HIV evolution in response to HLA-restricted CTL selection pressures: a population-based perspective

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Note: This is the non-copy-edited version of a paper appearing in the Journal of Microbes and Infection. Please cite as follows:
Jonathan M. Carlson and Zabrina L. Brume, HIV evolution in response to HLA-restricted CTL selection pressures: a population-based perspective(2008), *Microbes and Infection* (2008), **10**(5):455-61, doi:10.1016/j.micinf.2008.01.013

Abstract

Cytotoxic T-lymphocytes (CTL) recognize antigenic peptides displayed by HLA class I molecules on the infected cell surface and represent a major selective force driving HIV evolution through a phenomenon known as "immune escape". Here we summarize recent advances in our understanding of the consequences of CTL escape on HIV evolution at the population level and discuss its implications for HIV vaccine design.

Keywords: Human Immunodeficiency Virus (HIV), Human Leukocyte Antigen (HLA), Cytotoxic T-lymphocytes (CTL), immune escape, viral evolution, vaccine

Introduction

Since its identification as the pathogenic cause of Acquired Immunodeficiency Syndrome (AIDS) in the early 1980s, Human Immunodeficiency Virus Type 1 (HIV-1) has emerged as a major global pandemic with an estimated 33 million infected individuals worldwide at the end of 2007 [1]. Specifically targeting the CD4+ subset of T-lymphocytes, HIV-1 causes a progressive deterioration of immune function, leaving the infected individual susceptible to a range of opportunistic infections eventually leading to AIDS and death. Although improvements in antiretroviral therapy have dramatically reduced HIV-related morbidity and mortality among those with access to treatment [2], the search for an effective HIV-1 vaccine continues.

The extraordinary mutational capacity of HIV-1 represents a major challenge to vaccine development [3,4]. On average, the error-prone HIV-1 reverse transcriptase introduces one mutational "error" per replication cycle, while template-switching and recombination represent additional mechanisms for generating alternative viral species [3]. Within an infected individual, a progressive expansion of viral diversity occurs over the disease course [5], with multiple variants co-existing as a heterogeneous swarm or

"quasispecies" that is unique to each patient. On a global scale, HIV-1 has undergone dramatic diversification since its introduction into humans less than 100 years ago [6]: nucleotide sequences from the multiple viral subtypes and circulating recombinant forms comprising HIV-1 Group "M" strains (which account for the majority of infections worldwide) may differ by up to 35-40% [4]. Achieving a broader understanding of the factors driving viral evolution on both an individual and a global level is thus of paramount importance to vaccine design.

Over the natural course of infection, the host immune response acts as a major selective force driving HIV-1 evolution in a continuous dynamic process known as "immune escape" [7]. Directed against three-dimensional epitopes on the virion surface, the role of antibodies is to neutralize free-floating virus or to tag them for destruction by effector cells or complement. Escape from the HIV-1-specific antibody response thus takes the form of amino acid substitutions within the viral Envelope protein and represents a main driver of both intra-individual and global HIV Envelope diversity [8]. In contrast, the role of cytotoxic T-lymphocytes (CTL) is to eliminate virus-infected cells through recognition of short, linear peptides processed intracellularly and presented on the cell surface by Human Leukocyte Antigen (HLA) class I molecules. Since peptides from all viral proteins have the capacity to bind and be presented by class I molecules, HLA-restricted CTL select for escape mutations on a proteome-wide basis. This fact, combined with the observation that CTL likely contribute more to immune control of HIV-1 infection than antibodies [9], highlights CTL as a potentially major in vivo selective force driving genome-wide viral evolution.

It is generally agreed that a successful HIV-1 vaccine will require stimulation of an effective CTL-based immune response in addition to an antibody response [9]. The recent suspension of a major CTL-based HIV-1 vaccine trial [10] underscores the need to improve our understanding of host antiviral immunity, including the impact of immune-driven viral adaptation on HIV-1 sequence diversity and its potential consequences for future vaccine strategies. In this review, we summarize recent advances in our knowledge of CTL-driven HIV evolution at a population level and discuss the implications for vaccine design.

