Research Focus

Reversion of immune escape HIV variants upon transmission: insights into effective viral immunity

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Many viruses that cause chronic viremic infections, such as human immunodeficiency virus type 1 (HIV-1), mutate extensively to avoid effective control by the host immune system. However, each immune escape mutation probably results in some fitness cost to the virus. The most effective immune responses might be those that target the regions of the virus where escape mutation inflicts the largest fitness cost to the virus. A virus crippled by immune escape mutations would result in reduced viral load and delayed disease. Such knowledge could be used to rationally design more effective vaccines.

Introduction

T-cell escape variants have been well described in HIV-1 (human immunodeficiency virus type 1)-infected individuals and SIV (simian immunodeficiency virus)- or SHIV (simian-human immunodeficiency virus)-infected macaques [1–6], but their rate of development needs further study. We recently examined the kinetics of T-cell escape in macaques [7], carefully evaluating the proportion of wild-type and escape variant clones by sequencing individual clones over short time intervals (every 3–4 days following infection). Interestingly, for an immunodominant SIV Gag epitope KP9 in pigtail macaques, when escape variants emerge they rapidly dominate viral quasispecies within one week (Figure 1a, black line). The loss of wild-type viruses in this scenario reflects preferential cytotoxic T lymphocyte (CTL) killing of wild-type-bearing cells. Slower patterns of escape at other epitopes reflect lower rates of killing by CTL (Figure 1a, blue line). Thus, the rate of T-cell escape, once it starts, is an *in vivo* measure of the effectiveness of CTL targeting of different epitopes. Where escape does not occur (Figure 1a, red line), this reflects either (i) that there is no CTL pressure for loss of wild-type sequence, (ii) that the loss of fitness incurred by any potential escape mutation is even greater than the loss of fitness experienced by the wild-type virus as a result of CTL pressure, or (iii) that insufficient replication has occurred for the escape mutation to be generated (e.g. with effective drug therapy or effective T-cell responses at other epitopes).

When viruses are transmitted to new hosts that do not share the same major histocompatibility complex (MHC) alleles as the donor, redundant T-cell escape mutations (i.e. at epitopes restricted by MHC not present in the new host) will revert if they cause a significant fitness cost. Recent reports in humans with HIV-1 infection and macaques with SIV infection document reversion of some T-cell escape variants upon transmission to new hosts [8,9]. The fitness impact of T-cell escape variants can thus be estimated by the rate of reversion of escape mutant to wild-type virus [7], which will be proportional to the fitness impact of the mutation. T-cell escape variants with the highest fitness cost (Figure 1b, black line) will very rapidly – over the first 14 days of infection – revert back to wild-type, and those with minimal impact will revert more slowly or not at all (Figure 1b, red and blue lines). Thus, the rate of reversion can be used to calculate the *in vivo* fitness cost of the immune escape mutations.

When a T-cell escape virus is transmitted to a host that expresses the MHC allele restricting the wild-type epitope and is capable of responding to that epitope, the T-cell escape mutation is typically retained at late time points [8,9]. Less is understood about retention of escape variants very early after infection. We recently studied macaques vaccinated against a wild-type CTL epitope and that were subsequently challenged with a T-cell escape variant virus; the kinetics of virus reverting to wild-type early after infection were analyzed [7]. As previously described [8,9], at late time points after infection, the T-cell escape variant is the only detectable viral quasispecies, reflecting the selective pressure of CTLs initiated by vaccination and expanded by infection. However, in animals vaccinated ineffectively with low levels of CTLs, significant levels of reversion start to occur over the first 1–2 weeks of infection, initially with a similar rate to animals not capable of responding to this epitope (Figure 1c, black line). However, by 2–3 weeks, when effective immune responses are being rapidly expanded or generated [10], the selection for the escape mutant kicks in and wild-type viruses are rapidly eliminated. In animals vaccinated more effectively with higher levels of pre-primed immunity, such as with DNA prime/poxvirus boost vaccines [11], the escape mutant is strongly selected for at early time points, and initial reversion is minimal or absent (Figure 1c, red and blue lines). Barouch *et al.* [12] also recently showed that late transient reversions of SIV CTL escape mutants occur, which serve to expand memory CTLs, although the kinetics of such events were not studied in great detail.

