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A mechanism of immune escape by slow-replicating HIV strains

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Abstract

Strains of HIV differ greatly in their ability to replicate in T4 cells. Fast-replicating strains are observed during the early and late stages of HIV infection, while slow-replicating strains prevail during the intermediate, latent, stage. The prevalence of slow-replicating strains has been attributed to these strains' ability to escape the immune response. However, how these strains are able to avoid being eliminated by an immune response for a period of several years has not been explained. Recent experiments indicate that HIV may be selectively transferred from infected macrophages to T4 cells specific for HIV antigens. Thus HIV may preferentially infect those T4 necessary for generating a protective immune response. To determine the conditions under which an HIV-specific immune response can be blocked, we have developed a mathematical model incorporating the process of viral transfer from infected macrophages to HIV-specific T4 cells along with the known processes of macrophage-T4 interaction, immune stimulation, and viral infection of T4 cells and macrophages. Our model shows that the mechanism of viral transfer to HIV-specific T4 cells can allow slow-replicating strains of HIV to escape immune response, under conditions in which an immune response occurs against fast-replicating strains. The model also suggests that in addition to being slow/low the ability to reduce or block T cell activation may be an important characteristic of escape mutants.

The persistence of HIV infection through the long latency period of AIDS[1] demonstrates HIV's ability to avoid being eliminated by the host immune response. This is
an ability characteristic of the lentivirus family[2], to which HIV belongs. In the case of HIV, the necessity of designing effective immunization strategies makes it imperative to understand the virus' specific mechanisms of immune evasion.

Immune evasion has frequently been attributed to two viral mechanisms: latent cellular infection, and rapid mutation. As a retrovirus, HIV can persist integrated in a cell’s genome, in a completely latent fashion[3]; since latently infected cells display no viral antigens, they will not be removed by an immune response. It has been suggested that AIDS latency is a period of predominantly latent and low-level cellular infection[1]. However, more recent data indicates that substantial populations of free virus and actively infected T4 cells may be present during the latent period [4, 5]. A second possible mechanism of immune evasion is the virus’ rapid mutation, which allows mutational drift of its displayed antigens away from those targeted by the immune response [6, 7, 8].

Many strains of HIV-1 have been isolated [9, 10, 11], and new strains appear to be arising by mutation in infected individuals[12]. Strains of HIV-1 differ both in their antigens[9] and in their activity. Differences in activity among HIV strains include differences in the level of their replication in T4 cells, in their ability to infect macrophages [13], and in whether they induce syncytia formation [14]. With regard to replication in T4 cells, strains of HIV appear to fall into two main classes: “rapid/high” viral strains, similar to the initial LAV/HTLV-III isolate, which replicate rapidly and show high expression in many laboratory lines of T4 cells; and “slow/low” viral strains, which replicated poorly, if at all, in vitro T4 systems [15, 16].

The ability of HIV isolates to replicate in T4 cell cultures is correlated with the stage of the infection of the patient from which they were isolated. Virus isolated from patients in the earliest stages of infection may be of either a rapid/high or slow/low strain[17, 18, 19], the type probably being determined by the strain of virus in the infectious inoculum. In many patients, the earliest clinical manifestation of infection is a flu- or mononucleosis-like period of viremia occurring about two weeks after the infective event [20, 17, 18]. If an initial viremia occurs, it is soon followed by an asymptomatic latency period, lasting several years. Soon after patients enter this stage, HIV isolates are of the low replicating type [21]. Slow/low strains dominate isolates from patients through most of the latent phase of the disease, with rapid/high strains appearing in isolates as or shortly before patients move into the ARC and AIDS phases [22, 23, 24]. After seroconversion, which may occur during the initial viremia [25], patients often produce effective neutralizing antibody against many strains of HIV [26]. Neutralizing
antibody specific for strains present in the initial infection may appear rapidly [21]. However, early in the latency period, variant, slow/low strains may appear, to which the patients do not produce effective neutralizing antibody [21]. This may suggest some immune-evading mechanism by these strains of virus [21].

An experimental infection of chimpanzees with HIV-1, which allows a detailed analysis of viral strains and the immune response against them, showed a similar pattern for the early stages: the first viral isolates from the animals following infection were rapid/high, similar to the virus in the inoculum, but later isolates were slow/low[7, 27]. Significant antigenic variation occurred between the first and the later isolates, so that antibody produced by the chimpanzees, which was effective at neutralizing the viral strains of the inoculum and the initial isolates, was relatively ineffective at neutralizing virus from the later isolates.

