This is an appendix to the paper by Korthals Altes & Jansen 2000 Intra-host competition between *nef*-defective escape mutants and wild-type human immunodeficiency virus type 1. *Proc.R.Soc. Lond.* B **267**, 183-189.

Electronic appendices are refereed with the paper. However, no attempt has been made to impose a uniform editorial style on the electronic appendix.

Appendix A: Robustness of the results

1. Model with non-specific immune response.

In principle the non-specific immune response is directed against viral particles in the bloodstream. To make computations easier, we have assumed this immune response removes infected cells, a reasonable assumption, given the fact that steady state of virions is often assumed.

System 1 holds, with the following modifications and additions:

$$\frac{dI_1}{dt} = \beta V_1 T - \delta_I - kE_n I_1 - qEI_1$$

$$\frac{dI_2}{dt} = \beta V_2 T - \delta_I - kE_m I_2 - qEI_2$$

$$\frac{dE}{dt} = p(I_1 + I_2) - \delta E$$

We find:

$$R_0 = \frac{\delta_I + \left(\frac{kna}{\delta_E} + \frac{qp}{\delta}\right)\bar{I}_1}{\delta_I + \left(\frac{kma}{\delta_E} + \frac{qp}{\delta}\right)\bar{I}_1}\frac{p_2}{p_1}, \qquad \text{and } R_0' = \frac{p_1}{p_2}$$

2. Model with proliferation of CD8 cells not only dependent on the number of infected cells, but also on the number of CD8 cells available

System 1 holds, with the following modifications:

$$\frac{dE_m}{dt} = ma(I_1 + I_2)\frac{E_m}{q + E_m} - \delta_E E_m$$

$$\frac{dE_n}{dt} = (maI_2 + naI_1)\frac{E_n}{q + E_n} - \delta_E E_n$$

With this model, we find the following:

$$R_0 = \frac{\delta_I + \left(\frac{kna}{\delta_E}\bar{I}_1 - kq\right)}{\delta_I + \left(\frac{kma}{\delta_E}\bar{I}_1 - kq\right)}\frac{p_2}{p_1}, \qquad \text{and} \qquad R_0' = \frac{p_1}{p_2}$$

We can show for both models that when R_0=1 and p_2=p_1, m=n, and that R_0=1 corresponds to a line of p_2 as a function of m with a positive slope. Consequently, the same pattern of exclusion and coexistence as observed in our model is expected in these modified models, confirming the robustness of our findings.

Appendix B: Number of epitopes as a function of the killing rate

From (5), we obtain the following expression for the critical number of epitopes:

$$\mathrm{m_{crit}} = \frac{\left(\frac{p_2}{p_1}\right)(\delta_E\delta_I + kan\bar{I}_1) - \delta_E\delta_I}{ka\bar{I}_1} \qquad \Leftrightarrow \qquad \mathrm{m_{crit}} = n\left(\frac{\beta p_2\theta - \delta_I}{\beta p_1\theta - \delta_I}\right)$$

We are interested to see whether $\mathrm{m_{crit}}$ increases with increasing k. Thus, we determine the sign of $\frac{d\mathrm{m_{crit}}}{dk}$:

$$\frac{d\mathrm{m_{crit}}}{dk} = \frac{n\beta p_2(\beta p_1\theta - \delta_I) - n\beta p_1(\beta p_2\theta - \delta_I)}{(\beta p_1\theta - \delta_I)^2}\left(\frac{d\theta}{dk}\right) = \frac{n\beta\delta_I(p_1 - p_2)}{(\beta k_1\theta - \delta_I)^2}\left(\frac{d\theta}{dk}\right)$$

$p_1 > p_2$ by definition, so $\frac{d\mathrm{m_{crit}}}{dk}$ has the sign of $\frac{d\theta}{dk}$.

