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Recent Advances in Research of HIV Infection: Implications of Viral and Host Genetics on Treatment and Prevention

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Abstract

The genetic diversity among human immunodeficiency virus (HIV) subtypes as well as the variability of viral sequences found in HIV-infected individuals presents a number of difficult obstacles for the development of universally effective HIV treatment and prevention methods. Here, we present a brief summary of recent developments in the analysis of viral genetics and human genomics to provide insight into future methods for HIV treatment and prevention. Recent studies have mined viral sequences found in newly infected individuals to identify common features of all transmitted viruses that could provide potential targets for HIV vaccine development. Analysis of human immunogenetics has identified specific alleles associated with reduced virus loads in HIV-infected individuals providing valuable information that may influence individual responses to treatment and prevention methods. Increased sensitivity of antiretroviral drug resistance testing has improved the detection of hidden drug resistant virus but also highlighted the potential for drug resistant viruses to reduce the effectiveness of clinical treatment regimens. The rapidly expanding amount of data generated by studies of viral genetics and human immunogenetics will provide valuable information to guide the design of new strategies to improve clinical treatment and enhance HIV vaccine development.

Keywords

AIDS; Antiretroviral drug resistance; Human immunodeficiency virus; Immunogenetics; Transmission

Introduction

The pandemic of human immunodeficiency virus (HIV) infection continues to pose an enormously difficult public health challenge for a multitude of reasons. One of the most daunting aspects of the HIV epidemic is the extremely high level of genetic diversity among the 13 recognized global subtypes and at least 51 circulating recombinant forms [1]. The

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degree of global diversity observed originates with the error-prone nature of the viral replication machinery and the ability of the viral genome to incorporate multiple nucleotide substitutions which, within infected individuals, results in a population of closely related, but genetically distinct variants termed quasispecies. Evolving HIV genetic diversity presents numerous obstacles for both the human immune response as well as the implementation of antiviral therapies, vaccines and microbicides. Recent studies utilizing new sequencing technologies and genomics tools have begun to reveal a vast amount of data regarding the genetics of both HIV and the human immune system. In this review, we will present a brief summary of recent studies examining HIV genetics and human genomics as well as how this body of knowledge will impact the development and implementation of future effective methods to both treat and prevent HIV infection.

Genetic Analysis of Transmitted Variants

Although the HIV global epidemic is characterized by an extraordinarily high level of viral genetic diversity, the viral quasispecies initially found in most individuals early in infection tends to be remarkably homogenous. Recent studies have focused on sampling sequences of the viral *env* gene from the viral quasispecies within HIV-infected individuals. The viral *env* gene encodes the highly variable viral surface glycoprotein (Env) required for entry into HIV target cells. Several studies based on recently infected individuals have established that in 60–90% of cases, depending on the population studied, HIV infection is apparently established by a single viral variant [2]. In multiple variant infections, only 2–5 variants can be found, although higher infection multiplicities have not been ruled out because sampling for many tissue compartments is not readily available. Infection with multiple variants has been observed to occur more frequently when the barrier of the host mucosal surface is compromised or bypassed, as with sexually transmitted infections, anal intercourse or intravenous drug users [3]. Additionally, infection with multiple variants has been linked to certain markers of faster disease progression [4]. Therefore, methods aimed at reducing the likelihood of infection with multiple variants, such as treatment of sexually transmitted infections in HIV-uninfected persons, will likely slow disease progression in those individuals who become HIV-infected.

Advances in bioinformatics analysis have enabled the compilation and comparison of viral sequences collected from a large number of newly infected individuals to identify common sequences and phenotypes that may be shared in HIV transmission. A recent study by Gnanakaran et al. [5] examined over 7,000 *env* sequences obtained from 275 samples collected during acute or chronic phases of HIV infection to search for signature genetic patterns specifically associated with acute infection. This analysis yielded 25 promising signatures, including the absence of a specific sequence known to facilitate viral escape from antibodies and several signatures that may be essential to proper expression and/or processing of the Env glycoprotein. Asmal et al. [6] extended this finding by demonstrating that sequences associated with acute infection indeed produce higher levels of Env and increased incorporation of Env into virions in vitro. Together these studies highlight the potential utility of mining large data sets of viral sequences from acute and chronic HIV infection for genetic patterns uniquely related to HIV transmission before specific hypotheses are further tested in experimental systems. Thus, genetic analysis of viral

sequences has begun to identify specific properties of all HIV transmitted variants that may be targeted in future vaccine and microbicide methods to prevent sexual transmission of HIV.