The HLA-restricted CTL response is a major selective force driving HIV-1 evolution within an infected host

HLA-restricted CTL are major mediators of host antiviral control during HIV-1 infection [7]. A temporal correlation exists between the appearance of HIV-1-specific CTL in vivo and the decline of acute-phase viremia [11] (antibodies appear only later [7,9]), and experimental depletion of CD8+ cells in rhesus macaques prior to Simian Immunodeficiency Virus (SIV) infection results in inability to control virus levels [12]. In addition, a strong epidemiological link exists between specific HLA class I alleles and differential rates of HIV-1 disease progression [13], suggesting that the quality of the CTL response and/or the characteristics of targeted epitopes strongly influences the effectiveness of antiviral control [7]. However, the strongest evidence supporting CTL as a major determinant of HIV-1 control may be mutational immune escape. First described in 1997, selection of viral escape mutations within key CTL epitopes during primary [14] and chronic [7,15] HIV-1 infection identified immune-driven evolution as a continuous process occurring throughout the disease course. Although escape mutations are often

selected within CTL epitopes (thus disrupting peptide-HLA binding or recognition of the peptide/HLA complex by the T-cell receptor), escape is by no means confined to epitope boundaries. Mutations in epitope flanking regions, which impair intracellular peptide processing and presentation, have also been described [16], as have secondary or compensatory sequence changes that can stabilize escape mutations selected elsewhere [7,17].

Escape follows generally predictable patterns in response to specific immune pressures

In the past decade, observational studies have identified a large number of CTL escape mutations that are reproducibly selected in the context of specific HLA restrictions [7,14,15,17,18,19]. This has led to a monumentally important observation: HIV-1 evolution follows generally predictable patterns when specific immune pressures are applied. This phenomenon was most strikingly demonstrated in a unique case-report of monozygotic twins infected with the same virus on the same day through needle sharing: over a three-year follow-up period, the kinetics and patterns of CTL and antibody escape mutations were nearly identical in both twins [20]. Even among unrelated individuals, kinetics and patterns of HIV-1 evolution are broadly predictable based on HLA restriction. The majority of persons expressing the "protective" HLA-B*57 allele [13], for example, will select for a T to N mutation at position three of the TW10 epitope in the p24 Gag protein within the first weeks after infection [7,18]. In B*27-expressing individuals, the first mutation that arises in the immunodominant Gag epitope KK10 is an L to M change at position six, followed years later by an R to K change at position two [7,19]. The fact that sites and pathways of escape are broadly predictable indicates that despite the extensive worldwide sequence diversity of HIV-1,

substantial constraints govern the evolution of this virus [20,21]. This raises the possibility that immunogens incorporating knowledge of common escape pathways may be designed. Until relatively recently, however, identification of escape mutations have generally been limited to smaller observational studies, and largely biased towards "protective" HLA alleles associated with long term viremic control [7,14,15,17,18,19].

Immune selection pressures drive HIV evolution at the population level: but to what extent?

If within-host CTL escape patterns are broadly predictable based on HLA profile, the frequency and distribution of HLA alleles in humans likely shape viral evolution at the population level in a similarly predictable manner. Indeed, the "HLA footprinting effect" hypothesis states that the circulating HIV-1 consensus sequence reflects viral adaptation to the most commonly-expressed HLA alleles in a population [22,23]. One potential mechanism underlying this hypothetical footprinting effect is the repeated selection of fitness-neutral escape mutations in the context of frequently-observed HLA alleles, eventually leading to fixation of "inactive" forms of CTL epitopes in the circulating pool of viral strains and potentially rendering HIV less immunogenic as the epidemic progresses [22,23]. Alternatively, a bottleneck effect may have occurred early in the course of the pandemic. Under this hypothesis, escape mutations arising in the earliest patients persist in the circulating strain. In either scenario, persisting escape mutations must have minimal fitness cost in the absence of CTL to prevent reversion back to the susceptible form following transmission.

The role of CTL in shaping population HIV-1 sequence diversity is influenced by a complex interaction among multiple conflicting selective forces, one example being the

delicate balance between the benefits of escape versus the associated costs to viral fitness [24]. While escape mutations, by definition, confer a selective advantage under active CTL pressure, these mutants may not represent the most efficiently replicating species in the absence of immune pressure. For example, the dominant T242N escape mutation at position three of the B*57-restricted TW10 epitope in p24 Gag abrogates B*57-epitope binding [18], but also confers a substantial replicative cost in the absence of CTL pressure as demonstrated by rapid reversion to wild type following transmission to a B*57-negative individual [18] and in vitro assays measuring viral fitness [25]. Reversion of escape mutations following transmission to an individual lacking the HLA allele, however, does not necessarily occur in all cases: the fitness cost of the substitution, the presence of compensatory mutations, and a complex array of other host and viral selective forces influence which immune-selected mutations may reach appreciable levels in the population [18].

Estimating the extent of immune imprinting on HIV-1 is additionally challenging due to the lack of a comprehensive map of HLA-associated escape sites across the viral genome. Until recently, studies of CTL escape have focused on select alleles and/or HLA-restricted epitopes in small observational studies; however, recent advances in DNA sequencing technologies have facilitated the collection of HLA and HIV-1 sequence data in large cohorts of HIV-infected individuals, thus allowing the first population-based assessments of HLA-driven imprinting on the viral genome.