Nara and Goudsmit [7], and Miedema et al. [19] have incorporated these observations into models of HIV infection in which latency is the manifestation of a dynamic process, rather than being a static period of hidden cell infection. In these models, high-replicating strains may be present in the initial infection, but these strains are rapidly suppressed by an effective initial immune response. Before these or other initial strains are eliminated, antigenically distinct “escape mutant” strains are postulated to arise. The population of the mutant strains is thought to grow slowly over the several years of latency. In the Miedema model, the cytopathicity of the mutant strains causes a slow depletion of the T4 population, and particularly of the memory T4 cells, that eventually leads to a loss of immune function. In both models, as immune function is lost, high-replicating strains reappear, since the immune response no longer holds them in check.

The persistent survival of slow/low strains during a period in which the body’s immune system is still functional, along with the observed failure of the body to produce effective antibodies specifically against these emergent strains, points to some mechanism of immune evasion by the slow/low strains. Miedema et al.[19] suggest that simple low viral expression and monocyte tropism limit the antigenemia to a level too low to induce a full immune response. In the Nara model [7], continued mutational variation of the V3 loop, along with relatively low viral presence, keeps the immune response at a low level. In both cases low viral- and antigen presence are essential for the virus' avoidance of the immune response. These hypotheses encounter the same problem as the early, static theory of latency: the level of viral expression during the latent phase need not be notably low. This is a particular problem if the decline of the T4 population
during the latency period is attributed to direct viral action; considering the large size of the T4 population, this implies a substantial viral presence.

Nowak et al. [8] have presented a different dynamical model of the latency period. In this model, mutant strains of virus continually arise during latency; the immune system responds to each strain as it arises, keeping it in check. However, all strains are assumed to be able to kill T4 cells. At some point, due to T4 cell depletion, the system is unable to respond effectively to newly arising strains, and active disease rapidly follows. However, this model cannot explain the observed failure of the system to respond to mutant strains arising early in latency.

An alternative possibility, which we consider here, is that the slow/low strains avoid immune destruction by interfering with the immune system. It has been suggested that the recruitment of HIV-specific T4 cells to sites of anti-HIV immune reaction could lead to the “suicide” of these cells, since they become subject to HIV infection in these sites[28]. Recent experiments by Mann et al. [29] and by Manca et al. [30] point to a more specific mechanism by which the virus may target HIV-specific T4 cells, and in this way block the immune response against itself. This blocking would be strain-specific, resulting in a natural process of kin selection by which such activity could evolve. The effectiveness of immune blocking will depend on the rates of cell infection and the processes involved in generating an immune response. In this paper we present a mathematical model of these processes that illustrates the principle that slow/low strains can evade an immune response under conditions where a rapid/high strain will generate a response.

1 Background: transfer of HIV from infected macrophages to antigen-recognizing T4 cells

T cells only recognize antigen when it is presented by an antigen-presenting cell (APC). While B cells are important as APCs in secondary immune response, the predominant APC in primary immune responses is the macrophage. The intimate association of T4 cells and macrophages, coupled with the fact that HIV-infected macrophages produce new virus in a steady, noncytopathic manner, suggests the possibility of HIV transfer from macrophages to T4 cells[31]. In vitro experiments by Mann et al. [29] and Manca et al. [30] indicate that infected macrophages retain the ability to present antigen and that HIV may be transferred directly from infected macrophages to T4 cells. Both
groups observed a selective disappearance of subsequent T cell responsiveness to the antigens presented by the macrophages, indicating that T4 cells recognizing antigens presented by HIV infected macrophages were specifically deleted.

Mann et al. also obtained evidence that infected macrophages naturally present the antigens of the infecting HIV virus in association with MHC class II. Presentation of HIV antigen to T4 cells by HIV infected macrophages points to the significant possibility that HIV may selectively infect, and remove, T4 cells specific for HIV antigens. By selectively eliminating T4 cells specific for its own antigens, a strain of HIV could potentially escape generating an immune response.

Whether escape occurs is a complex matter, depending on a number of viral and immune system factors. In order to escape an immune response, a strain of HIV would have to infect and reduce the population of viral-specific T4 cells to a level too low to initiate an immune response before a response is triggered against the virus. Thus, the possibility of viral immune escape by selective T4 cell destruction involves a competition between different dynamical processes. An analysis of the possible outcomes is given below.

