Opinion

Cell PRESS

Rates of HIV immune escape and reversion: implications for vaccination

Miles P. Davenport[1], Liyen Loh[2], Janka Petravic[1] and Stephen J. Kent[2]

[1] Centre for Vascular Research, University of NSW, Kensington, NSW 2052, Australia
[2] Department of Microbiology and Immunology, University of Melbourne, VIC 3010, Australia

HIV-1 mutates extensively *in vivo* to escape immune control by CD8+ T cells (CTLs). The CTL escape mutant virus might also revert back to wild-type upon transmission to new hosts if significant fitness costs are incurred by the mutation. Immune escape and reversion can be extremely fast if they occur very early after infection, whereas they are much slower when they begin later during infection. Immune escape presents a significant barrier to vaccination, because escape of vaccine-mediated immune responses could neutralise any benefits of vaccination. Here, we consider the dynamics of immune escape and reversion *in vivo* in natural infection, and suggest how understanding of this can be used to predict optimal vaccine targets and design vaccination strategies that maximise immune control. We predict that inducing synchronous, broad CTL by vaccination should limit the likelihood of viral escape from immune control.

Studying the dynamics of immune escape and reversion

A confusing picture has emerged in recent years from studies of immune escape and reversion of HIV-1 in humans and SIV in macaques. Some macaque studies suggest fast rates of escape and reversion during or early after acute infection. Fast escape, where escape mutant (EM) virus first arises and becomes the primary quasi-species within a few weeks, implies strong immune pressure from CD8+ T cells (CTLs). Similarly, fast reversion from EM to wild-type (WT) virus over a few weeks indicates high fitness costs of the escape mutation [1,2]. By contrast, several studies of HIV-1-infected people suggest much slower rates of immune escape and reversion, where several months or longer is required to complete the change from one viral phenotype to the other [3–7]. The difference in escape rates in humans and macaques has been used to suggest that CTL pressure is lower in HIV than in SIV [4], whereas the difference in reversion rates was interpreted as escape mutations in humans having lower fitness costs. However, recent evidence indicates that transmission of escape mutant virus and the maintenance of escape mutant virus are associated with reduced viral loads in HIV, implying significant fitness costs associated with the CTL escape variants [8–10].

Understanding the differences in escape and reversion rates between SIV and HIV requires studying the dynamics of viral evolution within the host. Although mutational escape from HIV-specific CTL responses has been documented for over 15 years [11], sequencing technologies remain the primary method to follow escape. Bulk sequencing is insensitive to variants $<30\%$ of the population and even cloning and sequencing cannot reliably detect the presence of $\leq 10\%$ of minor species unless massive numbers of clones are sequenced. A viral subpopulation comprising 10% of the total represents a large amount of viremia where the total viral load exceeds 10^6 copies during early infection. This methodological limitation has made it difficult to perform detailed longitudinal studies of escape and reversion dynamics. Quantitative PCR-based methods have recently been developed and used to accurately measure the 'viral load' of both WT and EM quasi-species [12–14]. Specific forward primers modified with locked nucleic acids and minor-binding groove bases allow detection of $<0.1\%$ minor quasi-species [13,14]. This permits tracking EM or WT virus over time, even where large quantitative differences between WT and EM virus emerge as escape proceeds. These quantitative PCR approaches have allowed a dynamic understanding of immune escape, and have clarified the differences between HIV and SIV. These data now suggest that a dynamic interplay between viral mutation, host immune pressure, and the availability of host target (CD4+) T cells explains the observed differences and has important implications for vaccine design [3,4]

This article assesses the reasons why rates of CTL-driven immune escape and reversion are variable during the course of natural HIV infection of humans and SIV infection of macaques, and how vaccination is likely to impact rates of escape and reversion. These are topical issues given the failure of a recent CTL-based vaccination approach, which induced fairly narrow responses, to prevent or modify HIV infection of humans [15]. We predict that some apparently 'conserved' epitopes in HIV might escape slowly simply because immune responses to these epitopes develop late. Targeting of such epitopes by vaccination could be counterproductive, because it will simply drive early escape. However, we predict that inducing broad CTL responses that simultaneously target multiple epitopes in acute infection will reduce escape and facilitate more effective virologic control.

