

Intra-host versus inter-host selection: Viral strategies of immune function impairment

(viral evolution/mathematical models)

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ABSTRACT We investigate the evolution of viral strategies to counteract immunological attack. These strategies can be divided into two classes: those that impair the immune response inside or at the surface of a virus-infected cell and those that impair the immune response outside an infected cell. The former strategies confer a “selfish” individual selective advantage for intra-host competition among viruses. The latter strategies confer an “unselfish” selective advantage to the virus population as a group. A mutant, defective in the gene coding for the extracellular immune function-impairment strategy, may be protected from immune attack because the wild-type virus in the same host successfully impairs the host’s immune function. Such “unselfish” defense strategies are neutral with respect to intra-host competition. We present simple models of viral intra-host and combined inter- and intra-host evolution. We show that selfish strategies can evolve by intra-host evolution. Unselfish strategies may evolve if inter-host selection pressures outweigh intra-host selection, suggesting that such strategies can only evolve in viruses with low mutation rates.

The vertebrate immune system has developed a number of different mechanisms to combat virus infections. But in this evolutionary arms race viruses have answered with subtle countermeasures.

Adenoviruses, for example, are endowed with several countermeasures against the different arms of the immune response. They obstruct the interferon (IFN)-mediated inhibition of translation of double-stranded RNA in infected cells (1). Adenoviruses also encode three different proteins (2) that prevent cytolysis of virus-infected cells by tumor necrosis factor (TNF). TNF is a cytokine that is secreted by activated macrophages and lymphocytes and exerts several antiviral activities including cytotoxic effects on virus-infected cells. The detailed mechanism by which the viral proteins protect against TNF are not yet fully understood, but all three proteins act after the binding of TNF to its receptor on the infected cell. Another line of defense of human adenoviruses is a protein that reduces the cytolysis of infected cells by the cytotoxic T cells (2). These T cells specifically recognize and destroy virus-infected cells, which present viral peptides on their surface. The effect of the viral defense protein is to impair the translocation of viral peptides from the cytoplasm to the cell surface and, therefore, reduce the T-cell recognition of infected cells.

Poxviruses have also evolved several defense mechanisms against the immune responses. Vaccinia virus codes for a protein that inhibits complement function. The complement system is involved in the control of inflammation. When activated spontaneously by microorganisms (alternative pathway) or by antibody (classical pathway), the complement system leads to the lysis or phagocytosis of free virus or

virus-infected cells. Vaccinia virus inhibits the classical pathway of complement activation (3). Both cowpox and vaccinia virus developed proteins that interfere with interleukin 1 (4–6), a cytokine involved in a variety of inflammatory and immunological responses. Shope fibroma virus produces a soluble TNF receptor, which acts as a decoy, binding TNF before it reaches the infected target cell (7). Similarly, myxoma virus secretes IFN- γ receptors (8).

All these viral defense strategies seem advantageous for the defense against the immune responses, but there is an important difference between the adenovirus and the poxvirus strategies listed above. Although the defense mechanisms of adenoviruses all occur inside or on the surface of the infected cell, the above listed proteins of the poxvirus family act outside the infected cell. In order to understand this crucial difference, consider the following situation: During an ongoing infection a new mutant arises, which is defective in the gene coding for the viral defense mechanism. In the case of the adenovirus strategies, which occur inside the infected cells, such a defective mutant is inferior to the wild type because it lacks the protective viral protein. For the poxviruses strategies, however, where viral defense occurs outside the infected cell, a defective mutant can profit from the protective viral proteins produced by the wild type as much as the wild type itself. We therefore propose to call those viral defense mechanisms that confer an individual selective advantage to their carrier, “selfish,” and those that confer an unspecific selective advantage for the whole virus population, “unselfish.” Thus, virus-encoded defense proteins that are not secreted and act only inside (or at the surface) of the infected cell are selfish strategies. Virus-encoded defense proteins that are secreted and act outside the cell are unselfish strategies. More examples for selfish and unselfish viral countermeasures against the immune responses are listed in Tables 1 and 2, respectively (for reviews, see refs. 9–11).

The evolution of selfish viral defense mechanisms can be easily explained by intra-host selection. The evolution of unselfish strategies, however, is more intricate, as they do not equip an individual virus with a selective advantage within a host. Here we address the questions: How can unselfish strategies evolve in the first place, and how can they be maintained in the virus population?