2 Model: Interaction of cell infection, antigen presentation, and stimulation of immune response

Figure 1 schematically presents a model of HIV infection of macrophages and T4 cells. This model is based on the following assumptions:

Cell infection. Both T4 cells and macrophages may be infected by free virus. T4 cells which are specific for viral antigen may become infected in the process of recognizing antigen presented by an infected macrophage [29, 30]. After a delay, \( \tau \), of a few days, infected macrophages are assumed to begin releasing new virus at a steady rate. Also after a delay, infected T4 cells are assumed to produce \( N \) new virus that kill the cell and are then released. The number \( N \) of new virus produced will vary with the strain of virus. We ignore the possibility of latent infection in T cells and cell-to-cell transmission of the virus so as to focus on the more active mechanism of immune escape via depletion of HIV-specific T4 cells.

Antigen uptake, presentation, and immune stimulation. Antigenic material is shed by free virus and released from infected T4 cells undergoing lysis. This antigenic mate-
rial, along with whole virus, is taken up by macrophages, which then become antigen-presenting. Infected macrophages may also present HIV antigens.

Recognition of antigen presented by an APC leads to T4 cell activation, and production of cytokines. If there is a sufficient number of such antigen-recognition events per unit volume, the accumulation of IL-2 and other cytokines can lead to T4 division, the generation of help for B-cell activation, cytotoxic T cell activity and other processes of the immune response. We incorporate two aspects of this response process in our model. First, we suppose that the recognition of presented antigen by a T4 cell is the basic unit of immune stimulation; we thus define a quantity $S$, the effective rate of such events. Second, we suppose that as long as this rate is less than a critical value $S_c$, there will be insufficient accumulation of IL-2 and other cytokines to sustain the response process, and thus no specific immune response will occur. For a normal immune response, $S$ is given by

$$S = k_s \bar{M} \bar{T},$$

where $k_s$ is a constant determining the rate at which T4 cells encounter antigen-presenting macrophages and become stimulated, and $\bar{M}$, $\bar{T}$, are the populations of HIV antigen-presenting macrophages and antigen-specific T4 cells, respectively.

By our hypothesis, T4 cells may also recognize antigen presented by infected macrophages, $\bar{M}^*$, and become HIV infected in the course of recognizing antigen. Viral action may then affect the T4 cells' participation in immune response processes. Including antigen presentation by infected macrophages, $S$ becomes

$$S = k_s \bar{M} \bar{T} + k_s p_p (1 - p_t p_b) \bar{M}^* \bar{T}. \quad (2)$$

The contribution of antigen-presentation events by infected macrophages $\bar{M}^*$ is reduced by $p_p$, the probability that infected macrophages present viral antigens, and by a factor $1 - p_t p_b$, meant to model the probability that T cell function is not inhibited after encounter with an infected macrophage. Here $p_t$ is the probability that HIV is transferred to and infects an antigen-recognizing T4 cell during antigen-presentation, and $p_b$ is the probability that the immune activity of the infected T4 cell is blocked. The blocking probability $p_b$ may be interpreted as the fractional loss of normal immune activity, e.g., the fractional reduction in production of IL-2, by a population of infected T4 cells compared to the same number of uninfected cells.

**Factors affecting viral immune escape.**

As this model contains none of the processes of the immune response, except the initial stimulation of T4 cells, the model can describe only the period of viral growth
preceding an effective immune response. Thus our solution of the equations of the model terminates either when the immune stimulus $S$ reaches the critical level $S_c$, at which point an immune response will have been initiated, or when the population of HIV-specific T4 cells has been reduced to say 0.1% of its initial value, and thus effectively eliminated. At this point we assume that the strain of virus will have escaped, or will shortly escape, an immune response.

The ability of a strain of virus to escape an immune response, in this model, depends on the rate of viral killing of viral-specific T4 cells as compared with the rate of immune stimulation $S$. If immune stimulation occurs rapidly enough, a response will be triggered before viral specific T4 cells are eliminated. Viral traits which tend to maximize T4 cell killing or inactivation, and minimize immune stimulation $S$, will increase the likelihood that the virus will escape an immune response. We consider three viral factors in this regard.

**Viral tropism.** The mechanism of immune escape that we consider depends upon the transfer of virus from infected APC to T cells. Thus viral strains that are not macrophage tropic will not have this mechanism available.

**Viral immune blocking.** The proposed mechanism of immune escape depends on the effective elimination of viral-specific T4 cells. This requires that HIV either rapidly kills T4 cells or blocks their immune functioning. Even rapid T4 cell killing by HIV requires a few days. It is thus possible that infected cells will become activated and produce cytokines before they die. Indeed, in [29], a period of T4 IL-2 production followed exposure of T4 cultures to antigen-presenting infected macrophages, although a highly cytotoxic, rapid/high strain of virus was used, and the antigen-recognizing T4 cells were eventually eliminated. Immune activity by infected cells, prior to their elimination by viral cytotoxicity, will contribute to the immune stimulus $S$. $S$ will be minimized by a viral activity which rapidly blocks the immune activation of infected cells.