Reversion of transmitted escape mutant virus

To understand the dynamics of escape and reversion *in vivo*, it is easiest to first consider the dynamics of reversion; EM virus is often at a replicative disadvantage compared

Corresponding author: Kent, S.J. (skent@unimelb.edu.au)

0966-842X/$ – see front matter doi:10.1016/j.tim.2008.09.001 Available online 27 October 2008

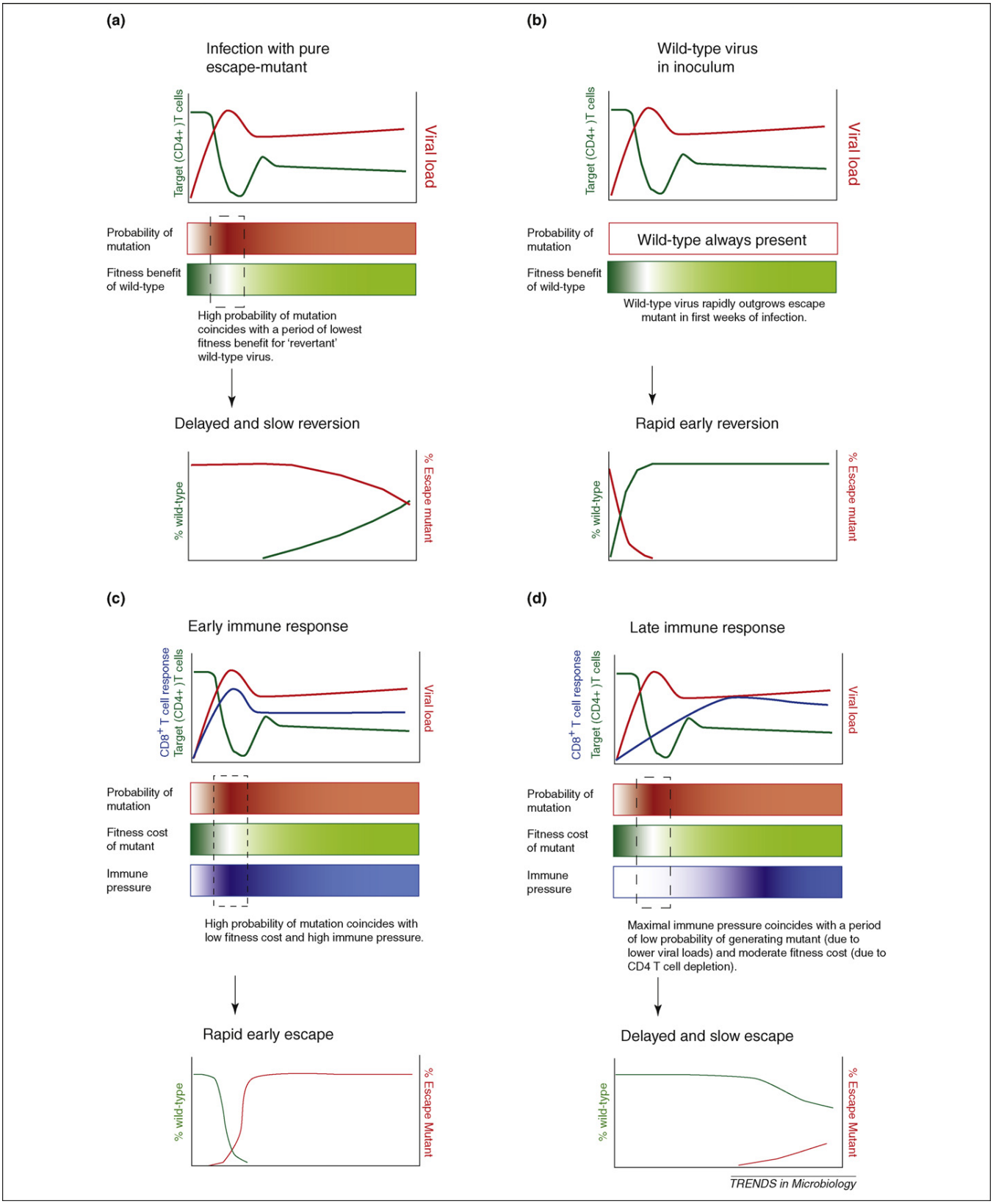

Figure 1. Factors determining immune escape and reversion. Wild-type (WT) virus can outgrow escape mutant (EM) virus in a host that is unable to recognize the epitope because of its higher replicative capacity, leading to 'reversion' from EM to WT **(a, b)**. When the host is inoculated with pure EM virus, the WT must arise by mutation. However, the optimal time for mutation (viral peak) coincides with the worst time for WT virus to compete because of the low number of target cells. Thus, reversion tends to occur late and slowly after infection with pure EM virus. When WT virus is present in the initial inoculum, it outgrows EM during the first weeks of infection, when high target cell number favor its growth. Immune escape **(c, d)** can occur at different rates depending on whether the immune response is early **(c)** or late **(d)**. Vaccination might speed up 'late responses,' leading to rapid early escape. If both the EM virus and strong immune pressure are present early in infection, high immune pressure and low fitness cost, due to the nadir in target cell numbers soon after the peak of virus, will lead to rapid escape. If the immune pressure (or mutation) is present only later in infection, then higher steady-state target cell numbers will increase the effective fitness cost of escape, leading to slower selection of the escape variant.

with WT virus, owing to the 'fitness cost' of escape mutation [6–8]. Thus, in the absence of immune pressure (for example in a host lacking the correct MHC to recognize the WT epitope), a transmitted EM virus reverts to WT (Figure 1a,b). There are several prerequisites to successful reversion: (i) the WT virus must have higher replicative capacity ('fitness') than the EM; (ii) the WT virus needs to be present (either in the initial inoculum, or generated through mutation from EM virus); and (iii) there must be sufficient available uninfected CD4 cells – 'target cells' – for the WT virus to grow in and outcompete the EM [13]. Simplistically, if there are very few cells available for infection, both WT and EM will grow slowly, and the WT virus will have difficulty 'outgrowing' the EM virus.

Thus, a critical driver of early 'reversion' is the presence or absence of the WT virus in the infecting inoculum. Where the WT virus is already present in the EM virus, the fitter WT virus can rapidly outgrow the EM virus during the early exponential phase of viral replication, when target cell levels are at their highest (Figure 1b). However, when infection occurs with pure EM virus, then mutation of EM virus is required to generate the original WT variant. Because mutation occurs only during infection of new targets, it will be more likely to occur as the number of infected cells increases. This first WT mutant to arise in