Intra-host competition cannot account for their evolution. But we show that unselfish viral strategies may be favored by inter-host competition if they confer a significant transmission advantage to all virus strains in a particular host.

This paper is about viral evolution. We investigate the evolutionary dynamics that govern intra- and inter-host competition. We outline which viral defense mechanisms can arise due to intra-host evolution, and we show that these are the mechanisms termed selfish above. We also explore circumstances under which inter-host selection can lead to the evolution and maintenance of unselfish defense mecha-

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Abbreviations: TNF, tumor necrosis factor; HIV, human immunodeficiency virus; IFN, interferon.

nisms. We show that intra-host competition need not maximize total virus loads. The section on intra-host versus inter-host evolution combines intra-host and inter-host competition and explains how unselfish strategies might have evolved.

INTRA-HOST EVOLUTION

In this section we focus on a simple model of intra-host evolution. Consider two virus mutants replicating in a single host. The two mutants differ in their replication rates and immunological properties, but they induce and are recognized by the same immune responses:

$$\frac{dv_1}{dt} = (r_1 - s_1z)v_1 \quad [1]$$

$$\frac{dv_2}{dt} = (r_2 - s_2z)v_2 \quad [2]$$

$$\frac{dz}{dt} = k_1v_1 + k_2v_2 - (u_1v_1 + u_2v_2)z - bz. \quad [3]$$

The variables v_1 and v_2 denote the population size of virus strains 1 and 2; the variable z represents the immune response against both strains. In the absence of an immune response, both virus strains grow exponentially with replication rates r_1 and r_2 . The terms $-s_1z$ and $-s_2z$ account for the killing of virus by the immune response, where s_1 and s_2 are the respective parameters for the strain-specific susceptibility to immune destruction. The growth rate of the immune response is stimulated at a rate proportional to the amount of each variant, $k_1v_1 + k_2v_2$, where the rate constants k_1 and k_2 specify the magnitude of immune response stimulation. The terms $-u_1v_1$ and $-u_2v_2$ represent virus-induced impairment of the immune responses. The natural decay rate of the immune response is given by the term bz .

In absence of strain 2, the system converges to the equilibrium

$$\hat{v}_1 = br_1/(s_1k_1 - r_1u_1) \quad \text{and} \quad \hat{z} = r_1/s_1, \quad [4]$$

if $s_1k_1 > r_1u_1$. Strain 1 cannot be regulated by the immune response and grows exponentially, if $s_1k_1 < r_1u_1$. From Eqs. 1 and 2 we see that both strains cannot coexist in equilibrium (except in the nongeneric case $r_2/s_2 = r_1/s_1$). Strain 2 can invade and outcompete strain 1 if

$$r_2/s_2 > r_1/s_1. \quad [5]$$

From this simple model we can make two important points.

(i) Selection does not simply favor the fastest replicating strain, but the strain with the largest ratio of replication rate r to immune recognition s . A mutant could be superior to its wild type by reducing the efficiency of a gene involved in replication if it gets, in turn, an advantage by decreased immune recognition (for example, by losing an epitope).

(ii) The selection of a new mutant is independent of the viral parameters determining the growth rate of the immune response k_1 and k_2 or u_1 and u_2 . Hence, a new mutant is selected regardless of its effect on the immune response. An interesting phenomenon can be observed. Strain 2 may be inferior to strain 1 when fighting alone against the immunological attack but may, nevertheless, outcompete strain 1. In mathematical terms, a mutant strain 2 that fulfills $r_2/s_2 > r_1/s_1$ outcompetes strain 1, but if additionally its immunological parameters k_2 and u_2 are such that $r_2/(s_2k_2 - r_2u_2) < r_1/(s_1k_1 - r_1u_1)$, then strain 2 eventually establishes a lower viral load

than strain 1 (see Eq. 4). Hence, intra-host selection need not maximize viral loads. Fig. 1 illustrates this situation.

At this point we want to give some justification for the simplifications in our model. (i) The competition criterion (see inequality 5) depends only on Eqs. 1 and 2. Therefore, the particular form of the dynamics of the immune response z does not determine selection. (In Eq. 3 we chose the simplest reasonable form.) (ii) The above model does not distinguish between humoral and cellular immune responses, nor does it include strain-specific immune responses. We checked that an extension of the model along these lines leads to qualitatively similar results.