**Level of viral antigen production.** An immune response can be stimulated by antigen presented by uninfected macrophages. Therefore, minimizing the stimulation $S$ also requires minimizing the population of uninfected HIV-antigen-presenting macrophages. This requires minimizing the level of viral antigen. Free virus in blood and extracellular fluid is a likely source of HIV antigens; free viral particles may be directly ingested by macrophages and free virus may shed its envelope proteins. Viral induced T4 cell lysis may also contribute large amounts of antigen since unassembled viral material will be released on cell lysis. A virus that grows slowly in T4 cells with low HIV
production will tend to minimize the source of antigen.

In order to determine the importance of these factors in immune escape, we analyze strains of virus with different levels of these traits.

3 Results

3.1 A non-macrophage tropic strain

We first consider a non-macrophage-tropic strain of virus. We model this by setting the rate parameter for macrophage infection to zero (see equations 3–9 in the appendix). In the absence of macrophage infection, the mechanism of selective viral transfer to HIV specific T cells does not operate. Hence this case serves as an illustration of the basic dynamics of viral growth and immune stimulation in our model, and as a baseline for comparison with macrophage-tropic strains.

Figure 2a shows the growth of the viral infection and rate of generation of a population of antigen-presenting macrophages for a situation in which 1000 infectious virus particles is given at time zero. Parameters are set for a rapid/high strain of HIV.

The figure shows that the population of free virus (Δ) initially drops, as virus is absorbed by T4 cells, and then rebounds due to production of new virus by infected T cells. The initial transients cause a series of small oscillations, but the dynamics tends towards steady growth of the infection. With the increased appearance of viral antigen, the population of HIV-antigen-presenting macrophages (□) also increases. These macrophages present antigen to viral specific T4 cells and stimulate an immune response on day 14 (Fig. 2b). Here the response is stimulated when the rate of antigen presentation events (○) reaches its critical value, depicted by the shaded region in Fig. 2b. The population of viral-specific T4 cells (●) is not depleted by the infection. The time required to reach the level of viremia needed to trigger a response is in general agreement with reported times (6 days [20], 13 days [32], and 20 days [17]) between exposure to HIV and the onset of the symptoms of the initial HIV viremia.
3.2 Macrophage tropic strains

We now consider macrophage-tropic viral strains that can infect both macrophages and T cells. By the process of viral transfer during antigen presentation, these strains have the potential ability to eliminate HIV specific T4 cells and thus escape generating an immune response. Whether escape occurs will depend on the detailed dynamics of the process. By varying parameter values determining the replication rate of virus in T4 cells, and the ability of infected T4 cells to participate in immune response, we test the effect of these viral traits on the dynamics of the infection. We compare strains that have the same ability to infect and replicate in macrophages but differ in their ability to replicate in T4 cells and block T4 cell activation.

3.2.1 A rapid/high strain induces an immune response

Figure 3 shows the dynamics of an infection by a strain of virus with rapid/high replication in T4 cells and low blocking activity. The growth of free virus is similar to that in the non-macrophage tropic case (Fig. 3a); the predominant source of free virus here is T4 infection. However, there is now a substantial population of infected macrophages that contributes to immune stimulation S. Because of this, S reaches the critical value $S_c$ slightly sooner than in the case of a non-macrophage tropic strain (Fig. 3b). There is no significant reduction in the viral-specific T4 population by the time S reaches $S_c$. Because of the high antigenemia induced by the high level of replication in T4 cells, and the significant immune stimulation from infected and non-infected macrophages, a strain with these characteristics is unable to escape an immune response.

3.2.2 A slow/low strain that partially blocks T cell activation escapes generating an immune response

Now consider a viral strain that is macrophage tropic, which does not reproduce well in T4 cells and which is 85% effective in blocking T cell activation. In Fig. 4 we illustrate an extreme case in which the virus does not replicate at all in T4 cells. The growth of infection is much slower in this case (Fig. 4a) (note the change in scale of the time-axis). There is a small population of uninfected antigen presenting macrophages and a relatively large population of infected macrophages (Fig. 4a). The immune stimulation from infected macrophages is limited since virus is transferred to HIV specific T4 cells and blocks their activity. Hence the total immune stimulation S is low, and before $S$
can reach the critical value $S_c$ the population of viral specific T4 cells begins to drop significantly (Fig. 4b). Since stimulation depends on these T4 cells, $S$ also drops and it never reaches $S_c$.