an infected cell must then avoid an early death before infecting another cell, and then grow and compete with the EM virus. However, WT revertants are most likely to be generated by mutation around the peak of infection, and this corresponds with the worst time for their growth and competition against EM virus because of the shortage of uninfected target cells. Indeed, in many circumstances there are predicted to be too few target cells for WT virus to grow at this time [13]. As a result, if a WT mutant is generated at peak, it will either fail to grow, or compete extremely poorly with EM and thus only slowly outgrow EM virus. Thus, after infection with pure EM virus reversion might often not occur in acute infection and instead arise during the chronic phase, where the daily probability of mutation is lower but the target cell availability in the chronic phase of infection is higher and will at least permit moderate WT virus growth rates.

Recent analyses of viral variability during HIV-1 infection by analysing single genomes predict that approximately three-quarters of new HIV-1 infections are initiated with a single strain, whereas a quarter of subjects acquire two or more variants [16,17]. In cases where WT and EM are both transmitted, it is also possible that the WT virus could outgrow the EM virus sufficiently rapidly that EM virus might not be detected at all by sequencing techniques by the time of the first sampling (usually 2–3 weeks after infection). Thus in humans rapid reversion might not be detected in acute infection, and those reversion events that are observed will be late and slow [9,10] – not necessarily because of the low fitness cost of escape, but due to infection dynamics following clonal inoculation with EM virus. It is noteworthy that where clonal EM virus has been used to infect monkeys and thus mutation is required to produce WT variants, slower reversion is also observed [2]. Recombination between WT and EM virus could further speed up reversion [18].

Understanding the dynamics of reversion *in vivo* is essential to understand immune escape. The selection of EM virus involves a balance of WT and EM advantages: the increased replicative fitness of the WT virus means that it is always trying to 'revert' and take over from EM, and the strength of this selection for WT virus is determined by target cell availability, as discussed above. The advantage of the EM virus is that it escapes CTL killing, which might also fluctuate over time. The net effect of the WT 'reversion advantage' and the EM 'immune escape advantage' determines the rate of immune escape, as discussed below.

Timing and rate of immune escape

The strong selection pressure exerted by CTL induces early and rapid escape at some epitopes in SIV [11]. Vaccination can accelerate this by applying higher immune pressure [19]. By contrast, escape in HIV is often relatively slow. Recently, it has been shown that the timing of immune escape is a major determinant of its rate; escape at the same epitope in macaques varies in rate depending on when it occurs [20]. Evidence from studies of extremely early infection in humans suggests rapid escape in early infection here also [14,15].