Evaluation of viral sequences in samples collected during clinical trials of HIV prevention strategies also afford the opportunity to examine breakthrough infections in treatment and control arms to identify characteristics of viral variants that manage to establish infection in the context of a specific intervention. The STEP trial, a double-blinded, phase 2b study of vaccine efficacy, attempted to induce immune responses against 3 HIV proteins, but the trial lacked efficacy in preventing infection [7]. Retrospectively, Rolland et al. [8] obtained and compared near full-length viral genomes from 68 HIV-infected individuals from both the vaccine and placebo arms of the STEP trial. Not surprisingly, breakthrough infections in the vaccine recipients were initiated by viruses with sequences divergent from the vaccine. Importantly, sequence differences between viruses establishing infection in vaccine and placebo recipients were almost exclusively restricted to the 3 viral proteins that comprised the vaccine. Thus, while this analysis highlights the challenges facing HIV vaccine development, the application of viral genetic analysis to breakthrough infections in clinical trials is being used to hone previously tested prevention methods in order to maximize efficacy. Taken together, novel strategies of analyzing HIV sequences are beginning to yield promising opportunities to develop new, and improve upon current, HIV prevention methods.

Insights from Analyses of Host Genomics

HIV diversity and its continuing evolution in human populations are dictated in part by host factors, including those that mediate innate and adaptive immune responses to HIV infection [9]. Comprehensive data from deep sequencing of the entire HIV genome [10] coupled with genome-wide search for host genetic factors important to HIV acquisition and control [11] are expected to facilitate a fine dissection of molecular mechanisms underlying the battle between HIV and host genomes.

To date, immunogenetics as a prominent frontier in AIDS research has already made important contributions to (i) identification of HIV-resistance factors (e.g. null *CCR5* alleles), (ii) the understanding of effective and durable immune control of HIV infection in elite or viremic controllers [12], (iii) mapping of viral epitopes for vaccine design, and (iv) evaluation of vaccine efficacy. Although human genomes have been scanned for patient populations with informative, HIV-related phenotypes [13, 14], several recent studies now offer new evidence that productive and transformative research may need to focus on systematic analysis of highly polymorphic human leukocyte antigen (HLA) molecules and killer cell immunoglobulin-like receptor (KIR) genes. Continued study of the complex virus/host interaction will inform development and implementation of treatment and prevention regimens.

For example, 2 recent studies have demonstrated that individuals infected with HIV subtypes B and C have relatively low viral load when they carry HLA-DRB1*13:03, an infrequent allele that might have been missed by earlier studies for lack of statistical power [15, 16].

Favorable HLA class II alleles are likely to target epitopes in HIV Gag instead of Env [16], which is consistent with the well-established notion that immune recognition of conserved epitopes in HIV Gag may be a common mechanism for effective immune control, as immune escape mutations in Gag typically lead to reduced viral fitness. In the AIDS vaccine trial (RV144) in Thailand, lack of neutralizing antibody (nAb) response was associated with HLA-DRB1 variants *11 and *16:02 [17]; vaccine recipients with the HLA-DQ heterodimer consisting of DQA1*05:01 and DQB1*03:01 were also unlikely to produce nAb after vaccination [17]. Future vaccine trials may need to consider HLA class II genotyping as another useful tool for deciphering correlates of immune protection, as supported by preclinical findings from an SIV-macaque model [18]. Of note, HLA class II alleles implicated by the RV144 trial bear no similarity to those (e.g. DRB1*01, *03, *07 and *15) reported earlier for several routinely applied vaccines, suggesting that nAb responses may differ by vaccine constructs and/or dosing regimens.

Unlike potent anti-HIV nAb that acts relatively late in the course of HIV infection, CTLs and NK cells presumably act early. As such, HLA class I and KIR gene variants with distinct functional properties can be analyzed against early phenotypes. This hypothesis has been tested in recent HIV seroconverters from sub-Saharan African countries [19]. In the acute-phase of HIV infection (duration of infection <3 months), the majority of seroconverters with effective control of viremia had HLA-B*44 and B* 57 – 2 favorable HLA variants in the cohort [19]. These allelic products are known to target multiple conserved HIV epitopes, but their functional advantages may also further reflect intrinsic differences in protein expression [20]. One study has also called for close attention to HLAC expression, as certain alleles (e.g. C*06:02) may be functionally advantageous through avoidance of down-regulation by microRNAs [21]. HLA-KIR interactions can provide another dimension for gauging the relevance of HLA function to HIV control, as at least 22 nonsynonymous mutations in the HIV genome are apparently attributable to several KIR genes (especially *KIR2DL2*) [22]. A clear picture about these multifactorial and multidimensional pathways may emerge soon to allow a fine-mapping of HIV mutations induced by CTL, nAb, NK, and other related effectors mediated by HLA and KIR gene products. However, it will require much more painstaking effort to determine the selection of these mutations relative to individual immune pressure, their patterns of reversion in the absence of immune pressure, and the replication capacity of mutated viruses. Further study will be necessary to understand the interplay between human immunogenetics and HIV in order to implement treatment and prevention methods with maximum efficacy.