In this model, both low viral production and immune blocking help viral strains escape. For the parameters assumed in our simulations both features are necessary. For a rapid/high strain that can also block stimulation, the dynamics are similar to those of Fig. 3 (data not shown); the effect of the immune blocking is only to delay the stimulation of the immune response, not prevent it. Rapid/high strains produce large amounts of antigenic material and thus antigen specific T cells are stimulated by noninfected APC before they can be destroyed or blocked by viral action. A strain that replicates poorly in T cells, rather than not at all, show dynamics similar to Fig. 4 but with slight faster dynamics and somewhat higher immune stimulation. Clearly, as the replication rate increases Fig. 4 type dynamics converts to the type shown in Fig. 3 for the rapid high strain. A more interesting case, is that of a slow/low strain that does not interfere with T cell activation. Such a strain shows dynamics similar to Fig. 4, but the immune stimulus $S$ is always larger than in the Fig. 4 (data not shown) because of the lack of immune blocking. For such strains $S$ can reach $S_c$ before viral-specific T4 cells are significantly depleted.

4 Discussion

Some strains of HIV avoid being eliminated by the immune system. Thus persistence of HIV through a long latent period is a characteristic feature of HIV infection and progression towards AIDS. Strains that escape immune elimination have been observed in humans [21] and chimpanzees [7, 27], and have all been of the slow/low type, i.e. characterized by low viral expression and slow replication in T4 cells.

Here we have considered from a theoretical viewpoint the properties of a strain that would make it a good escape mutant. Based on observations of Mann et al. [29] and Manca et al. [30] in which HIV appears to be transferred from infected macrophages to T4 cells during antigen presentation, we suggest that strains of HIV that grow in macrophages may be preferentially transferred to HIV-specific T4 cells. Infection and depletion of such T cells would prevent immune elimination of the virus and lead to immune escape.

The situation, however, may not be this simple. Presentation of antigen leads to
T4 cell stimulation; thus, the same event that can potentially lead to elimination of HIV-specific T cells can also trigger an immune response. In order to determine which outcome is more likely in a given set of circumstances, we have developed a mathematical model for the dynamics of HIV infection of T4 cells and macrophages, and the events leading to the stimulation of an anti-HIV immune response. Our model follows the generation of HIV-antigens that can potentially be presented by macrophages. Rapid/high strains of HIV should generate large amounts of antigen, due to shedding of gp120 from free virus and the release of unincorporated viral material upon the lysis of infected T cells. Thus our model predicts that with rapid/high strains HIV antigenic material, to a large extent, will be presented by uninfected macrophages. Hence, transfer of HIV upon antigen presentation should not be common and stimulation of an immune response should occur. Contrastingly, slow/low strains should produce little antigenic material. Thus uninfected APC should do little presentation. However, if such a strain were macrophage or dendritic cell tropic it could grow in APCs. Further, if as suggested by Mann et al. [29], infected APCs process and present HIV antigens, then transfer of infectious virus from APC to viral-specific T4 cells could preferentially eliminate immune responsiveness rather than stimulate it.

Using our model we find that even slow/low strains may stimulate an immune response. Presentation of HIV-antigen by an infected APC can stimulate a T cell. Further, even if virus is transferred to the T cell during the encounter, the T cell may still function and lead to the generation of an immune response. Thus, we find that viral strains which in addition to being slow/low interfere with the ability of a T cell to become activated are more likely to be escape mutants than strains that do not have this propensity.

The prediction that strains which escape immune response have low levels of replication in T4 cells is in agreement with observation [21, 7, 27], and has been suggested by other theoretical models [7, 19]. In these other models the effect of low T4 replication is to limit viral antigenemia to a level too low to stimulate an effective immune response. In our model this too is an important effect. However, our model differs from these earlier models in that low viral expression works together with the mechanism of selective elimination of viral-specific T4 cells to allow HIV to escape an immune response.

Our model also strongly suggests that a strain of virus cannot escape generating an immune response unless T4 cells infected with the strain show a low level of immune activity. This prediction is new. There have been a number of reports of suppression of T4 response by HIV factors, including whole inactivated virus [33], tat protein [34],
and gp120 [35, 36]. Most of these reports have addressed the question of the effect of HIV infection on the T4 cells' long-term immune functionality. Here we have been concerned with the cells immune functionality in the days following infection, in a situation where the T4 cell receives the stimulus for activation at approximately the same time as it is infected due to antigen presentation by an infected macrophage. To our knowledge, the only experiments that directly address this question are the cited experiments of Mann et al. and Manca et al. In these experiments significant immune function by T4 cells infected by HIV in the course of antigen recognition was indicated by IL-2 production[29] or thymidine incorporation[30] by these cells. The viruses employed in these experiments were rapid/high, which we would not expect to be adapted for immune escape. We propose here that escape mutants possess as an adaptive mechanism, in addition to slow T4 replication, an ability to block the immune activity of T4 cells.