Immune escape again requires several factors: (i) the presence of EM virus (in inoculum, or generated by mutation); (ii) the presence of immune pressure on WT; (iii) sufficient number of target cells at the time of appearance of EM, so that it is able to grow when it emerges; and

Box 1. Factors influencing reversion and escape rate

Selection of immune escape variants is driven by immune pressure favouring unrecognized epitope variants. Thus, the higher the CTL response, the higher the escape rate. However, escape mutation usually also involves a 'fitness cost' because of the lower replicative capacity of the escape mutant virus. The effective fitness cost experienced by the virus is influenced by the number of available target cells for the viruses to grow in [13]. Early in infection, target cell numbers are high and the immune response is weak (point A), so the net balance favours reversion to wild-type virus [19]. However, just after the peak in virus the CTL response is at its peak, and the target cell number is at its lowest point (point B). This set of factors promotes rapid escape. In chronic infection, CTL levels have dropped and target cell numbers are partially recovered, leading to a slow rate of immune escape (point C) (Figure I).

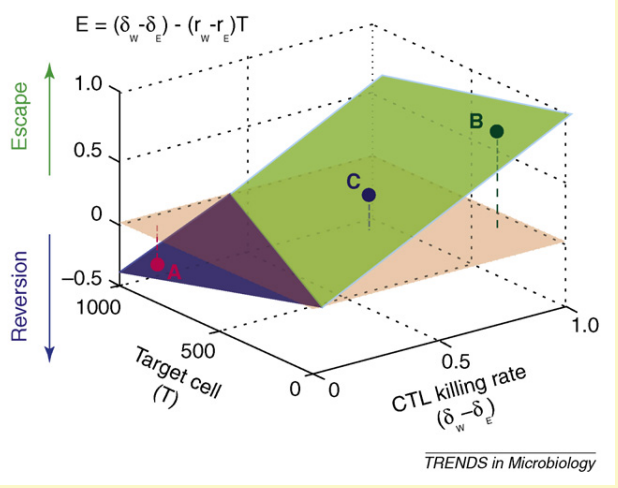

Figure I. Factors influencing reversion and escape rate.

(iv) that the fitness cost of escape is less than the immune pressure (Box 1). As described above, the fitness cost of escape varies with the availability of target cells for infection. Early in infection the growth advantage of the WT virus is maximised because of the large number of target cells, and the selective pressure of CTL is low because of a delay in their activation [16,17]. The optimal time for the EM to appear is just before the peak of viraemia; at this time the probability of mutation is close to its highest level, there is already some CTL response reducing the WT fitness, and target cells are low enough to reduce the WT growth advantage, but not yet so low that the EM virus is unable to grow, as might occur at maximum depletion. If the EM emerges at this time, it is well-established just after the peak of total viral load. This time represents the perfect combination of factors for promoting escape (Figure 1c): a peak in CTL response (to many epitopes), and a nadir in CD4+ T cell numbers leading to a low 'fitness cost' of escape. At this time there is little growth of either EM or WT virus because of low target cell numbers, but the rapid decay of WT virus due to CTL control leads to the rapid selection of EM virus.

This rapid early escape in the first few weeks of infection is often a predictable factor of early SIV/HIV infection. Early escape occurs as a natural consequence of viral and immune dynamics, and might be hard to alter by vaccination. However, escape later in infection is more variable in its timing and might not occur in all individuals. Understanding why early escape is often so stereotyped and delayed escape is often variable or absent at some epitopes could provide insights into improved viral control.

Understanding early versus late escape during natural infection

The discussion above indicates why we might often see early, rapid escape. Why then is escape delayed at some epitopes (Figure 2)? A simple explanation for this is that the mutation is rare, so that there are too few infected cells during acute infection to guarantee production of the EM, and it instead tends to arise later in chronic infection (Box 2). In this case, many rounds of infection might be required before the mutant arises. This is most likely the case when multiple mutations are required to produce escape [18]. An alternative mechanism is that the host immune response tends to arise late, after the peak of viremia [18–20]. In this latter case, even though the mutation might be produced during acute infection, the immune pressure driving its selection might be absent or minimal so it is likely to be lost. Once the immune response