Assessing the extent of HLA-driven HIV-1 evolution at the population level: challenges and controversies

The first study investigating HLA-mediated imprinting on HIV-1 at the population level was published by Moore et al. in 2002 [22]. Specific HLA alleles associated with the presence or absence of the consensus amino acid over codons 20-227 of the Reverse Transcriptase protein were identified in a cross-sectional analysis of >400 clinically-derived HIV-1 sequences from Western Australia, using Fisher's exact test and logistic regression. A total of 64 "positive" and 25 "negative" correlations (where the HLA was respectively associated with divergence from, or preservation of, the consensus residue) were identified. Positive correlations confirmed the predictable selection of HLA-restricted escape mutations and highlighted the substantial portion of viral codons whose evolution is driven by escape. Negative associations, on the other hand, were interpreted as evidence to support the HLA footprinting effect; in other words, confirmation that the predominant circulating HIV-1 sequence had arisen in response to selection pressures imposed by the most commonly-expressed HLA alleles in the population [22].

There is one major concern, however, with this type of statistical association study. Evolutionary biologists have pointed out that standard tests of association that assume independence (such as the Fisher's exact test or logistic regression) are inappropriate for the analysis of inter-species (or in this case, inter-strain) data, because sequences with a shared phylogenetic history do not represent statistically independent observations [26]. This problem is most apparent in the analysis of heterogeneous cohorts, where standard tests will identify correlations between subtype-specific viral polymorphisms and HLA alleles prevalent among individuals infected with those subtypes. In this case, results do not necessarily indicate that these polymorphisms are

selected under contemporary immune pressures; rather, they more likely reflect a correlation between HLA alleles enriched in particular human populations and a subtype (or lineage)-specific viral polymorphism shared by all sequences in this branch of the tree (a so-called "founder effect") [27]. Even in relatively homogeneous cohorts, failure to account for the evolutionary relatedness among HIV-1 sequences leads to increased variance in traditional statistical tests and thus uncertainty in interpreting the results [28].

To address this issue, Bhattacharya et al. [27] proposed two novel methods to account for the underlying phylogenetic structure of HIV-1 sequences when identifying sites of CTL-mediated selection. These approaches attempted to identify associations that were unusual in the context of a shared lineage while statistically downplaying associations that could have been explained by neutral evolution. Their methods were used to identify HLA-associated polymorphisms in the Gag and Protease proteins in a mixed-clade dataset of 96 sequences from the same cohort investigated by Moore et al. [27]. By comparing associations identified using standard vs. lineage-corrected analyses, Bhattacharya et al. [27] demonstrated that a number of associations identified by the original logistic regression method actually represented spurious associations that were better explained by founder effects. The authors noted however that phylogenetic correction achieves more than simply the elimination of spurious associations due to lineage effects. Consideration of tree structure also identified novel associations that were overlooked by the uncorrected analysis, thus allowing the potential for increased power compared to standard methods (though a formal power analysis was not undertaken) [27]. Overall, however, in the 96 sequences analyzed by Bhattacharya et al., only 14 strong HLA allele/HIV-1 sequence associations were identified [27]. The relative paucity of

associations identified by Bhattacharya et al. [27] compared to Moore et al. [22] led some to question the contribution of HLA-mediated selection pressures to viral evolution [29].

What followed was a heated scientific debate regarding the extent of HLA-mediated viral imprinting at the population level. Was the data we previously accepted to be strong evidence of immune-driven viral evolution simply explained by founder effects? Not necessarily. Not only did the Moore [22] and Bhattacharya [27] analyses feature different HIV-1 gene regions sequenced from different patient groups, but the latter was based on a dataset less than one-quarter the size of the former, and thus had substantially reduced power to detect associations. Indeed, mathematical modeling experiments suggest that the false-negative rate (i.e. the % of true associations missed) may be over 80% in a dataset of N=100 (J. Carlson et al., unpublished data), and Bhattacharya et al. themselves emphasized that their results should not be interpreted as evidence that immune pressure is a weak force in HIV-1 evolution [27]. Rather, the results underscore the importance of disentangling the effects of immune selection from founder effects and emphasize the need for larger datasets to determine the extent to which immune imprinting shapes HIV-1 diversity at the population level.

