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HIV CTL escape: at what cost?

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Abstract

Recent data have established the HIV-1 and SIV escape CTL through epitope mutation. However, three novel studies prove that such escape comes at a "cost" to overall viral fitness. Understanding how HIV-1/SIV escape CTL and the impact of the escape mutations has tremendous importance in developing CTL based vaccines. Further, a CTL based HIV-1 vaccine is likely to have long-term protective effect against disease only if the escape virus is significantly weakened compared with wild type.

Data on humans infected with human immunodeficiency virus type 1 (HIV-1) and macaques infected with simian immunodeficiency virus (SIV) suggest that cytotoxic T-lymphocytes (CTL) are important in controlling virus replication [1]. CTL target infected cells by recognizing viral peptides (8–10 residues in length) bound to class I major histocompatibility complex (MHC) molecules. Different MHC alleles bind viral peptides with different affinities. MHC alleles, which bind particular viral peptides well, often result in strong cellular responses against those viral epitopes. In theory, if CTL are important in controlling or reducing HIV-1 replication *in vivo*, then virus mutants that avoid this cellular response should have a replicative advantage in the host. Indeed, several studies in both macaques and humans have documented the presence of virus mutants that escape the dominant CTL response and become the major replicating strain *in vivo* [2,3]. In most examples to date, the mutant or escape virus replicates efficiently and viral loads remain considerable. However, the impact of CTL escape mutations on viral fitness has not been extensively examined.

Recently, two groups addressed whether CTL escape mutants are virologically less fit than their wild type counterparts [4,5]. In side-by-side papers published in the March 2004 issue of Nature Medicine, Friedrich et al. [4] examined SIV in the macaque model, while Leslie et al. [5] studied HIV-1 in human mother-infant pairs. In the SIV/macaque model, earlier reports have established three discrete CTL escape epitopes associated with two MHC class alleles [6]. Two of these epitopes, located in Gag and Tat, respectively, are bound by Mamu-A*01, while the third epitope, located in Nef, binds Mamu-B*17. In rhesus macaques with the cognate class I alleles, each of these epitopes is seen at high frequency following infection with SIVmac239 [6]. None is seen in SIVmac239-infected macaques that lack Mamu-A*01 or Mamu-B*17 alleles.

Studies by Friedrich and colleagues demonstrated that *in vitro* replication of SIVmac239 encoding escape epitopes in Gag, Tat, and Nef (termed 3x SIV) is impaired compared to wild type virus [4], suggesting a relatively lower level of fitness. Infection of two rhesus macaques negative for either Mamu-A*01 or Mamu-B*17 also yielded interesting results. In the first four weeks after infection with 3x SIV, mutations in all three, escape epitopes were maintained. By week 16 the Gag mutant had reverted towards wild type and by week 20 the Nef mutations had also reverted. The Tat peptide did not revert. During these 20 weeks, the viral loads of these animals did not change

appreciably. However, these reversions to wild type after 4 to 5 months of infection strongly suggest that these CTL escape mutants are less fit *in vivo* than wild type viruses in the absence of immune pressure.

Similar conclusions were reached in analyses of HIV-1 vertical transmission in mother-infant pairs, in which specific Class I alleles are not shared [5]. In HIV-1 infection of humans, two closely related HLA molecules, B57 and B5801, are associated with relative control of virus replication [7-10]. The dominant CTL response associated with HLA-B57 positive individuals is against a Gag peptide, TW10. Significant variation in this peptide is seen after HIV-1 transmission from HLA-B57/B5801 negative individuals to those who carry either allele, although not all clinical progressors, who carry HLA-B57, have virus with escape mutations in TW10 [11]. Analysis of two mother-infant pairs after vertical transmission of HIV-1 revealed that the mothers, who were each HLA-B57/B508 positive, had CTL escape mutations in TW10. The two infants, each negative for HLA B57/B508, were initially infected with virus carrying these mutations. However, over the ensuing months, the escape mutations in TW10 reverted to wild type in both infants. Taken together, these results strongly support the idea that at least some CTL escape mutations come at a cost to overall viral fitness, and this cost may be enough to prevent any lasting impact on overall viral evolution.