Using the implicit function theorem in equation (3), we find:

$$\frac{d\theta}{dk} = \frac{\lambda an - \delta_T anc\theta}{2\beta^2 p_1^2 \delta_E \theta + \delta_T kanc - \delta_E \delta_I p_1 \beta}$$

Using (3), this can be rewritten as:

$$\frac{d\theta}{dk} = \frac{\delta_E p_1 \beta\theta(\beta p_1 \theta - \delta_I)}{k\left(\beta^2 p_1^2 \delta_E \theta + \frac{\lambda kan}{\theta}\right)},$$ which is positive if the equilibrium (6) exists.

Thus, $\frac{d\mathrm{m}_{\mathrm{crit}}}{dk}$ is positive.

Appendix C: Model 2

In the first model, we focused on the function of Nef in enhancing replication. Here, we proceed to the case in which the crippled form of *nef* also has a reduced ability to activate latently infected cells. Two variables are added: latently infected CD4+ T cells carrying the full-length (L_1) or crippled *nef* genotype (L_2). We also add a couple of parameters: f, the fraction of cells infected going into latent infection, δ_L, the death rate of latently infected cells, and α_1 and α_2, the rates of activation of cells latently infected with full-length and crippled virus respectively. $\alpha_1 \geq \alpha_2$, as the second variant has impaired function. An additional assumption is made here: activation of latently infected CD4+ T cells is solely driven by the virus.

Model 2.

$$\begin{cases} \dfrac{dT}{dt} = \lambda - \delta_T T - \beta(V_1 + V_2)T \\ \dfrac{dI_1}{dt} = (1-f)\beta V_1 T - \delta_I I_1 - kE_n I_1 + \alpha_1 L_1 \\ \dfrac{dI_2}{dt} = (1-f)\beta V_2 T - \delta_I I_2 - kE_m I_2 + \alpha_2 L_2 \\ \dfrac{dV_1}{dt} = p_1 I_1 - cV_1 \\ \dfrac{dV_2}{dt} = p_2 I_2 - cV_2 \\ \dfrac{dE_m}{dt} = ma(I_1 + I_2) - \delta_E E_m \\ \dfrac{dE_n}{dt} = maI_2 + naI_1 - \delta_E E_n \\ \dfrac{dL_1}{dt} = f\beta V_1 T - \delta_L L_1 - \alpha_1 L_1 \\ \dfrac{dL_2}{dt} = f\beta V_2 T - \delta_L L_2 - \alpha_2 L_2 \end{cases} \qquad \text{(i)}$$

The default parameter values are:
$\lambda = 1$, $\delta_T = 0.01$, $\delta_I = 0.5$, $\beta = 0.05$, $k = 0.1$, $p_1 = 100$, $c = 3$, $a = 0.01$, $\delta_E = 0.02$, $p_2 = 75$, but later $p_1 = p_2 = p = 100$, $n = 8$, m varies but $m \leq n$, $\delta_L = 0.01$, $\alpha_1 = 0.001$, $\alpha_2 = 0.00075$ when it does not vary between 0 and α_1, $f = 0.5$.

As for the first model, we investigate the possibilities for the crippled variant to invade a viral population consisting solely of full-length genotypes. Thus, we look at the invasion criterion for a crippled gene in a population of full-length viruses. R_0 can be expressed, as previously, in terms of the life span of the cell infected with crippled virus multiplied by the number of infected cells generated by this infected cell.

A fraction f of cells become latently infected upon infection, a phase which lasts on average $\frac{1}{\delta_L + \alpha_2}$. The remaining fraction (1- f) goes into productive infection, a phase lasting on average $\frac{1}{\delta_I + k\bar{E}_m}$. A fraction α_2 of latent cells then goes into productive infection, where it has the life span given earlier.