Detection of Antiretroviral Drug Resistance

A complication in HIV clinical care stemming from the high mutation rate and genetic diversity of HIV is the emergence and persistence of mutations that confer resistance to drugs used in antiretroviral therapy (ART). Because primary or transmitted drug resistance mutations are associated with poorer treatment outcomes, a Department of Health and Human Services panel recommended that all persons newly diagnosed with HIV infection receive an HIV genotype prior to initiating therapy [23]. The conventional method for virus genotyping is Sanger sequencing; however, the sensitivity of this technology is limited in that it is unlikely to detect in individuals a variant present at a frequency of less than 20–

30% of the quasispecies [24]. This limitation can lead to underestimating the burden of resistance in drug-naïve individuals, particularly for persons diagnosed long after becoming infected when transmitted resistant viruses may have become supplanted by more fit wildtype virus. Resistance can also be underestimated in ART-experienced individuals who may have low-expressing archived variants selected by prior treatment regimens [25]. The development of sensitive genotyping tools that allow for finer resolution of quasispecies subpopulations has uncovered hidden drug-resistant variants that are undetected by conventional sequencing. Some of the more common molecular methods used for resistance mutation detection include allele-specific or mutation-specific real-time PCR [26, 27], parallel allele-specific sequencing [28], ultra- deep (pyro) sequencing [29], and single-genome sequencing [30], each with inherent technical complexities and resource requirements.

In a study of newly diagnosed ART-naïve individuals from North America, who had either wildtype HIV infections or who had at least one resistance-associated mutation as determined by conventional genotyping, allele (mutation)-specific PCR testing found previously undetected low-frequency transmitted resistance mutations in both groups [31]. The detection of minority resistance mutations in 17% of the conventionally genotyped wildtype virus samples and the identification of additional mutations in 10% of those with evidence of other resistance mutations suggested that bulk genotyping missed at least 40% of transmitted mutations in this population. Furthermore, the finding that some individuals had evidence of HIV resistant variants of distinct genotypes implied that it was possible for infections to involve more than one variant with the potential to impact treatment outcomes. Sensitive genotyping for surveillance, however, may not offer substantially more information than conventional genotyping in geographic regions with less-extensive treatment histories and lower prevalences of transmitted resistance.

In a case-control study also by Johnson et al. [31], mutation-specific PCR testing identified that 7/95 (7%) persons who experienced virological failure on first-line efavirenz-based ART had low-frequency drug resistance mutations at baseline; whereas, low-frequency resistance was found in only 2/221 (0.9%) treatment successes ($p = 0.0038$). A study by Goodman et al. [32], using allele-specific PCR at a mutation lower cut-off of 0.5%, found that minority-level detection of K103N, a non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation, when present at 2,000 copies/ml was associated with a significant ~47-fold higher risk of virological failure in first-line NNRTI-based ART [32]. This suggested that higher resistant variant loads within low-level populations were more strongly associated with treatment failure. Simen et al. [33] employed ultra-deep sequencing with a mutation detection lower limit of 1% to assess resistance in drug-naïve patients starting ART within the Flexible Initial Retrovirus Suppressive Therapies (FIRST) study of 3 initial regimens. Twice as many participants were identified to have resistance mutations with ultra-deep sequencing than was detected by standard sequencing, 28% versus 14%, respectively ($p < 0.001$). The presence of NNRTI resistance detected only by ultra-deep sequencing was significantly associated with virologic failure (HR 2.50; 95% CI, 1.17–5.36). Taken together, these findings strongly support that, at least for NNRTI combination therapies with dual NRTIs, NNRTI-resistant variants may retain their impact even if present at a low frequency within the quasispecies.

Low-frequency mutations, however, do not universally impact ART. Lataillade et al. [34] reported that in persons virologically failing on ritonavir-boosted protease inhibitor (PI/r) regimens, ultra-deep sequencing identified major protease inhibitor mutations at low levels only sporadically, and those variants remained susceptible to the boosted PI. Moreover, Codoñer et al. [35] retrospectively assessed the value of sensitive testing on predicting the success of salvage therapy for HIV-infected persons with heavy treatment histories and who have experienced virologic failure with deep salvage therapy. Here, again the additional few PI resistance mutations identified by deep sequencing prior to salvage therapy were inconsistently predictive of PI susceptibility.

Finally, another potential use for sensitive genotyping was revealed in the deep sequencing of the *env* V3 region by Swenson et al. [36] from participants who received either the R5-specific entry inhibitor maraviroc or the NNRTI efavirenz in the MERIT trial. In this analysis, identification of low-level non-R5-tropic viruses at study entry were associated with poorer maraviroc treatment outcomes than those in the efavirenz arm. Limiting maraviroc to those with only R5-tropic virus would have resulted in maraviroc having a noninferior response to efavirenz. Therefore, because minority-level resistance class and design of antiretroviral regimen can impact treatment success, the benefit of sensitive genotyping in clinical management requires further exploration.

Conclusion

The advent of new sequencing and bioinformatics tools are beginning to benefit the development of improved methods for treating and preventing HIV infection. Despite the complicated genetic variability of HIV, sequencing and bioinformatics tools provide promise for identification of future viral targets of HIV prevention methods. Comprehensive studies of immunogenetics and viral sequences are beginning to understand the complicated interplay between virus and host, whereby specific alleles are associated with faster or slower disease progression. Increasingly sensitive ARV drug resistance testing methods have made it possible to detect minority drug resistance mutations which may impact the success of clinical patient management. In summary, data generated by studies of viral genetics and human immunogenetics provide valuable information to guide development of new strategies to improve HIV treatment and prevention.

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