The existence of immune blocking ability is consistent with current ideas about the molecular mechanisms of HIV in T4 cells. A number of instances of trans activity between cellular factors regulating T cell activation, and HIV genes regulating viral replication, have been reported[37, 38, 39, 40]. The existence of such trans-activity raises the possibility that a viral factor could regulate cell activation. One possible candidate is Nef.

While the nef gene has been implicated in the negative regulation of HIV, whether it directly controls HIV gene transcription is still unresolved [41, 42, 43, 44]. However, nef has been observed to down-regulate the expression of CD4 molecules on T4 cells[45, 46], suggesting that it could block or reduce T4 cell activation. Thus nef could act as our theory requires - blocking both T cell activation and HIV expression. If nef blocks viral replication by means of blocking cell activation, then its effect on replication may depend on the degree of cell activation. Thus an experimental technique that forces cell activation sufficiently strongly might be able to override any activation blocking by nef, while a milder induction of cell activation might not. A similar effect of rpt-1 has been noted[38]: rpt-1 blocked viral LTR-controlled gene expression in resting, but not in activated, T4 cells. This is a possible explanation of some of the inconsistencies in reports of nef activity.

Viral factors responsible for immune disruption in AIDS have generally been sought separately from factors responsible for low viral expression. This may reflect a general belief that low expression is an adaptive mechanism of HIV [45], while immune disruption is a side effect of viral action. Our model points to the possibility that the low
expression and immune disruption function together as an adaptive mechanism. It is thus plausible that they are linked together evolutionarily. Such an adaptation would have been more likely to evolve if a single HIV mechanism could both induce low HIV expression in T4 cells, and block T4 cell immune activity. This is possible since HIV replication has consistently been observed to be associated with T4 activation; thus, a single viral factor which blocked T4 activation would block both T4 immune activity and HIV replication.

The model upon which our conclusions of immune escape are based is necessarily simplified, and one should ask how relevant is it to the actual process of HIV infection. The possibility of viral action reducing or eliminating the viral-specific T4 population follows logically from the selective transfer of virus to these cells upon antigen presentation. The extent to which the viral-specific T4 population is reduced is necessarily dependent on parameter values and details of the model. The regulation of T4 population levels is undoubtedly more complex than in our model. For this and other reasons, the total elimination of viral-specific T4 cells is probably less likely in reality than in the model. Indeed, the fact that an immune response can occur against slow/low strains in the initial viremia [21] indicates that the adaptions of the slow/low strains are not by themselves sufficient to avoid immune response. However, the traits of immune blocking, and low T4 expression, should remain adaptive for HIV even if they result only in a reduction, rather than a total elimination, of the immune response against the virus. The ability of slow/low mutants to escape immune response could be due to a combination of the mechanism proposed here and other mechanisms, such as latency, mutational drift, and the immune deficiencies that arise once the HIV infection is established.

Our proposed mechanism of viral escape is particularly interesting from an evolutionary standpoint. The ability of HIV to escape immune response depends on slow or no replication and immune blocking in T4 cells. These traits are important only for those virions which infect T4 cells as opposed to macrophages, and in the case of a strain with no T4 replication, such virions have no progeny. In spite of this, these traits are potentially adaptive for the entire viral strain, since as our model shows such strains can escape generating an immune response. The success of such an adaptive mechanism would be an example of kin selection [47] among virions.

Our model of HIV immune escape makes three testable predictions: that escape mutants will be macrophage tropic, will be slow/low with regard to growth in T cells, and will block T cell activation or at least reduce lymphokine production. If the last
prediction can be verified, it suggests that stimulation of the immune system at the onset of flu like symptoms and early viremia might prevent the generation of escape mutants.

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6 Appendix: Equations

Here we give the equations defining our model. The parameters in these equations are defined in Table 1.