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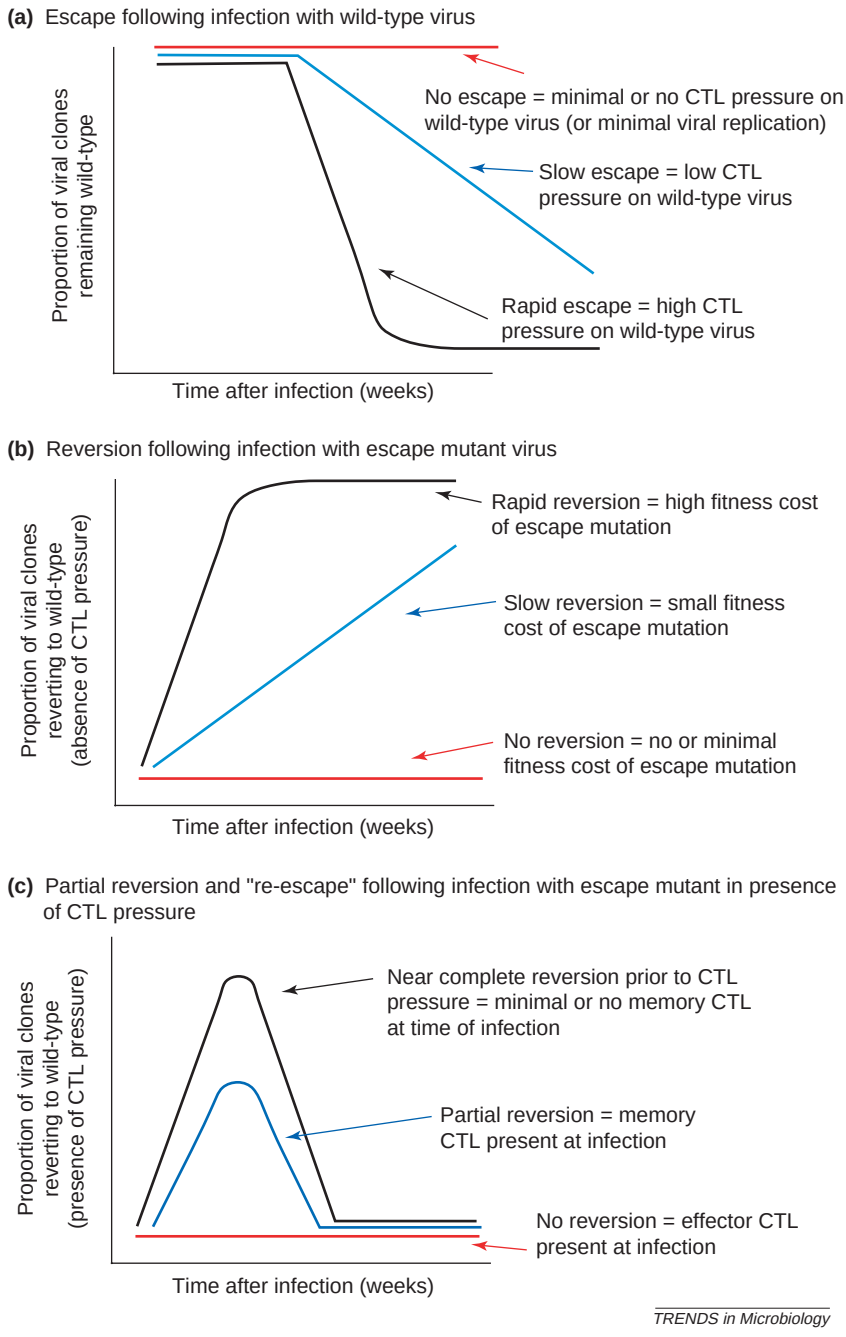

Figure 1. Scenarios where the effectiveness of simian immunodeficiency virus (SIV)-specific CTL responses *in vivo* in macaques can be estimated from kinetic analyses of the proportion of wild-type and escape mutants identified by sequencing viral clones. Three examples of effective, partially effective or ineffective CTLs are presented in each scenario. **(a)** Escape from wild-type sequence in the presence of CTL pressure. The rapidity of outgrowth of escape variants, once escape begins, reflects the efficiency of killing of cells infected with the wild-type epitope. **(b)** Reversion of escape mutations in the absence of CTL pressure. The rapidity of reversion reflects the fitness cost of the mutations. **(c)** Reversion and re-escape of CTL escape mutations following transmission of escape variants in the presence of CTL pressure (e.g. by prior vaccination). The degree of reversion occurring reflects the presence and effectiveness of immunity at the time of infection.