In a recent study of SIV pathogenesis, we observed similar, striking results for CTL escape mutants [12]. To evaluate the role of the second coding exon of Tat *in vivo*, we engineered a mutant of SIVmac239 with dual stop codons at the end of the first exon of Tat (SIVtat1ex). These stop codons had no effect on virus replication *in vitro* and resulted in minor amino acid changes in Rev that did not affect its function. Comparison of four rhesus macaques (L840, L855, L882, N200) infected with SIVtat1ex with control macaques infected with wild type SIVmac239 showed no differences in viral load or $CD4^+$ T-cell counts during acute infection. Subsequently, we observed that two of the macaques infected with SIVtat1ex (L840, L855) completely controlled viremia, while two (L882, N200) had appreciable viral loads. Three months after infection, we examined the stop codons at the end of the first exon of Tat. In one of the animals with persistent viremia (L882), the virus had mutated the stop codons to encode the second exon of Tat. This animal had a very high viral load and low $CD4^+$ T-cell counts. Nine months post-infection (p.i.) it became ill with simian AIDS and was euthanized.

Analysis of viruses from the other three macaques indicated that the stop codons in Tat remained intact for 8 months p.i. At this time, the *tat* reading frame from one of these animals (L840) opened up and this reversion was associated with a decrease in $CD4^+$ T-cell counts and a modest increase in viral load. However, at day 548 p.i., the stop codons in *tat*'s first exon re-emerged and this reversion was coincident with an increase in $CD4^+$ T-cell counts and a reduction in viral load to undetectable levels. The stop codons at the end of the first exon of *tat* persisted for four years and this animal remains healthy.

Viruses from the other macaque that initially controlled viremia (L855) maintained the stop codons in *tat*'s first exon for over one year p.i. However, between days 413–485 p.i., the viral load in this animal increased from undetectable to $\sim 10^5$ copies/ml. Analysis of virus during this period revealed that the stop codons in *tat* had opened up and the virus now encoded both exons of Tat. The $CD4^+$ T-cell count, which had been stable in this animal, began to steadily decrease and its viral load increased to 10^6 copies/ml. The stop codons in *tat*'s first exon did not re-emerge. This animal eventually developed simian AIDS and was euthanized.

We next sought to determine why virus from one infected macaque (L840) transiently opened up the stop codons in *tat*, whereas viruses from two other infected macaques (L882 and L855) permanently reverted to encode both Tat exons. Western blot analysis showed similar anti-SIV antibody responses in L840 and L855 (L882 had only a weak antibody response). Using a γ-interferon ELISpot assay, we found that L840 had a much stronger cellular response against Tat second exon peptides than L882 or L855. These data, taken together, suggest the following:

1. SIV encoding two-exon Tat is more fit than SIVtat1ex *in vivo*.

2. Reversion from SIVtat1ex to SIV encoding two-exon Tat is associated with increased viral loads and decreased $CD4^+$ T-cell counts.

3. In L882 and L855, a limited CTL response specific for the second exon of Tat developed and when the virus reverted, little immune pressure was placed on the new virus. On the other hand, L840 mounted a strong CTL response against epitopes in the second exon of Tat. The immune selection favored the re-emergence of SIVtat1ex as the predominant virus.

4. In the absence of immune pressure, SIV encoding two-exon Tat is more fit than SIVtat1ex (Figure 1), but in the setting of strong immunologic pressure, SIVtat1ex is more fit, albeit less pathogenic (Figure 2).

Curiously, virus from macaque L840 evaded Tat second exon-specific CTL by truncating the protein, rather than