6.1 Equations for uninfected, unactivated cells:

\[
\frac{dT}{dt} = \sigma_T - (k_{VT}V + \omega_T)T, \\
\frac{d\tilde{T}}{dt} = \sigma_{\tilde{T}} - (k_{spp}p_{i}M^* + k_{VT}V + \omega_T)\tilde{T}, \\
\frac{dM}{dt} = \sigma_M - (k_{VM}V + k_u(V + A) + \omega_M)M + \omega_{\tilde{M}}\tilde{M}.
\]

For each uninfected cell type, there is a constant source term \(\sigma\), and a normal death rate constant \(\omega\). Non-HIV specific and HIV-specific T4 cells are denoted \(T\) and \(\tilde{T}\), respectively. The cells of each type may be infected, and thus removed from the uninfected cell population, as described below. Macrophages, \(M\), become HIV-antigen presenting \(\tilde{M}\) upon uptake and processing of viral antigen \(V + A\), and return to the resting macrophage pool at rate \(\omega_{\tilde{M}}\tilde{M}\).

6.2 Equations for free virus and infected cells:

\[
\frac{dV}{dt} = k_{MV}M^* + NL_T - (\omega_V + k_{VT}(T + \tilde{T}) + (k_{VM} + k_u)M)V, \\
\text{where} \quad L_T(t) = k_{VT}V(t - \tau)(T(t - \tau) + \tilde{T}(t - \tau)) + k_{spp}p_{i}\tilde{T}(t - \tau)M^*(t - \tau). \\
\frac{dM^*}{dt} = k_{VM}V(t - \tau)M(t - \tau) - \omega_{M^*}M^*
\]

Free virus \(V\) (eqn. 6) are produced continuously by infected macrophages. After T4 cell infection, an average of \(N\) new virions are produced in each cell lysis event; these events occur at rate \(L_T\). Free virus decays—i.e. loses infectivity, or is absorbed by cells not in our model—at rate \(\omega_V\). It also is removed as it infects cells and as it is taken up as antigen by macrophages.
The course of T cell infection is assumed to take time $\tau$ until cell lysis; thus the rate of lysis at time $t$, $L_T(t)$, is determined by the rate of infection at time $t - \tau$. Any T4 cells may be infected by free virus; viral-specific T4 cells may also be infected by transfer of virus from infected macrophages.

Macrophages are infected by free virus, and after a delay $\tau$ enter the population of virus-producing macrophages $M^*$. Infected macrophages die at rate $\omega_M^* M^*$.

### 6.3 Equations for antigen and activated cells:

\[
\frac{dA}{dt} = A g_L N L_T + A g_V \omega V - (k_u M + \omega_A) A \tag{8}
\]
\[
\frac{d\tilde{M}}{dt} = k_u (V + A) M - \omega_{\tilde{M}} \tilde{M} \tag{9}
\]

Antigenic material $A$ is released as unassembled viral material upon T4 cell lysis and is shed by free virus as they lose infectivity. It is absorbed by macrophages, and by unspecific body processes. Macrophages $M$ become antigen-presenting macrophages $\tilde{M}$ as they take up antigenic material $A$ and live virus $V$. 

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References


Figure 1. Model of macrophage presentation of viral antigen to T4 cells and infection of these cells. Viral antigen, $A$, (along with free virus, $V$, not shown) is taken up by macrophages, $M$, at rate $k_uAM$, and stimulates the macrophage to become an activated, antigen-presenting macrophage, $\tilde{M}$. Viral-antigen-specific T4 cells, $T$, are stimulated by antigen-presenting macrophages at rate $k_s\tilde{T}\tilde{M}$. Macrophages, $M$, become infected by virus, $V$, at rate $k_{VM}VM$; after a delay of time $\tau$ they become virus-producing macrophages, $M^*$, which produce free virus at a rate $k_{MV}M^*$. With probability $p_v$ virus-producing macrophages present viral antigens. Thus viral-specific T4 cells may also be stimulated by infected macrophages; this occurs at rate $k_s\tilde{T}p\tilde{M}M^*$. In this case, with probability $p_v$ virus is transferred from the macrophage to the T4 cell, leading to infection and ultimate removal of the viral-specific T4 cell.

Viral-specific T4 cells are also infected by free virus, at rate $k_{VT}VT$; also T4 cells not specific for viral antigens (and not shown on the diagram) are infected at rate $k_{VT}VT$. After of delay of $\tau$, infected T4 cells undergo cell lysis and produce $N$ new virus particles. The rate of T4 cell lysis is denoted $L_T$. Also in the course of T4 lysis, unassembled viral antigen is release, at rate $A_gLN_L$. Free virus, $V$, lose infectivity at rate $\omega_VV$; this involves shedding of viral antigen, $A$, at rate $A_g\omega_VV$.

Antigen-presenting macrophages lose their activation at rate $\omega_\tilde{M}\tilde{M}$, and infected macrophages $M^*$ die at rate $\omega_{M^*}M^*$. Uninfected macrophages and T4 cells die, and the population is replenished, at steady rates (processes not shown).