The strategies of selfish immune function impairment listed in Table 1 can be divided into two classes: those that increase replication rate r and those that decrease immune recognition s . The mechanisms of disruption of IFN function found in adeno-, pox-, herpes-, retro-, orthomyxo-, and reoviruses correspond to an increased replication rate r . These viruses produce proteins that prevent the IFN-induced inhibition of translation of viral RNA. A mutant, blocking IFN function, should therefore have an evolutionary advantage within a host due to an increased replication rate. On the other hand, the reduction of the presentation of viral antigens on the surface of an infected cell corresponds to a decrease in the parameter for immunological killing, s . Similarly, the evasion from the cytolytic effects of TNF corresponds to a decrease in the parameter s . From inequality 5 we see that increased r or reduced s confers an advantage for intra-host competition. Thus intra-host competition can account for the evolution of selfish strategies of immune function impairment.

The unselfish strategies listed in Table 2 all obstruct immune responses outside the infected cells. A mutant with such a strategy has an increased negative effect u on the immune responses. As inequality 5 shows, intra-host competition does not depend on u and, hence, such a mutant does not have a selective advantage within a host.

Our model does not consider spatial heterogeneity, which can, in principle, facilitate the evolution of unselfish strategies within a host (25). Nevertheless, for the maintenance of unselfish strategies the mutation rate has to be sufficiently low to guarantee that the viral populations at particular sites in the tissue are mainly clonal.

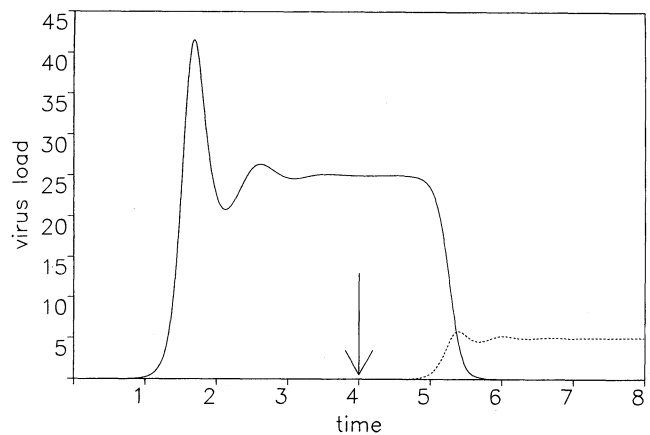


FIG. 1. A counterintuitive example of intra-host competition. The virus load is shown as a function of time (in arbitrary units). The solid line and the dotted line represent strain 1 and strain 2, respectively. Strain 1 is introduced at time $t = 0$ (the beginning of the infection), induces an initial peak of viremia, and is then regulated to (persistent) equilibrium. Strain 2 is introduced at time $t = 4.0$. Strain 2 outperforms strain 1 but, nevertheless, establishes a smaller viral load. Eqs. 1–3 have been integrated with the following parameters: $r_1 = 10$, $s_1 = 2$, $k_1 = 1$, $u_1 = 0$, $r_2 = 20$, $s_2 = 2$, $k_2 = 10$, $u_2 = 0$, and $d = 5$. Strain 2 can establish arbitrary low viral loads, depending on the choice of the parameters.

Table 1. Selfish viral strategies to counteract immunological attack

| Family | Virus | Viral action | Ref. |
|------------------|---------------------|--|------|
| Adenoviridae | | Obstruction of double-stranded RNA translation inhibition of IFNs | 1 |
| | | Escape from cytotoxic T cells by translocation inhibition of viral antigens on cell surface | 2 |
| | | Production of three different proteins that interfere with TNF after its binding to the receptor on cell surface | 2 |
| Poxviridae | Vaccinia | Inhibition of IFN function similar to adenoviruses | 12 |
| | Vaccinia | Interference with the presentation of viral antigens | 13 |
| Herpesviridae | Epstein-Barr virus | Impairment of IFN function similar to adenoviruses | 14 |
| | Cytomegalovirus | Inhibition of antigen presentation to cytotoxic T cells | 15 |
| | Herpes simplex I/II | Reduced recognition by cytotoxic T cells | 16 |
| Retroviridae | HIV | Inhibition of IFN function similar to adenoviruses | 17 |
| Orthomyxoviridae | Influenza | Inhibition of IFN function similar to adenoviruses | 18 |
| Reoviridae | Reovirus type 1 | Inhibition of IFN function similar to adenoviruses | 19 |

All viral proteins act inside or at the surface of the infected cell, indicating selfish immune function impairment.