We find:

$$R_0 = \left[(1-f)\left(\frac{1}{\delta_I + k\bar{E}_m} \right) + f\left(\frac{\alpha_2}{\delta_L + \alpha_2} \right)\left(\frac{1}{\delta_I + k\bar{E}_m} \right) \right] p_2 \frac{1}{c} \beta \bar{T}$$

$$\Leftrightarrow R_0 = \left[\frac{(1-f)\delta_L + \alpha_2}{\delta_L + \alpha_2} \right]\left(\frac{1}{\delta_I + k\bar{E}_m} \right) p_2 \frac{1}{c} \beta \bar{T} \qquad \text{(ii)}$$

In the equilibrium with only full-length virus, the following relations hold:

$$(1-f)\beta \frac{p_1}{c} \bar{I}_1 \bar{T} + \alpha_1 \bar{L}_1 = (\delta_I + k\bar{E}_n)\bar{I}_1 \qquad \text{(iii)}$$

$$\bar{L}_1 = \frac{f\beta p_1 \bar{I}_1 \bar{T}}{c(\delta_L + \alpha_1)}$$

$$\bar{E}_m = \frac{ma}{\delta_E} \bar{I}_1$$

$$\bar{E}_n = \frac{na}{\delta_E} \bar{I}_1$$

Replacing $\bar{E}_n$ and $\bar{L}_1$ in (iii), and $\bar{T}$ and $\bar{E}_m$ in (ii), we obtain :

$$R_0 = \frac{p_2}{p_1}\left(\frac{(1-f)\delta_L + \alpha_2}{\delta_L + \alpha_2} \right)\left(\frac{\delta_L + \alpha_1}{(1-f)\delta_L + \alpha_1} \right)\left(\frac{\delta_E \delta_I + kan\bar{I}_1}{\delta_E \delta_I + kam\bar{I}_1} \right)$$

The critical number of epitopes can be derived from the invasion boundary:

$$m_{crit} = \frac{1}{ka\bar{I}_1}\left[\frac{p_2}{p_1}\left(\frac{(1-f)\delta_L + \alpha_2}{\delta_L + \alpha_2} \right)\left(\frac{\delta_L + \alpha_1}{(1-f)\delta_L + \alpha_1} \right)(\delta_E \delta_I + kan\bar{I}_1) - \delta_E \delta_I \right] \qquad \text{(iv)}$$

where $\bar{I}_1$ is not a function of α_2 nor m

Figure A1 draws the boundary condition ($R_0 = 1$) as a function of m and α_1, for several values of f, while burst size is the same for the two variants. This figure can be interpreted as

follows: for α_2=7.5x10^{-4} (default value), m≈7 for the curve corresponding to f = 0.5. The same conclusion can be drawn for the second model as for the first model, only the critical values are slightly different. When the crippled form has fewer than 6 epitopes, it is in the majority compared with the wild-type when activation levels are reduced to 75% of the wild-type activation (figure A2(a)). When the defective virus presents two epitopes and has retained more than 25% of its function, it is in the majority (figure A2(b)).