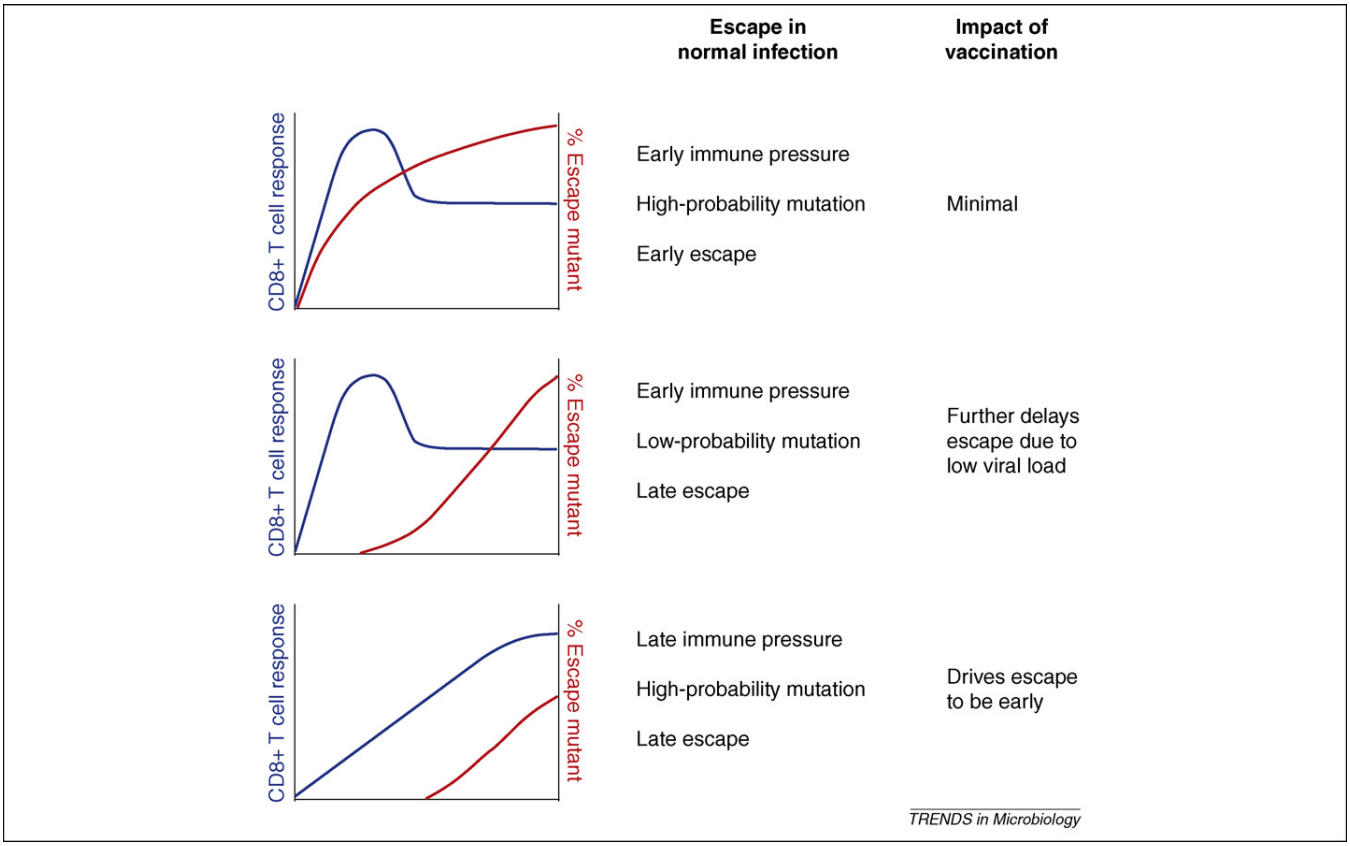

Figure 2. Choosing epitopes where vaccination will reduce escape. Vaccination is predicted to have different effects on the rate of viral escape, depending on the dynamics of escape in natural infection. **(a)** Epitopes that escape early do so because of a combination of early immune pressure and a 'high probability' escape mutation. Vaccination might reduce the probability of mutation by reducing viral load and increase immune pressure, but will likely have little effect on escape at these epitopes unless the reduction in viral load is very high. **(b)** Epitopes that escape late might do so because the mutation is a 'low probability' event, despite an early immune response, or because of a late-arising immune response **(c)**. In the case of a low-probability mutation, if vaccination reduces viral load it will further delay, and maybe even prevent, escape. However, if late escape occurs because of a late-arising immune response, vaccination will lead to an earlier 'memory' response to the epitope and simply precipitate early escape.