The levels of virus present in chronic infection are determined by the net effects of the suppression of wild-type virus because of CTL activity and growth restriction of escape mutant virus as a result of impaired viral fitness. Where CTL activity is very weak, then only a relatively fit escape mutant virus can compete with it (Figure 2). By contrast, in the presence of a strong CTL response, then even a severely impaired mutant will dominate over wild-type virus. Detailed analysis of the kinetics of escape mutant and revertant viral quasispecies thus provides a basis for understanding the effectiveness of a particular immune response. Measuring the rate of reversion of escape mutant virus to wild-type virus provides a good estimate of the relative fitness of the mutant compared with the wild-type virus, and indirectly provides a measure of the strength of the CTL response to the wild-type virus *in vivo*. Immunization with the aim of inducing the most effective immune responses, and ignoring those that have no or minimal impact on viral replication, should rationally improve the efficacy of vaccines for chronic viral infections, such as HIV.

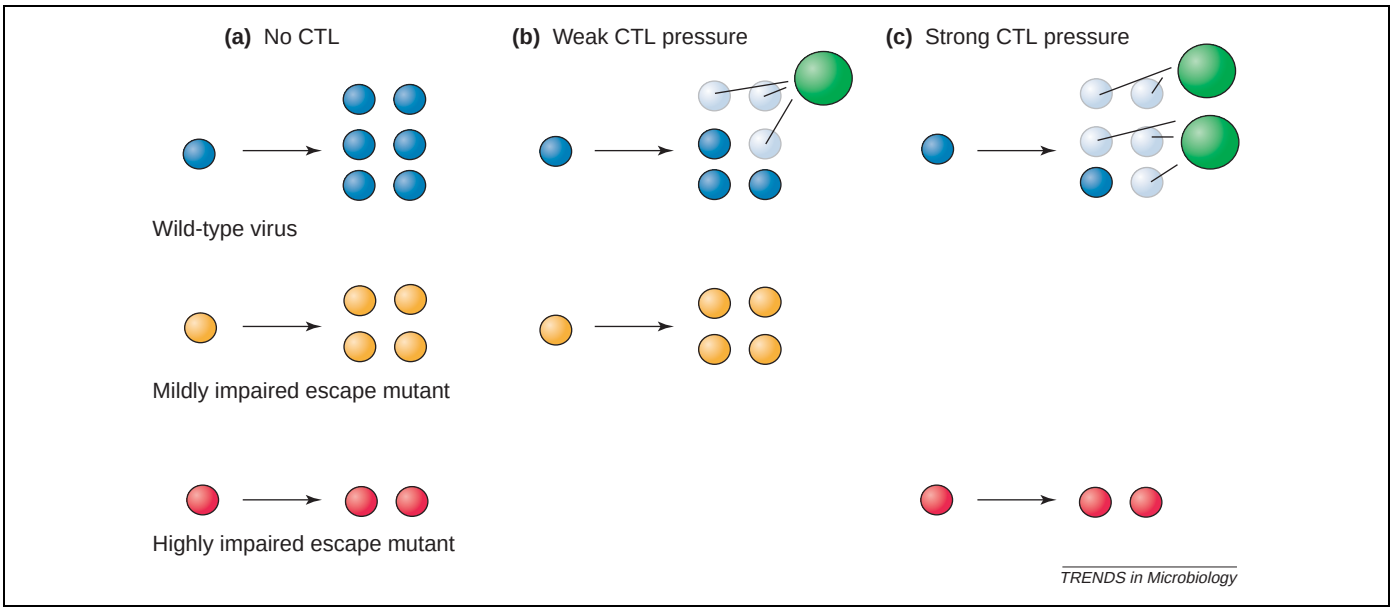

Figure 2. The degree of permissible fitness impairment in T-cell escape viral variants is proportional to CTL pressure. **(a)** Wild-type virus dominates in the absence of CTL pressure. **(b)** A mildly impaired virus can be selected in the presence of weak CTL pressure, whereas a strongly impaired escape mutant cannot. **(c)** A strongly impaired mutant can only be selected in the presence of strong CTL pressure.