HLA-associated immune pressures influence population HIV diversity at up to 40% of positions in some proteins

Soon after the Bhattacharya et al. publication [27], the first large-scale, population-based analysis of HLA-mediated imprinting on multiple HIV-1 genes was published [30]. Using the newly-developed lineage corrected methods [27], nearly 500 HLA-associated polymorphisms in the HIV-1 Protease, Reverse Transcriptase, Vpr and Nef proteins were identified in a cohort of ~700 chronically-infected, antiretroviral naïve

individuals [30]. Polymorphisms were dichotomized based on the direction of selection pressure: “escape” and “reversion” associations represented amino acids enriched in the presence or absence of a specific HLA allele, respectively. As expected, “escape” and “reversion” associations generally represented non-consensus and consensus residues, respectively, although some exceptions were noted. In addition, HIV-1 codons under diametrically opposed HLA selection pressures (where the “escape” amino for one allele represented the “immunologically susceptible” form for another, and vice versa) were identified, highlighting a dynamic tug-of-war of immune selective pressures influencing HIV-1 diversity at the population level [30]. Of note, the fact that the majority of identified associations fell outside the boundaries of known epitopes indicated that escape is not confined to single point mutations selected within regions directly targeted by CTL; rather, immune pressures select for a broad range of polymorphisms on a protein- (and genome-) wide level.

In addition, substantial differences in the number of immune selection events were observed among HIV proteins [30]. Nef exhibited the greatest evidence for immune adaptation, with ~40% of its codons harboring at least one HLA association. In contrast, ~10-15% of codons in Protease, Reverse Transcriptase, and Vpr exhibited evidence for HLA-mediated selection. More recent work suggests that the portion of Gag codons exhibiting HLA-associated substitutions is slightly higher than Pol (Brumme et al, unpublished data). Taken together, data indicate that amino acid variation at minimum of 10-40% of HIV-1 subtype B codons is driven by HLA class I-associated immune pressures, even after correction for lineage effects [30]. This re-confirms that CTL immune selection substantially shapes HIV-1 diversity at the population level.

Remaining challenges and future directions

Despite recent advances in the field, a genome-wide map of HLA-associated polymorphisms in HIV-1 remains far from complete. Although the importance of addressing the confounding effects of HIV-1 population structure are now recognized [27,30], three important issues will need to be addressed in future studies: statistical power, linkage disequilibrium (LD) among HLA alleles and co-evolution of amino acids in viral sequences. Although formal power studies will be required to determine the cohort size needed to establish the true extent of HLA-mediated adaptation in HIV, preliminary estimates suggest that a cohort of N=700 provides power to detect only ~50% of HLA-associated viral polymorphisms (J.C. et al., unpublished data). This result underscores the importance of extending these types of cross-sectional studies to even larger cohorts, as well as to assess other HIV-1 subtypes, where levels and patterns of HLA-mediated imprinting may differ. Indeed, one advantage of lineage-corrected methods may be the ability to combine heterogeneous cohorts to increase power, though the extent of shared epitopes between clades remains incompletely characterized.

Linkage disequilibrium (LD) among HLA alleles and HIV amino acid co-evolution also confound analyses of CTL-mediated viral evolution. The concern over LD arises due to the fact that HLA class I alleles are situated in close proximity on the human genome and are not inherited independently; therefore, an escape mutation driven by HLA-B*57, for example, may also be detected as being associated with the tightly linked Cw*06 allele. Whereas Moore et al. [22] addressed the issue of LD using logistic regression and a backwards elimination technique to identify the allele(s) that best

explained the escape polymorphism [22], current lineage-corrected methods [27,30] do not directly address this issue.

None of the current approaches account for the effects of amino acid co-evolution, such as when an amino acid substitution at one site preferentially occurs in the context of a secondary (or compensatory) mutation at another site [17]. Failure to account for this issue may result in both the primary and compensatory mutations being identified as correlated with the restricting HLA allele, when in fact only the former is directly selected. Although Moore et al. attempted to include co-varying amino acids as regressors [22], the confounding effects of phylogeny are even greater when two amino acids share the same evolutionary history; indeed, a substantial body of literature exists to address the problem of covarying amino acids, though it is not clear how these methods can be extended to incorporate HLA allele information or even correlations among multiple codons [28]. Similarly, although Bhattacharya et al. [27] found evidence of compensatory mutations using a modified version of the evolutionary history reconstruction method proposed by Ridley in 1983 [31], the results are difficult to interpret due to the confounding of long and short range effects that occur when only pairs of variables are considered. Clearly more comprehensive approaches are needed to disentangle the complex interactions that govern HIV-1 escape pathways.