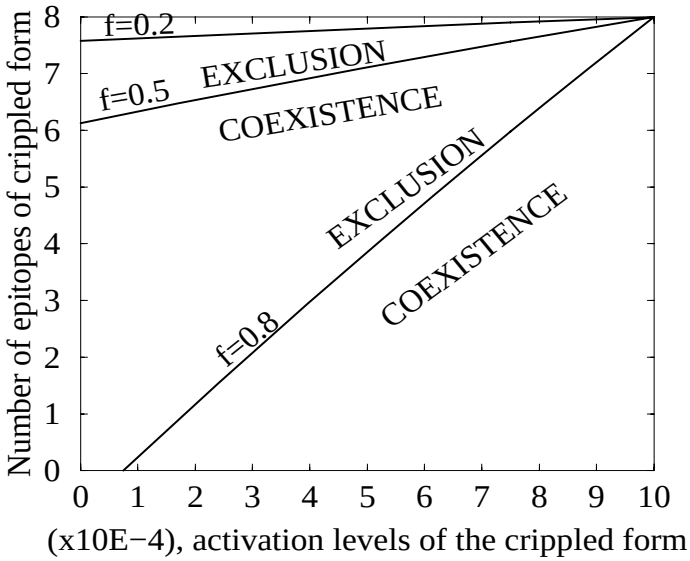

Figure A1. Outcome of the competition between the two genotypes as a function of the number of epitopes and activation levels of cells infected with the defective variant, using model 2 (see (i)). Either the two viral forms coexist ("coexistence") or the full-length form outcompetes the crippled variant ("exclusion"). The boundary is given by the condition R_0 = 1 (equation (iv)). Curves are drawn for different fractions of cells going into productive infection (f = 0.2, 0.5, 0.8).

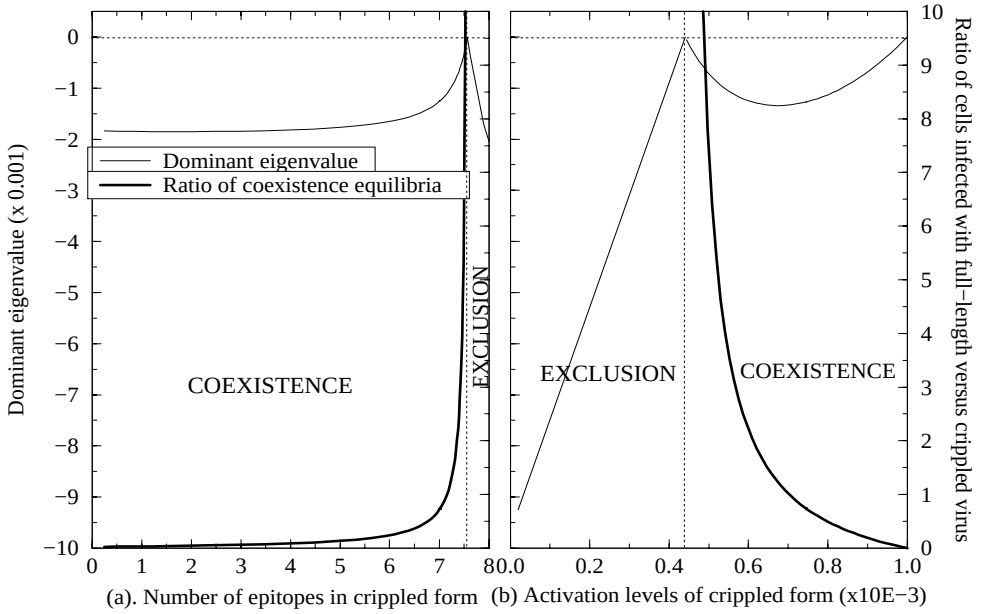

Figure A2. (a). Ratio of cells productively infected with full-length virus over cells productively infected with crippled virus in the coexistence equilibrium, against the number of epitopes in the crippled form (model 2, see (i)); $\alpha_2 = 0.00075$. Dominant eigenvalues are given for both equilibria. (b). Ratio of cells productively infected with full-length virus over cells productively infected with crippled virus in the coexistence equilibrium, against the activation levels of the crippled form (model 2, see (i)); m = 7; f = 0.5.. Dominant eigenvalues are given for both equilibria. In the exclusion steady state dominant eigenvalue and dominant transversal eigenvalue overlap.

Let us look at the effect of the proportion of cells going into productive infection. When f = 0, there is no latent phase, and loss of function has no effect, as there is no disadvantage to low activation levels. Thus, there is always coexistence. As f increases, more cells go into latent state upon infection, and the parameter region in which coexistence occurs becomes smaller, as the loss of function of the crippled form has increasing effect.

We now wish to know what the effect is, more generally, of the latent phase on coexistence of the two viral types. Thus, we look at model 2, but instead of altered activation level, the crippled variant has a reduced replication potential, as in our first model. Figure A3 gives the coexistence and exclusion regions for different levels of latency. When f = 0 (no latency), we find the line shown in figure 1. Figure A3 shows that when the proportion of cells going into productive infection becomes smaller (f increasing), the parameter region in which only the full-length gene can survive becomes larger.