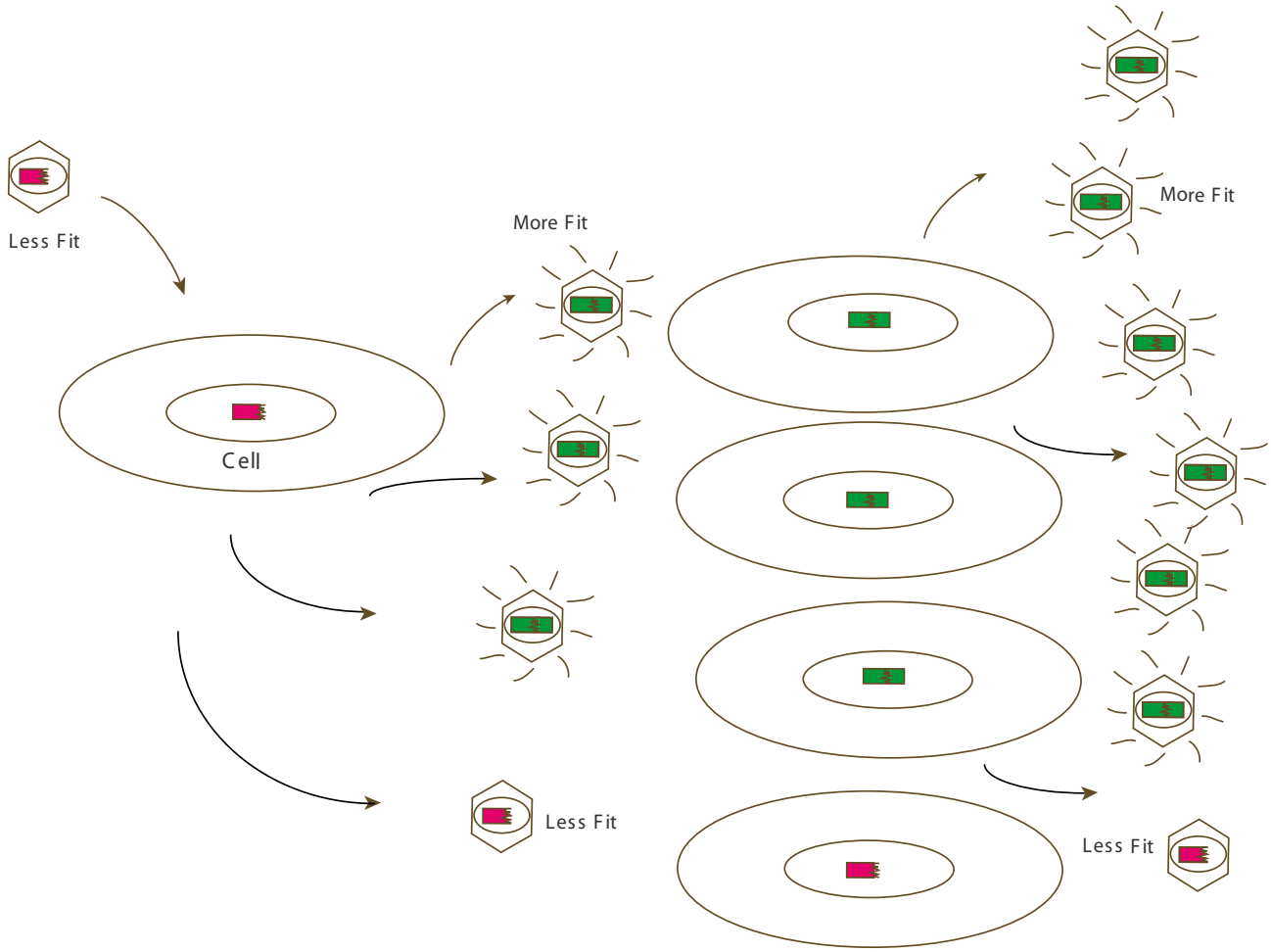

Figure 1
Cartoon representation of SIVtat1ex fitness *in vivo*. In the absence of CTL against Tat second exon epitopes (Tat 2 ex), SIV encoding two-exon Tat are more fit and replicate to higher levels. The single exon Tat genome is represented by a pink bar with a jagged edge; the two-exon Tat genome is depicted by a green rectangle. Viruses are illustrated as hexagons with inner ovals.

by simple amino acid mutations within the epitope. As in the papers described above, most HIV/SIV escape mutants have 2 to 3 amino acid changes in the dominant epitope [3]. Presumably, amino acid escape mutations in the Tat second exon epitope(s), targeted in L840, resulted in a substantial decrease in viral fitness. To our knowledge, this observation is unique in the HIV/SIV CTL literature. The second exon of *tat* is overlapped completely by *rev* and *env* and, as a consequence, any nucleic acid mutation in this region affects all three genes. Perhaps the necessity of maintaining these overlapping reading frames constrained the ability of Tat to sustain point mutations in the second exon and prevented escape from CTL.

Many current vaccine strategies for HIV and SIV in the non-human primate model are CTL-based (reviewed in [13,14]). Given what is known about CTL, it is probable that HIV and SIV can evade any specific CTL clone or clones. The purpose of a CTL-based vaccine against HIV-1 is, therefore, not to prevent viral escape but rather to force the virus to mutate to a less fit phenotype. We are currently studying this hypothesis by evaluating CTL against Rev, Tat and Env in the SIV/macaque model. By inducing immune responses against these overlapping regions, we hope to demonstrate that mutations associated with CTL escape will result in a virus that is significantly less pathogenic than wild type. In almost all cases, changing a base