INTRA-HOST VERSUS INTER-HOST EVOLUTION

In the following we investigate how genes coding for unselfish immune function impairment can evolve and be maintained in the interplay between inter- and intra-host selection. Let S be the density of susceptible hosts. There are two different viral infections: I_1 denotes hosts infected by a strain that has lost its ability of unselfish immune function impairment; I_2 are hosts infected by a strain that has retained its mechanism of unselfish immune function impairment. The simplest model combining inter-host and intra-host selection is as follows:

$$\frac{dS}{dt} = a - hS - b_1 I_1 S - b_2 I_2 S \quad [6]$$

$$\frac{dI_1}{dt} = b_1 I_1 S - (h + e_1) I_1 + q I_2 \quad [7]$$

$$\frac{dI_2}{dt} = b_2 I_2 S - (h + e_2) I_2 - q I_2. \quad [8]$$

Susceptible hosts immigrate (or are born) at a constant rate a . Their natural death rate is given by h . Susceptibles can be infected either with strain 1 or 2, the transmission rates being b_1 and b_2 . Infected hosts have a natural and a disease-induced death rate given by $h + e_1$ and $h + e_2$, respectively. Equivalently e_1 and e_2 can also account for the recovery and subsequent immunity of hosts (in which case immunity is assumed to be cross-reactive between the two viral strains). We assume that strain 2 with its ability to interfere with the immune response enjoys an advantage for inter-host selection—i.e., $b_2/(h + e_2) > b_1/(h + e_1)$. The natural assumption is that it has an increased transmission rate, hence $b_2 > b_1$.

But the above condition can also be fulfilled if $e_2 < e_1$. This can be interpreted in two ways: strain 2 leads to a longer infectious period because of a lower rate of recovery (and subsequent immunity against infection) or because of a lower disease-induced death rate (which seems likely only in virus infections, where the immune response is the cause of pathogenic effects).

The parameter q represents the rate at which infections initially dominated by strain 2 are taken over by strain 1. During an infection by strain 2 errors in viral replication will produce a large number of mutants, which are defective in the gene coding for unselfish immune function impairment. Such defective mutants are *neutral* on the level of intra-host competition. Their fixation occurs by neutral drift, and the rate of fixation will be (roughly) equivalent to the mutation rate. The drift in the opposite direction—namely, from a defective to a functioning unselfish gene—will be much less likely and can be neglected in first approximation. (There are many more ways of disrupting a gene function than restoring one.)

Strain 2 can be maintained by inter-host selection if

$$q < q_c = (h + e_1) \frac{b_2}{b_1} - (h + e_2). \quad [9]$$

This equation defines a threshold value for the maximal mutation rate q_c , at which unselfish strategies of immune function impairment can be maintained in a virus population by inter-host competition.

Inequality 9 can be read in a different way. For a given mutation rate q , a new mutant with unselfish immune function impairment (strain 2) has to come up with a transmission advantage, satisfying

Table 2. Unselfish viral strategies to counteract immunological attack

| Family | Virus | Viral action | Ref. |
|---------------|---------------------|---|------|
| Poxviridae | | Secreted viral protein inhibits interleukin I function | 4, 5 |
| | Cowpox | Suppression of interleukin $I\beta$ response | 6 |
| | Vaccinia | Viral protein inhibits the antibody-mediated complement function | 3 |
| | Shope fibroma | Virus produces soluble TNF receptors acting as a decoy for TNF | 7 |
| | Myxoma | Virus produces soluble IFN- γ receptor as a decoy for IFN- γ | 8 |
| Herpesviridae | Herpes simplex I/II | Inhibition of classical and alternative pathway complement activation | 20 |
| | Herpes saimiri | Inhibition of alternative pathway of complement activation | 21 |
| | Epstein-Barr virus | Interference with complement components | 22 |
| | Epstein-Barr virus | Virus produces homolog of interleukin 10, which inhibits cytokine synthesis | 23 |
| Retroviridae | HIV I/II | Virus induces killing of CD4 helper cells | 24 |