Ultimately, a comprehensive map of HLA-driven HIV-1 polymorphisms would represent an invaluable resource for the scientific community. Such a genome-wide list could serve as a reference whereby observed mutations in independent longitudinal studies could be classified as HLA-driven escape or reversion events. This may allow researchers the ability to determine exactly what proportion of observed within-host viral

evolution is driven by immune selection, as well as to predict what mutational patterns are expected in a patient with a given HLA profile. Until recently, the total evolutionary impact of immune selection has been difficult to address, due in large part to a lack of consistent methods to classify observed mutations (as well as a lack of longitudinal sequence data from individuals with known infection dates). Consequently, current estimates vary greatly: while one study of nine patients reported that 13.5% of nonsynonymous substitutions in the first month of HIV-1 infection represented escape mutations [32], another study on four patients reported that up to two-thirds of substitutions in the first five years of infection represented CTL escape and reversion events [21]. The initiation of larger longitudinal HIV-1 seroconverter cohort studies, where observed amino acid changes can be classified as HLA-attributable escape or reversion events according to a standard definition, is critical to address this important question.

Clinical consequences of immune-mediated evolution

Immune escape is believed to be a major factor limiting the immune system's ability to control HIV-1 in the long term [7]. However, with the exception of documented loss of viremia control following escape within the B*27-restricted KK10 epitope in Gag [15], a clear relationship between CTL escape and HIV-1 disease progression has been difficult to demonstrate. A multitude of factors influence the frequency and kinetics of viral escape, making the clinical consequences of immune-driven HIV-1 evolution challenging to quantify. Even among individuals expressing the same HLA allele, substantial differences in the magnitude and frequency of epitope targeting are often observed, rendering it problematic to study the clinical consequences

of escape on a population basis. A second complication is the issue of "immunodominance", an incompletely understood phenomenon describing the fact that, despite the expression of up to six HLA class I alleles (and the potential to target multiple epitopes per allele), an individual's CTL response is often initially directed against a single or few epitopes with a single HLA restriction [33]. Moreover, the breadth and specificity of targeted epitopes changes throughout the disease course. While the acute-phase CTL response is generally narrowly directed, a progressive broadening of the epitopic repertoire occurs over time, likely as a consequence of viral escape within the early immunodominant epitopes [34]. In addition, the balance between escape vs. fitness costs [24], the fact that "escape" is not always absolute (such that, in many cases the selected variant retains partial HLA binding and/or CTL recognition capacity [35]), and the ability of the immune response to adapt to a continuously evolving target (as demonstrated by the emergence of de novo CTL responses against newly-selected escape variants [36]), all contribute to the complexity of this issue. Finally, the fact that escape does not occur in isolation must also be considered, because mutations are likely to be selected in the context of a variety of compensatory changes and at the same time that reversion of transmitted mutations is occurring. Taken together, it seems unlikely to expect that the selection of any single mutation would be accompanied by a clinically detectable impact on HIV-1 disease progression.

The identification of HLA-associated polymorphisms in large clinically-derived datasets will allow, for the first time, the characterization of the relationship between escape and markers of HIV-1 disease progression on a broader level. In their 2002 study, Moore et al. reported that the presence of HLA-associated polymorphisms in RT

predicted pre-treatment plasma virus load (pVL) on a population basis [22]; however, this observation was not confirmed in a subsequent population-based study [30]. Further research is needed to assess whether there may be gene-specific differences in the contribution of CTL escape mutations to markers of HIV disease, the answer to which may greatly inform vaccine design.

Strategies to cope with viral diversity in HIV-1 vaccine design

Numerous strategies have been proposed to address the challenge of global sequence diversity in HIV-1 vaccine design. Immunogens based on consensus or phylogenetic reconstructions of ancestral and/or 'center-of-tree' sequences attempt to minimize genetic distance between the vaccine and circulating HIV-1 strains [4,37]. Polyvalent vaccine immunogens featuring maximal coverage of viral diversity and potential epitopes in compact sequence space have also been proposed [38,39,40]. To address the substantial mutational capacity of HIV-1, one proposed approach is to limit vaccine design to immunogenic yet highly conserved regions, such that escape could only occur at a substantial fitness cost [41]. A complimentary strategy would be to design immunogens that incorporate both "wild-type" and "escaped" variants (as long as the variant retains its ability to bind HLA), thereby blocking preferred routes of escape in infected individuals [42].

The development of phylogenetically-informed methods to accurately identify viral sites under active immune selection pressure using large clinically-derived HIV-1 sequence datasets represents a major advancement to the study of how viral genomes are shaped by human immunogenetic selection pressures. Achieving a complete picture of the sites, pathways and kinetics of immune escape will not only help us gain an

understanding of the extent to which host immunity shapes HIV-1 evolution, but will also inform the rational design of future vaccine immunogens.

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