Compensatory secondary mutations might subsequently emerge to partially restore virus fitness, overcoming the functional and structural constraints of the virus [13–15]. Antigen-processing mutants that also avoid CTL responses have been described recently [16]. Although antigen-processing mutations could also result in some fitness cost, these mutations might dominate viral quasispecies over other simpler escape mutants if they occur at a lower fitness cost. The eventual selection of dominant escape mutants, even at the RNA level [17], reflects the mutants with the most favourable combination of the low fitness cost, high level of immune evasion and greatest flexibility.

Although we have primarily discussed fitness costs surrounding T-cell escape mutant viruses, similar issues are probably faced for envelope mutations reverting during transmission [before the generation of effective neutralizing antibody (NAb) responses]. Earlier publications have demonstrated evolution of envelope changes during early infection [18], probably reflecting escape [19] and reversion at NAb epitopes. Derdyn *et al.* [20] recently demonstrated that many viruses isolated during acute infection are neutralization-sensitive [20], suggesting a reversion to neutralization resistance in the absence of antibody pressure, and thus a fitness cost to neutralization resistance. Envelope-specific T-cell responses have been shown to be particularly effective in control of HIV, SIV and SHIV infections in several publications [21–24], raising the intriguing possibility that there might be constraints on viral escape from both T-cell epitopes and NAb epitopes unique to the envelope.

In summary, the kinetics of escape and revertant SHIV viruses in vaccinated macaques provide insight into the *in vivo* efficacy of CTL responses. The rate of *in vivo* escape reflects how effective CTLs are at killing wild-type viruses, and the rate of reversion reflects the resultant *in vivo* fitness cost of the T-cell escape mutant. Whether escape mutant viruses transiently revert when passaged to a host capable of responding to the wild-type epitope will depend on the *in vivo* level of pre-primed T-cell responses before infection. This set of information, when applied across various T-cell responses, should rationally select the most effective T-cell immune responses to induce by vaccination.

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Blueprinting the regulatory response of *Escherichia coli* to the urinary tract

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Recent work has shown how comparative genomic and microarray analyses can provide insights into the transcriptional state of uropathogenic *Escherichia coli* (UPEC) during infection. This study will serve as an important platform from which to identify virulence determinants and the principle mechanisms of adaptation to the urinary tract.

Introduction

Transcriptional control is a central regulatory mechanism in bacterial–host interactions [1–4]. Microarray analyses have provided powerful insights into the expression states of tissues and cells, both eukaryotic and prokaryotic in origin. Expression analyses can demonstrate important genes that respond to environmental cues and can also provide clues as to how a tissue or cellular population will correspondingly respond. Importantly, microarray expression analyses provide a blueprint for future experimental studies by identifying temporally important factors for specific molecular investigations.

In vitro transcriptome analyses have been routinely successful because of the large number of organisms that can be recovered for RNA isolation. By increasing the scale of tissue culture infections, microarray analyses have been performed on infected epithelial and macrophage cell lines using *Neisseria meningitidis*, *Mycobacterium tuberculosis* and *Salmonella enterica*, among others [5–7]. However, most of the previous *in vivo* microarray analyses have been hindered by the difficulty of recovering sufficient bacterial mRNA from a host during the appropriate stages of the infections. Therefore, the majority of the previous *in vivo* studies have come from animal models in which the bacterial load becomes large in localized tissue regions, such as for *Streptococcus pyogenes* abscess formation in a skin infection model [8]. Additional successful *in vivo* studies include enteric infection with *Vibrio cholerae* [9,10].

Microarray analysis of UPEC *in vivo*

In their recent paper entitled 'Transcriptome of uropathogenic *Escherichia coli* [UPEC] during urinary tract infection', Snyder *et al.* describe a microarray analysis of bacterial populations in the urinary tract during a mouse bladder infection model [11]. This report is important because it provides a blueprint of the transcriptome of bacteria residing in the luminal compartment throughout the urinary tract. Information gleaned from this analysis has important ramifications on the potential involvement of diverse bacterial programs during infection, including

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