All viral proteins act outside the infected cell. In the case of HIV, CD4 helper cells are depleted, regardless of what antigen they are primed for. This result suggests that these mechanisms against immunological attack help their producers as much as their competitors and are therefore unselfish.

$$b_2 > b_1 \left(1 + \frac{e_2 - e_1 + q}{h + e_1} \right), \quad [10]$$

in order to persist in competition with a mutant (strain 1), which does not impair immune function. The evolution of genes coding for unselfish immune function impairment cannot go over intermediate transmission advantages. An unselfish mutant has to overcome this evolutionary hurdle (inequality 10) in a single mutation event. Therefore, viruses with low mutation rates cannot only maintain unselfish genes better, but they can also evolve unselfish genes easier because viruses with low mutation rates have to overcome a smaller evolutionary hurdle.

Once a gene coding for unselfish immune function impairment has been established in a viral genome, further optimization may proceed in small steps. Competition between two unselfish virus mutants will always favor the one with the larger transmission advantage. Fig. 2 shows a computer simulation of a simple extension of the above model.

Finally, we discuss the origin of unselfish mutants. How can single infections arise initially, which are dominated by unselfish mutants? We propose three possibilities: (i) neutral drift in an infected host may by chance favor the unselfish mutants; (ii) an unselfish strain is by chance the only one surviving a transmission event; and (iii) the gene for unselfish immune function impairment can "hitch-hike" on a second mutation that confers an intra-host selective advantage. All these pathways have a small likelihood and will occur only rarely but may be sufficient to lead occasionally to single hosts predominantly infected with the unselfish strain. From

these initial conditions the evolutionary dynamics (Eqs. 6–8) then start to work.

DISCUSSION

Competition between viral strains occurs on two different levels: within a single host and within the host population. We showed that intra-host evolution leads to an increased ratio of replication rate over immune recognition by cross-reactive responses. Viral strategies of immune function impairment that act inside or at the surface of an infected cell confer a selfish selective advantage within a host and evolve by intra-host selection. Viral mechanisms that act outside an infected cell are selectively neutral for intra-host competition. Therefore intra-host selection alone cannot account for their evolution. We showed that inter-host competition can optimize such unselfish mechanisms of immune function impairment only in viruses with low mutation rates. We derived a threshold for the mutation rate, above which viruses are not able to maintain genes coding for unselfish properties. Hence examples for unselfish strategies of immune function impairment should only be found in viruses with low mutation rates.

In Tables 1 and 2 we compiled a list of selfish and unselfish strategies of immune function impairment. The viruses using unselfish strategies belong to three families: the poxviridae, the herpesviridae, and the retroviridae. Pox- and herpesviruses are DNA viruses. Retroviruses are RNA viruses. As a rule, RNA viruses have much higher mutation rates than DNA viruses. Thus intra-host competition should be more important in the evolution of RNA viruses than in the evolution of DNA viruses. The literature on mutation frequencies of DNA viruses is sparse, but there are other lines of evidence that pox- and herpesviruses have low mutation rates even compared with other DNA viruses. (i) Herpesviruses encode at least one DNA-repair enzyme (26). (ii) Pox- and herpesviruses are among the largest known DNA viruses, and genome size has been shown to correlate strongly with mutation rate (27).

Human immunodeficiency virus (HIV) induces a general immune function impairment, which falls in the unselfish category because it does not appear to confer a specific advantage to specific HIV variants but confers advantage to the whole HIV population in a host. But HIV does not seem to fulfill the requirements for the evolution of an unselfish strategy of immune function impairment. It has a very high mutation rate. There is a large turnover of different genetic material infection (see, for example, refs. 28–30). This makes it unlikely that HIV could have evolved an unselfish strategy of immune function impairment. There is an alternative explanation: HIV replicates in CD4-positive T-helper cells and kills them. But these cells are vital for an efficient immune system in general. Thus, HIV replication and immune function disorder go hand in hand. We presume that the impairment of the immune system in HIV infection is a side-product of intra-host evolution for faster replication. Hence, the virulence of HIV may be determined by intra-host selection and may not represent an evolutionary adaptation to inter-host competition.

