the condition $g_{12} < d$.

A.8 Proof of Theorem 4.4

The Jacobian of system (3) with $n = 2$ evaluated at $(0, 0, 0, 0, \frac{a_1}{c_1}, \frac{a_2}{c_2})$ is

$$\begin{bmatrix} (1-\varepsilon)^2 g - d & 0 & 0 & 0 & 0 & 0 \\ -\kappa_1 \frac{a_1}{c_1} - \kappa_2 \frac{a_2}{c_2} & & & & & \\ \varepsilon(1-\varepsilon)g & (1-\varepsilon)g_1 - d & 0 & 0 & 0 & 0 \\ & -\kappa_2 \frac{a_2}{c_2} & & & & \\ \varepsilon(1-\varepsilon)g & 0 & (1-\varepsilon)g_2 - d & 0 & 0 & 0 \\ & & -\kappa_1 \frac{a_1}{c_1} & & & \\ \varepsilon^2 & \varepsilon g_1 & \varepsilon g_2 & g_{12} - d & 0 & 0 \\ 0 & 0 & 0 & 0 & -c_1 & 0 \\ 0 & 0 & 0 & 0 & 0 & -c_2 \end{bmatrix},$$

giving the conditions given in Theorem 4.4.

A.9 Proof of Theorem 4.5

Stability of the boundary infection equilibrium of type 1. The Jacobian evaluated at $(0, T_1^*, T_2^*, T_{12}^*, K_1^*, K_2^*)$ has eigenvalues

$$\lambda_1 = (1-\varepsilon)^2 g + d - (1-\varepsilon)g_1 - (1-\varepsilon)g_2, \quad \lambda_2 = g_{12} - d,$$

$$\lambda_{3,4} = \frac{A \pm \sqrt{A^2 + B}}{2} \text{ and } \lambda_{5,6} = \frac{C \pm \sqrt{C^2 + D}}{2},$$

with

$$A = -\frac{a_1 \kappa_1}{(1-\varepsilon)g_2 - d},$$

$$B = 4m \frac{[a_1\kappa_1 - c_1((1-\varepsilon)g_2 - d)][a_1\kappa_1 - (c_1 - s)((1-\varepsilon)g_2 - d)]}{s((1-\varepsilon)g_2 - d)},$$

$$C = -\frac{a_2 \kappa_2}{(1-\varepsilon)g_1 - d}$$

and

$$D = 4m \frac{[a_2\kappa_2 - c_2((1-\varepsilon)g_1 - d)][a_2\kappa_2 - (c_2 - s)((1-\varepsilon)g_1 - d)]}{s((1-\varepsilon)g_1 - d)}.$$

Under conditions for existence of boundary infection-equilibria, $g_{12} - d < 0$. Under conditions for existence of the boundary infection equilibrium of type 1, $A, B, C, D < 0$. Therefore, if $A^2 + B < 0$, $\lambda_{3,4} \in \mathbb{C}$ with $\Re(\lambda_{3,4}) < 0$. If $A^2 + B > 0$, both $\lambda_{3,4}$ are real and negative. Also, if $C^2 + D < 0$, $\lambda_{5,6} \in \mathbb{C}$ with $\Re(\lambda_{5,6}) < 0$. If $C^2 + D > 0$, both $\lambda_{5,6}$ are real and negative. Therefore, the local asymptotic stability of the boundary infection equilibrium of type 1 depends only on the sign of λ_1, giving the condition

$$(1-\varepsilon)^2 g < d + (1-\varepsilon)(g_1 + g_2).$$

Stability of the boundary infection equilibrium of type 2. The Jacobian evaluated at $(0, 0, T_2^*, T_{12}^*, K_1^*, K_2^*)$ has eigenvalues

$$\lambda_1 = (1-\varepsilon)^2 g - (1-\varepsilon) g_2 - \kappa_2 \frac{a_2}{c_2}, \quad \lambda_2 = (1-\varepsilon) g_1 - d - \kappa_2 \frac{a_2}{c_2} < 0,$$

$$\lambda_3 = g_{12} - d < 0 \text{ (existence condition)}, \quad \lambda_4 = -c_2 < 0,$$