Box 2. Mutation rate and the expected time of appearance of the escape mutant

HIV mutation occurs during the infection of new cells. The probability of a mutation emerging is therefore determined simply by the number of infected cells (and scales with viral load). The 'cumulative probability' of a mutation having occurred before a certain time is thus proportional to the total number of cells infected from the start of infection until this time and is proportional to the area under the viral load curve.

The cumulative probability of mutation grows exponentially until the peak of infection; the growth then slows down until the chronic phase, when it grows slowly and fairly linearly in time (Figure I). If the probability that an infected cell will produce an escape mutant (EM) is μ, then we can expect the EM to appear after $1/\mu$ cells are infected. If we consider two possible escape scenarios, one with a high probability of mutation (μ is high) and the other with a low probability of mutation (μ is low), they will be expected to occur at different times. Here, the mutation rate for the first mutant μ_1 is large, and in natural infection it appears early, at t_1 before the viral peak. The second mutant has a lower probability μ_2 and would appear in the chronic phase of natural infection at t_2.

Vaccination will act to reduce viral loads, and thus reduce the probability of mutation (although the effect of vaccination is always delayed for several days, during which the number of infected cells grows in the same way in vaccinated and unvaccinated individuals [29]. The 'easy' escape mutation (μ_1) will still occur, and any slight delays in its occurrence will have little effect. However, the emergence of the 'difficult' mutant (μ_2), that would have normally appeared during the chronic phase, will be delayed till much later or completely suppressed.

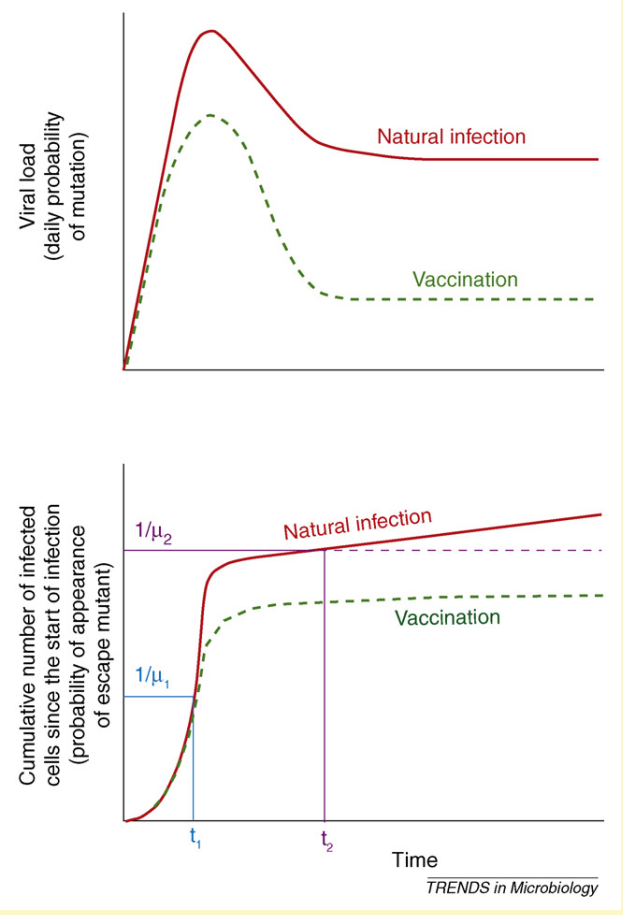

Figure I. Mutation rate and the expected time of appearance of the escape mutant.

develops in chronic infection, the rate at which mutants arise is again low, and the fitness cost has risen because the number of target cells is higher than just after the peak, so escape is slow and late (Figure 1d). Other immune factors could also influence viral load and escape dynamics; for example, during acute infection, innate immune responses might reduce viral growth of both EM and WT strains. Similarly, during chronic infection changes in T cell repertoire [21] or loss of CD4 T cell help might facilitate escape [22]. Overall, we expect a fundamental difference in escape timing and rates between those epitopes that experience high immune pressure early versus late.

Impact of vaccination on escape dynamics

Immune escape is a major mechanism by which the virus could neutralise the impact of vaccination [23]. What is the effect of vaccination on the rate of immune escape? Vaccination might act to reduce the escape rate by reducing the viral levels and the probability of generating a mutant, and by preserving CD4+ T cells, which increase the 'fitness cost' of escape (Box 2). However, vaccination might also make escape faster by increasing the immune selection pressure, particularly if this occurs in acute infection. The balance of these forces will be strongly dependent on the timing and mechanisms of escape in natural infection.