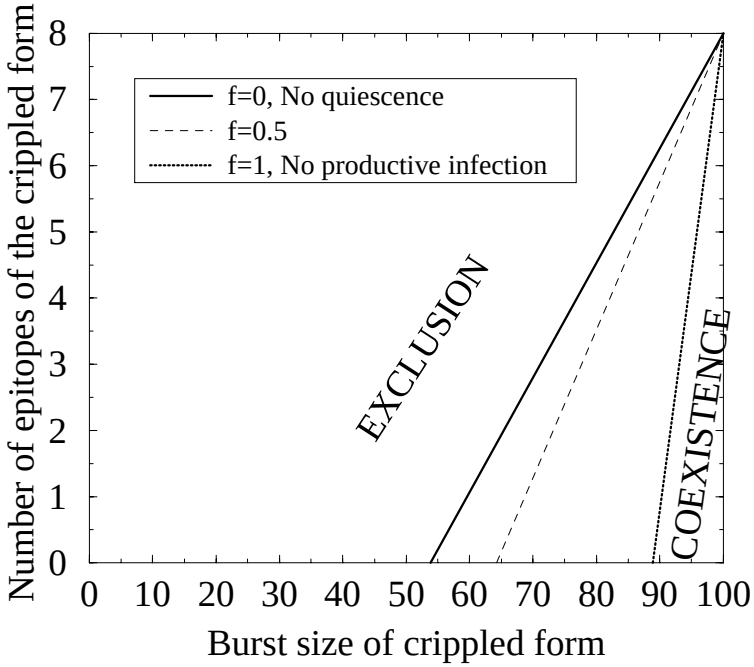

Figure A3. Outcome of the competition between the two genotypes as a function of the number of epitopes and burst size of cells infected with the defective variant, using model 2 (see (i)). Either the two viral forms coexist ("coexistence") or the full-length form outcompetes the crippled variant ("exclusion"). The boundary is given by the condition $R_0 = 1$. Curves are drawn for different fractions of cells going into productive infection (f= 0, 0.5, 1; $\alpha_2 = \alpha_1 = 0.001$).

This is the same as when we look at different activation levels, but the explanation is different. When all cells go into latency before eventually going into productive infection, the pool of productively infected cells is considerably smaller than when all cells immediately go into productive infection. The specific immune response acts on productively infected cells. The presumed advantage of the crippled protein is that it is less susceptible to attack by the immune system. However, this advantage will be reduced when the cells are less exposed to the immune response. Hence, when all cells go into latency before going into productive infection (f = 1), the parameter range for coexistence of the two variants becomes comparatively smaller.

Appendix D: Dominant eigenvalues for the steady states

Figure A4 gives the dominant eigenvalue associated with the equilibria of system 1, for varying immunogenicity (fig. A4(a)) and burst size (fig. A4(b)) of the crippled form. They give an indication of how fast the system regains its equilibrium value after a perturbation. The

characteristic return time, the time needed for the system to return half-way between the perturbation value and the equilibrium value is denoted $t_{1/2}$. We know that $1/2\ I_0 \approx I_0\ e^{\Lambda t1/2}$, where I_0 represents the value of the variables at perturbation, Λ the dominant eigenvalue. So $t_{1/2} = (\ln 2) / (-\Lambda)$.

An indication of how fast the system approaches the exclusion equilibrium is given by the dominant transversal eigenvalue (Hofbauer & Sigmund 1989). This corresponds to the characteristic return time of the mutant virus in a wild-type population.

Finally, the dominant transversal eigenvalue for a wild-type virus in a completely mutant population is given to indicate how fast the wild-type can invade.