and

$$\lambda_{5,6} = \frac{A \pm \sqrt{A^2 + B}}{2},$$

with A and B defined as above. We have $\lambda_2 < 0$ since $T_1^* = 0$ (existence condition in Theorem 4.3). Under the existence conditions of the boundary infection equilibrium of type 2, $A < 0$ and $B < 0$. Therefore, if $A^2 + B < 0$, $\lambda_{5,6} \in \mathbb{C}$ with $\Re(\lambda_{5,6}) < 0$. If $A^2 + B > 0$, both $\lambda_{5,6}$ are real and negative. Therefore, the conditions for the local asymptotic stability of the boundary infection equilibrium of type 2, $(0, 0, T_2^*, T_{12}^*, K_1^*, K_2^*)$, is $(1-\varepsilon)^2 g < (1-\varepsilon) g_2 + \kappa_2 \frac{a_2}{c_2}$.

Stability of the boundary infection equilibrium of type 3. The Jacobian evaluated at $(0, T_1^*, 0, T_{12}^*, K_1^*, K_2^*)$ has eigenvalues

$$\lambda_1 = (1-\varepsilon)^2 g - (1-\varepsilon) g_1 - \kappa_1 \frac{a_1}{c_1}, \quad \lambda_2 = (1-\varepsilon) g_2 - d - \kappa_1 \frac{a_1}{c_1} < 0,$$

$$\lambda_3 = g_{12} - d < 0 \text{ (existence condition)}, \quad \lambda_4 = -c_1 < 0,$$

and

$$\lambda_{5,6} = \frac{C \pm \sqrt{C^2 + D}}{2},$$

with C and D defined as above. We have $\lambda_2 < 0$ since $T_2^* = 0$ (existence condition in Theorem 4.3). Under the existence conditions of the boundary infection equilibrium of type 3, $C < 0$ and $D < 0$. Therefore, if $C^2 + D < 0$, $\lambda_{5,6} \in \mathbb{C}$ with $\Re(\lambda_{5,6}) < 0$. If $C^2 + D > 0$, both $\lambda_{5,6}$ are real and negative. Therefore, the conditions for the local asymptotic stability of the boundary infection equilibrium of type 3, $(0, T_1^*, 0, T_{12}^*, K_1^*, K_2^*)$, are $(1-\varepsilon)^2 g < (1-\varepsilon) g_1 + \kappa_1 \frac{a_1}{c_1}$.

A.10 Proof of Theorem 4.6

System (3) is considered in the general case with n epitopes. The infection-free equilibrium is defined as $T^* = T_i^* = T_{ij}^* = \cdots = T_{1\cdots n}^* = 0$, giving $K_e^* = \frac{a_e}{c_e}$, $\forall e$, to satisfy $\frac{d\mathbf{X}^*}{dt} = \mathbf{0}$, where $\mathbf{X}^*$ is the $(2^n + n)$-vector,

$$\mathbf{X}^* = [T^*, T_1^*, \ldots, T_n^*, T_{12}^*, \ldots, T_{1\cdots n}^*, K_1^*, \ldots, K_n^*]^T.$$

The Jacobian evaluated at the infection-free equilibrium is a lower triangular matrix; its eigenvalues λ_i, $i = 1, \cdots, 2^n + n$ are:

$$
\begin{aligned}
\lambda_1 &= (1-\varepsilon)^n g - d - \sum_{e=1}^{n} \kappa_e \frac{a_e}{c_e} \\
\lambda_2 &= (1-\varepsilon)^{n-1} g_1 - d - \sum_{e=2}^{n} \kappa_e \frac{a_e}{c_e} \\
\lambda_3 &= (1-\varepsilon)^{n-1} g_2 - d - \sum_{\substack{e=1 \\ e \neq 2}}^{n} \kappa_e \frac{a_e}{c_e} \\
&\vdots \\
\lambda_\cdot &= (1-\varepsilon)^{n-j} g_{i_1 \cdots i_j} - d - \sum_{\substack{e=1 \\ e \neq i_1, \cdots, i_j}}^{n} \kappa_e \frac{a_e}{c_e} \\
&\vdots \\
\lambda_{2^n} &= g_{1 \cdots n} - d \\
\lambda_{2^n+1} &= -c_1 \\
\lambda_{2^n+2} &= -c_2 \\
&\vdots \\
\lambda_{2^n+n} &= -c_n.
\end{aligned}
$$

If $\Re(\lambda_i) < 0$, $\forall i$, we obtain the conditions in Theorem 4.6.

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