For epitopes where escape occurs rapidly in natural infection, vaccination is likely to have relatively little effect. The probability of mutation arising could be reduced, but unless there is a large reduction in viral load, this might be insufficient to prevent the mutant arising early. By contrast, because the natural level of immune response is sufficient to produce escape, boosting this by vaccination is unlikely to produce more rapid escape because the escape is so fast that this is of little consequence.

Where vaccination is likely to have the most impact is in epitopes that normally escape late. However, the result of vaccination will be determined by the reason for the late escape. If an epitope escapes late because of the low probability of mutation, then anything that reduces viral load will delay escape in proportion to the cumulative viral load reduction (Box 2). Otherwise, if an epitope escapes late because of a delayed immune response, then vaccination against that epitope will drive the immune response to occur in acute infection. In this case, the phenotype of escape after vaccination will be early and rapid during acute infection and much faster than in natural infection. This contention is supported by the observation that although CTL responses restricted by HLA-B*27 provide a benefit during chronic natural HIV infection with late escape [24], in a vaccinated subject infected with HIV, escape occurred early and the subject progressed rapidly [25].

Identifying epitopes that don't escape until late in infection is one way of choosing immune targets for vaccination. However, we should first confirm that slow escape occurs despite immune pressure; that is, because of difficulties in producing the mutations rather than because of the absence of early immune pressure [18–20]. If vaccination can reduce viral loads in acute infection it should prevent early escape at these epitopes and facilitate sustained immune control.

Concluding remarks and future directions

How does the foregoing help us design better potential vaccination strategies for HIV? Immune escape from CTL responses is indeed an Achilles heel for HIV vaccination, and understanding and controlling the forces that determine escape is an important aim. The timing and rate of escape and reversion are determined by the complex interplay among viral load, target cell number, and immune pressure, and do not in themselves predict the fitness cost at an epitope or the likely effectiveness of vaccination. A significant issue clouding our understanding of escape is our 'one epitope at a time' approach to studying escape. Because of the technical complexities of measuring and comparing escape, we often tend to think about it 'one epitope at a time.' It is likely that escape *in vivo* conforms somewhat to this pattern, where a flurry of early escape events is followed by a slow sequential escape in chronic infection.

Targeting vaccination to epitopes that are stable and only escape late in natural infection could either precipitate or slow immune escape. The major advantage of targeting such 'late escaping' epitopes might come not from the effects on that epitope alone, but from bringing to bear a large number of responses simultaneously rather than have them arising sequentially [18–20,26] and permitting 'one escape at a time.' If we can synchronise multiple responses to occur simultaneously early in infection and reduce the peak viral load and target cell depletion, then we could reduce the probability of mutation and increase fitness cost at multiple epitopes. Moreover, the more epitopes that are simultaneously targeted, the more difficult it becomes to produce EM virus with multiple mutations. This presents significant challenges in overcoming the phenomenon of immunodominance and 'clonal succession' [27]; There is a natural tendency for highly presented epitopes or high-avidity T cell clones to dominate the response, and this might be exacerbated by vaccination [28]. Thus, the benefits of eliciting responses to multiple epitopes might be undermined if there is a strong immunodominance hierarchy, leading to sequential escape from successive waves of immunodominant CTL responses to different epitopes. The diversity of the response in terms of the number of epitopes equally targeted in acute infection rather than the total magnitude of the response could be the key consideration.

Acknowledgements

Supported by Australian National Health and Medical Research Council grants and a James S McDonnell Foundation Award. M.P.D. is a Sylvia and Charles Viertel Senior Medical Research Fellow.