Examples for selfish viral strategies, which interfere with the immune responses can be found in DNA viruses (adeno-, pox-, and herpesviruses) and in RNA viruses (orthomyxo-, reo-, and retroviruses) of largely different size and mutation rate. As we have shown, selfish immune function impairment may evolve by intra-host selection alone, regardless of mutation frequencies. More generally, selfish strategies may be favored by both intra- and inter-host selection, whereas unselfish strategies can only evolve by inter-host selection.

The evolution of viral genes for unselfish immune function impairment sheds light on other issues of viral evolution.

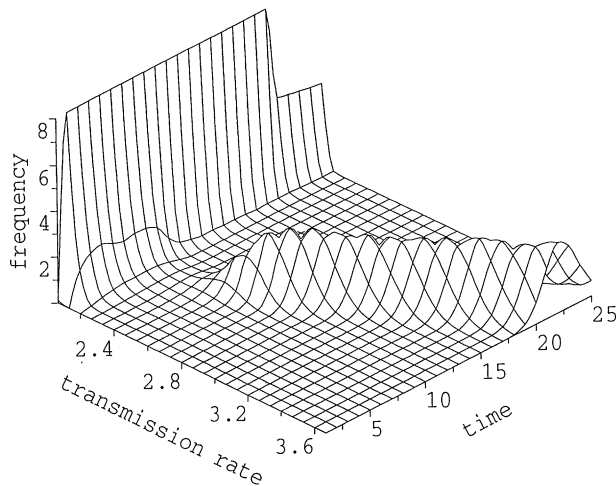


FIG. 2. A computer simulation of inter-host versus intra-host evolution, which shows the "evolutionary hurdle." Eqs. 6–8 were extended to: $\dot{S} = a - hS - S \sum_{i=0}^N (c + ib) I_i$, $\dot{I}_0 = cI_0S - hI_0 + q \sum_{i=1}^N I_i$, $\dot{I}_i = (c + ib) I_i S - hI_i - qI_i$. All hosts are assumed to die at the same rate h . I_0 denotes hosts predominantly infected by a virus without immune function impairment, I_1 to I_N denotes hosts infected by virus with increased unselfish immune function impairment; the transmissibility of I_i infections is given by $c + ib$. I_1 to I_N infections can lose their immune function-impairment mechanisms by random drift. This happens at rate q . Rare mutational events may produce unselfish virus infections with increased transmissibility. This corresponds to transitions $I_i \rightarrow I_{i+1}$, which are modeled stochastically. Along the x axis the virus strains are ordered by increased transmissibility $c + ib$. The z axis gives their frequency in the host population, and the y axis represents the time (in arbitrary units). The simulation is started with the I_0 infections only ($I_0 = I, I_i = 0$). At time $t \approx 8$ the first strain overcomes the evolutionary hurdle given by inequality 9. From then on inter-host selection optimizes immune response impairment in small steps (parameters: $a = 100$, $h = 5$, $q = 1/2$, $c = 2$, $b = 2/30$, and $N = 30$).

Theoretical considerations of the evolution of virulence are often based on the optimization of transmission between hosts, assuming some correlation between virulence and transmissibility (31–33). Obviously, transmissibility as such is a selectively neutral property in intra-host evolution. The presence of viral mechanisms for unselfish immune function impairment indicates whether one can expect other viral properties to be optimized that are neutral with respect to intra-host evolution.

Quite obviously, intra-host evolution drives viruses toward an increased ratio of replication rate over immune recognition, regardless of effects on the host or the transmission rate. Highly mutating viruses might therefore end up at a degree of virulence that is larger than optimal for their transmission (34). More surprisingly, this intra-host competition does not necessarily lead to higher viral burden. Strains establishing high viral loads can lose in competition with strains that are suppressed to lower levels because intra-host selection depends only on replication rate and immune recognition but not on the parameters that determine the stimulation of cross-reactive immune response. It is conceivable that slow replicating (less pathogenic) strains could outcompete fast-replicating (more pathogenic) strains within a host (35). This may provide a new perspective for postexposure treatment of viral infections.

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