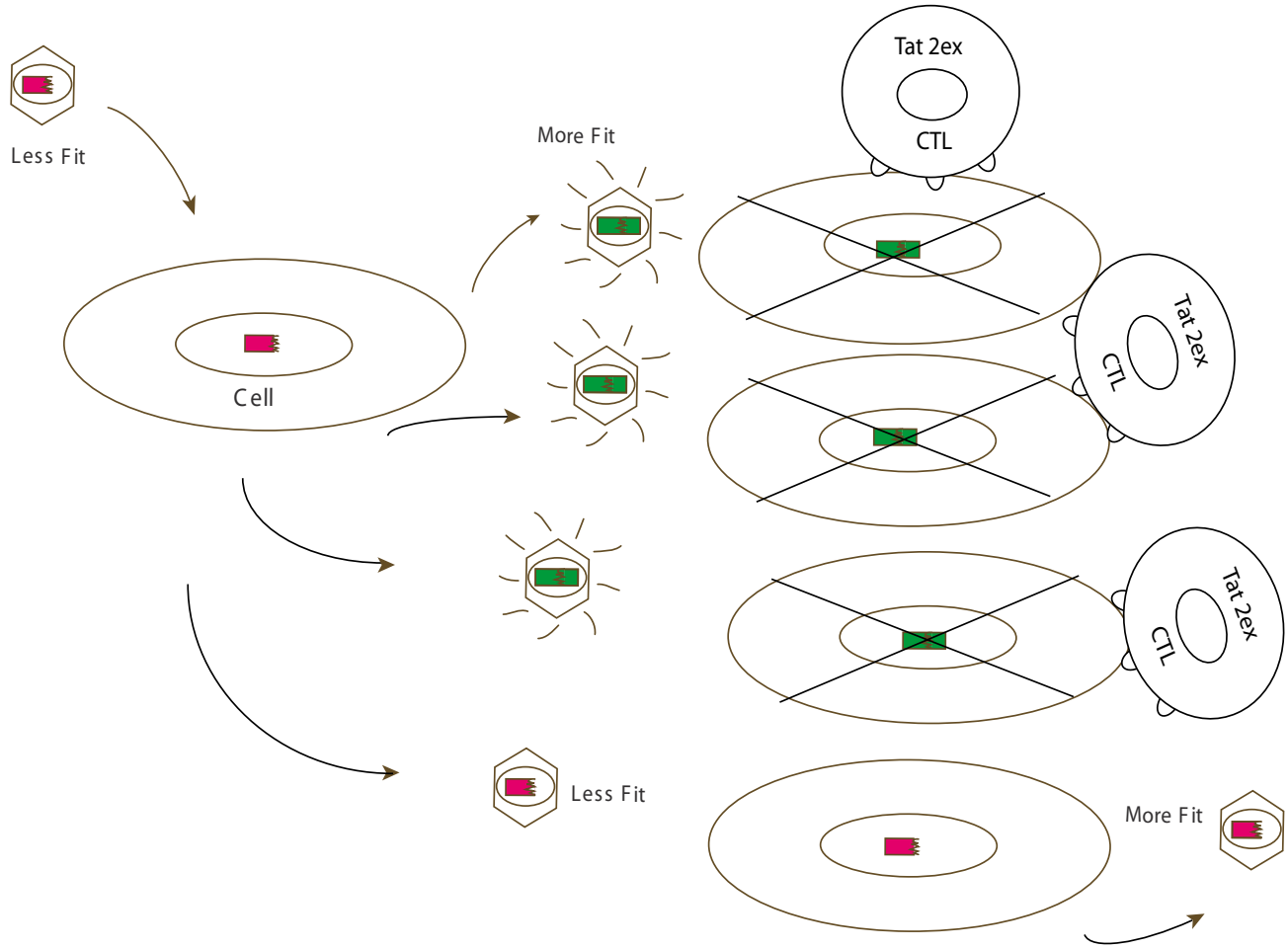

Figure 2
Cartoon representation of SIVtat1ex fitness *in vivo*. In the presence of specific CTL response against these epitope(s), cells expressing two-exon Tat are eliminated and SIVtat1ex becomes relatively more fit, albeit at lower level overall. The single exon Tat genome is represented by a pink bar with a jagged edge; the two-exon Tat genome is depicted by a green rectangle. Viruses are illustrated as hexagons with inner ovals.

pair in this region of HIV or SIV changes the encoded amino acid in at least two reading frames. For example, changing the nucleotide in the first position of any *tat* second exon codon also changes the translation of the overlapping *env* codon. Consistent with this approach, the goal of all CTL-based vaccine strategies should not only be to raise significant cellular responses against viral epitopes, but also to choose epitopes of which mutation results in a significant decrease in viral fitness. Thus far, encouraging results in our and these recent studies [4,5] suggest this may be possible; however, determining which epitopes have these properties is an empirically difficult task.

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