References

1 Fernandez, C.S. *et al.* (2005) Rapid viral escape at an immunodominant simian-human immunodeficiency virus cytotoxic T-lymphocyte epitope exacts a dramatic fitness cost. *J. Virol.* 79, 5721–5731
2 Friedrich, T.C. *et al.* (2004) Reversion of CTL escape-variant immunodeficiency viruses *in vivo*. *Nat. Med.* 10, 275–281
3 Leslie, A.J. *et al.* (2004) HIV evolution: CTL escape mutation and reversion after transmission. *Nat. Med.* 10, 282–289
4 Asquith, B. and McLean, A.R. (2007) *In vivo* CD8+ T cell control of immunodeficiency virus infection in humans and macaques. *Proc. Natl. Acad. Sci. U. S. A.* 104, 6365–6370
5 Goulder, P.J. *et al.* (2001) Evolution and transmission of stable CTL escape mutations in HIV infection. *Nature* 412, 334–338
6 Kelleher, A.D. *et al.* (2001) Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. *J. Exp. Med.* 193, 375–386
7 Brumme, Z.L. *et al.* (2008) Marked epitope- and allele-specific differences in rates of mutation in human immunodeficiency type 1 (HIV-1) Gag, Pol, and Nef cytotoxic T-lymphocyte epitopes in acute/early HIV-1 infection. *J. Virol.* 82, 9216–9227
8 Chopera, D.R. *et al.* (2008) Transmission of HIV-1 CTL escape variants provides HLA-mismatched recipients with a survival advantage. *PLoS Pathog.* 4, e1000033
9 Goepfert, P.A. *et al.* (2008) Transmission of HIV-1 Gag immune escape mutations is associated with reduced viral load in linked recipients. *J. Exp. Med.* 205, 1009–1017
10 Rousseau, C.M. *et al.* (2008) HLA class I-driven evolution of human immunodeficiency virus type 1 subtype c proteome: immune escape and viral load. *J. Virol.* 82, 6434–6446
11 Phillips, R.E. *et al.* (1991) Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 354, 453–459
12 Peyerl, F.W. *et al.* (2005) Use of molecular beacons for rapid, real-time, quantitative monitoring of cytotoxic T-lymphocyte epitope mutations in simian immunodeficiency virus. *J. Clin. Microbiol.* 43, 4773–4779
13 Loh, L. *et al.* (2008) Vaccination and timing influence SIV immune escape viral dynamics *in vivo*. *PLoS Pathog.* 4, e12
14 Loh, L. and Kent, S.J. (2008) Quantification of simian immunodeficiency virus cytotoxic T-lymphocyte escape mutant viruses. *AIDS Res. Hum. Retroviruses* 24, 1067–1072
15 Watkins, D.I. *et al.* (2008) Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. *Nat. Med.* 14, 617–621
16 Salazar-Gonzalez, J.F. *et al.* (2008) Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. *J. Virol.* 82, 3952–3970
17 Keele, B.F. *et al.* (2008) Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7552–7557
18 Streeck, H. *et al.* (2008) Immune-driven recombination and loss of control after HIV superinfection. *J. Exp. Med.* 205, 1789–1796
19 De Rose, R. *et al.* (2007) Comparative efficacy of subtype AE simian-human immunodeficiency virus priming and boosting vaccines in pigtail macaques. *J. Virol.* 81, 292–300
20 De Rose, R. *et al.* (2008) Delivery of immunotherapy with peptide-pulsed blood in macaques. *Virology* 387, 204–210
21 Davenport, M.P. *et al.* (2007) The T cell repertoire in infection and vaccination: implications for control of persistent viruses. *Curr. Opin. Immunol.* 19, 294–300
22 Grakoui, A. *et al.* (2003) HCV persistence and immune evasion in the absence of memory T cell help. *Science* 302, 659–662
23 Barouch, D.H. *et al.* (2002) Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature* 415, 335–339
24 Gao, X. *et al.* (2005) AIDS restriction HLA allotypes target distinct intervals of HIV-1 pathogenesis. *Nat. Med.* 11, 1290–1292
25 Betts, M.R. *et al.* (2005) Characterization of functional and phenotypic changes in anti-Gag vaccine-induced T cell responses and their role in protection after HIV-1 infection. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4512–4517
26 Davenport, M.P. *et al.* (2002) Cell turnover and cell tropism in HIV-1 infection. *Trends Microbiol.* 10, 275–278
27 Davenport, M.P. *et al.* (2002) Clonal selection, clonal senescence, and clonal succession: the evolution of the T cell response to infection with a persistent virus. *J. Immunol.* 168, 3309–3317
28 Sen, P. *et al.* (2007) Clonal focusing of epitope-specific CD8+ T lymphocytes in rhesus monkeys following vaccination and simian-human immunodeficiency virus challenge. *J. Virol.* 82, 805–816
29 Davenport, M.P. *et al.* (2004) Predicting the impact of a nonsterilizing vaccine against human immunodeficiency virus. *J. Virol.* 78, 11340–11351