Figure 2. Model predictions for the infection dynamics of a non-macrophage tropic strain of HIV ($k_{MV} = 0$). $N = 1000$; other parameters are as in Table 1. (Parameters referring to macrophage infection and viral blocking are not relevant to this case). Initially $V = 1000$, $M^* = \tilde{M} = A = 0$, and initial values of $T$, $\tilde{T}$, $M$ are given in Table 1. (a) Growth of populations of free virus, $V$ ($\triangle$—$\triangle$), and viral-antigen-presenting macrophages, $\tilde{M}$ (□—□). (b) Population of uninfected, viral-specific T4 cells, $\tilde{T}$, (●—●; left hand scale), and immune stimulus $S$ in events/hour (◊—◊; right hand scale). The shaded region at the top represents values of $S \geq S_c$, i.e. the region in which an immune response is triggered. The dynamics are followed until $S$ enters this region.

Figure 3. Model predictions for the infection dynamics of a macrophage-tropic strain of HIV with fast replication in T4 cells ($N = 1000$) and minimal blocking of T4 response ($p_b = 0.1$). Other parameters are as in Table 1. Identification of curves as in Fig. 2. Also, in (a), infected macrophages $M^*$ (○—○) are plotted. As in Fig. 2, the dynamics are followed until $S = S_c$, implying that an immune response has been
triggered.

Figure 4. Model predictions for the infection dynamics of a macrophage-tropic strain of HIV with no T4 replication ($N = 0$) and substantial immune blocking ($p_b = 0.85$). Other parameters are as in Table 1. Identification of curves as in Fig. 3. In this case the immune stimulus, $S$ ($\diamond — \diamond$), never reaches the critical value $S_c$; the dynamics are followed until the population of uninfected viral-specific T4 cells $\tilde{T}$ ($\bullet — \bullet$) is effectively destroyed, i.e. falls to 0.1% of its initial value.
Table 1: Parameters and initial values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value Used</th>
<th>Basis; References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau$</td>
<td>time from cell infection to new virus production</td>
<td>100 h</td>
<td>T4 cells;[46, 47]; macrophages;[48]</td>
</tr>
<tr>
<td>$A_{gL}$</td>
<td>fraction of viral material unassembled on viral lysis</td>
<td>0.15</td>
<td>assume half of viral loss is decay to antigenic fragments</td>
</tr>
<tr>
<td>$A_{gv}$</td>
<td>Ag production accompanying viral decay</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>$N$</td>
<td>number virus produced per T4 cell</td>
<td>varies</td>
<td></td>
</tr>
<tr>
<td>$p_p$</td>
<td>probability that infected macrophage presents HIV antigen</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>$p_t$</td>
<td>probability of viral transfer to T4 from HIV Ag presenting macrophage</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>$p_b$</td>
<td>probability of viral action blocking T4 response</td>
<td>varies</td>
<td></td>
</tr>
<tr>
<td>$S_c$</td>
<td>critical immune stimulus</td>
<td>$8 \times 10^9$ h$^{-1}$</td>
<td>assume 1% of specific T4 cells stimulated in 5 h.</td>
</tr>
</tbody>
</table>

Rate constant for:

- $\omega_T$: death of T4 cells
- $\omega_M$: death of macrophages
- $\omega_V$: loss of infectivity by virus
- $\omega_{dA}$: cessation of antigen presentation by macrophages
- $\omega_A$: nonspecific absorption of antigen
- $\sigma_T$: production of (unspecific) T4 cells
- $\sigma_F$: production of viral-specific T4 cells
- $\sigma_M$: production of macrophages
- $k_{VT}$: infection of T4 cells by free virus
- $k_{VM}$: infection of macrophages by free virus
- $k_{MV}$: production of virus by infected macrophage
- $k_s$: stimulation of Ag-specific T4 cell by Ag-presenting macrophage
- $k_u$: stimulation of macrophage to become Ag-presenting on uptake of Ag

Initial values:

- $T_0$: normal T4 population
- $T_0$: normal T4 population specific for viral antigens
- $M_0$: normal macrophage population

- $T_0 = 4 \times 10^{11}$ cycles
- $T_0 = 4 \times 10^8$ cycles
- $M_0 = 1 \times 10^9$ cycles

[57] ~one out of $10^5$ T4 cells recognizes antigen of given virus
Fig. 1

cell/virus pathway

antigen pathway
VIRAL-SPECIFIC T4 CELLS $\times 10^{-6}$ vs IMMUNE STIMULATION EVENTS/HOUR vs DAYS

DAYS

Fig 4b