Fig. A4 shows that the fastest process is the invasion of a wild-type in a mutant population: when $0<m<8$, $1.98<t_{1/2}<3.15$ days, and when $20<p_2<80$, $0.46<t_{1/2}<6.93$ days. Then comes the process of disappearance of the crippled form in the equilibrium with only wild-type virus: when $4<m<8$, $3.46<t_{1/2}<34.65$ days, and when $0<p_2<60$, $1.13<t_{1/2}<13.81$ days. Coexistence is attained slowest, the dominant eigenvalue having very low absolute value, leading to $t_{1/2} = 63$ days. This pattern seems remarkably robust against changes in parameters.

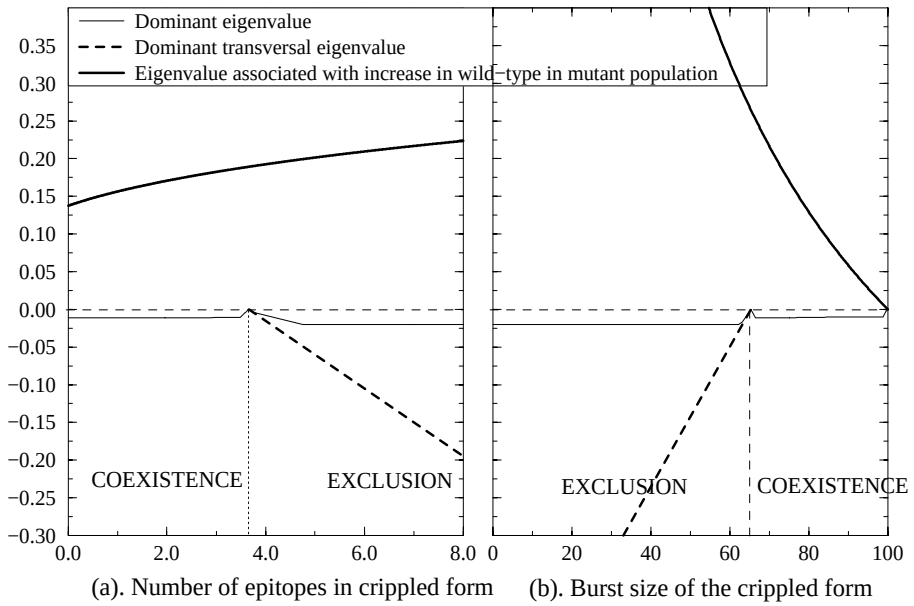

Figure A4. (a). Dominant eigenvalues and dominant transversal eigenvalues of the steady states against the number of epitopes in the crippled form, system (1); $p_2 = 75$. (b). Dominant eigenvalues of the steady states against the burst size of the crippled form (system (1)); $m = 2$. The dominant transversal eigenvalue associated with the rate of change of wild-type virus in a mutant population (bold curve) goes off the scale, and tends to infinity for burst sizes approaching zero.

For model 2, dominant eigenvalues are around -0.001 for the exclusion equilibrium and -0.0018 for coexistence (as a function of m, fig. A2(a)); it is -0.0045 for the exclusion equilibrium as a function of activation level of the defective virus, and -0.0009 for coexistence (fig. A2(b)). Characteristic return times vary on average between 5 months and around 2 years. This slower process is a consequence of the introduction of viral latency in the model, delaying evolution of the system. If a change in the function of Nef is only expressed as a change in activation level, viral latency obviously is the rate-limiting factor. However, if the burst size and activation levels of the defective genotype are decreased in combination, it yields a much faster rate of appearance. We have done these computations, and for a p_2=75 and α_2=0.00075, we found dominant transversal eigenvalues, for varying m, between 0.107 and 0.15, yielding a doubling time between 6.47 and 4.62 days (calculations not shown). Hence, wild-type virus will grow rapidly in a population of only defective variants.

Literature

Hofbauer, J. & Sigmund, K. 1989 On the stabilizing effect of predators and competitors on ecological communities. *J. math. Biol.* **27**, 537-48.