ADAPTATION, CO-EVOLUTION, AND HUMAN SUSCEPTIBILITY TO HIV-1 INFECTION

Amalio Telenti

«The closer you look at life, the more rapid and intense the rate of evolutionary change.»

Jonathan Weiner, The Beak of the Finch

Darwin believed evolution occurred over very long periods of time and generally moved in a set direction toward fitness, in the same direction the environment was heading. HIV-1, by means of its inherent capacity to mutate, and thus evade and adapt, serves to assess co-evolution with humans over the short period that elapsed since the estimated entry of HIV-1 into the human population during the first half of the last century (Korber, 2000). In this paper, I review the selective pressures exerted by humans on the virus, and potential evolutionary influences exerted by the virus on the human population under the weight of the current pandemic. I will pay special attention to the growing knowledge on the elaborate innate defense mechanisms against retroviruses, and on the potential role of human genetic variants of antiviral genes and of host proteins needed for the viral life cycle in modulating human susceptibility to HIV-1 infection.

Adaptation of HIV-1 to the human population

Humans exert powerful selective pressures on HIV-1. Some are exogenous in nature, such as the pressure exerted by antiretroviral drugs leading to the selection of drug resistance, and others are of endogenous nature, such as the pressure generated by the adaptive immune system, leading to selection of immune escape mutants. These two processes shape the virus in a fashion that may lead to population-specific HIV-1 variants, and that generally result in diminished viral fitness. In population biology, fitness is defined by the relative reproductive capacity of an individual based on its contribution to the next generation. In viro-

logical terms, fitness is best described by the relative replication rates of viruses in competition kinetics (Bleiber, 2001b).

Adaptation to drug pressure. Although the fitness of resistant HIV-1 strains overlaps that of susceptible clinical isolates, resistant strains are overall less infectious and replication competent (Telenti, 1999, Kaufmann, 2000, Bleiber, 2001a, Bleiber, 2001b). Mutations selected by reverse transcriptase (RT) and protease (PR) inhibitors frequently involve changes of enzyme active site residues. Reduction in primer extension activity by mutant RT has been demonstrated in vitro (Back, 1996, Back, 1997). Mutant PR enzymes exhibit diminished catalytic efficiency in the processing of viral Gag and Gag-Pol polyproteins (Schock, 1996, Zhang, 1997, Rayner, 1997, Croteau, 1997, Carrillo, 1998). These modifications in enzyme processivity may translate into the accumulation of immature viral particles. Kinetic analysis of consecutive proteolytic cleavages of the Gag-Pol polyprotein suggests that HIV-1 would cease being viable when the efficiency of a mutant protease is less than 61% of the wild type activity for each step of cleavage (Rasnick, 1997). Mutation in both PR and RT can contribute to diminished viral fitness, and either enzyme may display a profound impairment (Bleiber, 2001a). Resistance to HIV-1 fusion inhibitors is also associated with impaired fitness, while resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) modifies viral physiology only minimally (Schmit, 1996, Rayner, 1997). This has been attributed to drug binding remote from the RT active site (Esnouf, 1995), and explains the occasional identification of NNRTI resistance mutations as natural polymorphisms (Havlir, 1996).

Given the remarkable plasticity of the HIV-1 genome, selection of compensatory mutations leading to improving enzyme function takes place. This process involves structurally relevant amino acid substitutions in target enzymes (e.g., in the hinge or flap regions of the viral protease) (Schock, 1996, Borman, 1996), changes that improve processing of enzyme substrate (e.g., Gag cleavage sites) (Doyon, 1996, Zhang, 1997, Carrillo, 1998, Bally, 2000), or by compensation at distance (Nijhuis, 1999, Peters, 2001). However, these pathways of compensation may not provide an immediate or full remediation of viral fitness deficits (Mammano, 1998)

as there could be evolutionary and adaptive limits of HIV-1 (Yuste, 1999, Nijhuis, 1999). Diminished fitness underlies the lower pathogenicity of multidrug-resistant strains (Kaufmann, 1998). Fitness cost can also be translated into transmissibility cost. Resistant strains, and in particularly multidrug-resistant viruses are less transmissible in a population (Yerly, 2004), a phenomenon that can be ascribed to adaptive attenuation.

Adaptation to immune pressure. Immune selective pressure forces the emergence of viruses with escape mutations that result in infected cells not being recognized by cytotoxic T lymphocytes (CTL) (Lieberman, 2002). Viruses adapt to the host they infect through a process of HLA-associated selection (Moore, 2002). This carries a fitness cost, as proven by reversion after viral transmission to a different host, when the CTL pressure exerted on the virus shifts (Leslie, 2004). This process, when brought to the scale of the population, implies that after many cycles of viral replication and passage in human hosts, the viral population evolves to a consensus virus with mutations in genes that encode dominant immunogenic peptides (Scherer, 2004). MHC-restricted immune responses may shape the viral genetic diversity over time, providing a selective pressure that is a function of the frequency of various alleles as the virus is passed from one host to the next. In this scenario, it may be predicted that the most common viral population (ie the 'population consensus sequence') is likely to represent the genotype best adapted to the most frequently encountered disease-modifying MHC alleles (or haplotypes). Trachtenberg et al. (Trachtenberg, 2003) proposed the occurrence of frequency-dependent selection according to HLA supertypes, an attractive hypothesis (Telenti, 2003) that has recently gained support with the identification of a higher capacity to elicit CTL responses by rare HLA supertypes (Scherer, 2004). Eventually, pathogen diversity and discrete strain structure may be determined by different host networks (Buckee, 2004).

Adaptation of the human population to HIV-1

While the response of the virus to selective pressures imposed by the host can be readily observed, it is much more difficult to define the evo-

lutionary pressures of the virus on the host because of the recent nature of the epidemic. However, this topic can be revisited if what is described is the adaptation of humans to retroviruses. To approach this topic, I will focus on the determinants of human susceptibility to HIV, and the genetic basis of inter-individual differences in susceptibility to disease progression.

Interindividual levels of permissiveness. The genetic make-up of an individual plays a role in his or her susceptibility to HIV-1 infection and progression of disease. Some of the observed variation has been attributed to immunogenetic diversity (MHC homozygocity, specific HLA types), polymorphism in chemokines, chemokine receptors and in cy–tokine genes *(CCR5, CCR2, CX3CR1, SDF1, MIP1a, RANTES, IL-10, IL-4)* (Carrington, 2001, Telenti, 2002, O'Brien, 2004). Isolated primary human cells from different individuals also vary in their permissiveness – the ability of purified cells to be infected and to sustain replication of HIV-1 (Williams, 1991, Spira, 1995, Eisert, 2001, Ciuffi, 2004). Infection of macrophages derived from pairs of identical twins displays a high concordance in the kinetics of HIV-1 replication (Chang, 1996, Naif, 1999), underscoring the role of genetic factors in determining susceptibility to infection.

The HIV-1 life cycle is characterized by numerous interactions with host cellular proteins (Greene, 2002, Peterlin, 2003) *(Figure 1)*. Restriction at entry plays a key role in determining infection kinetics (Reeves, 2002, Butticaz, 2003). However, host proteins involved in later steps of the viral life cycle also contribute to interindividual susceptibility to HIV-1 infection (Ciuffi, 2004). Genetic polymorphism in host genes participating in the viral life cycle could result in differences in the levels of expression or in functional differences of protein variants that may lead to differences in permissiveness to HIV-1 infection. The cell would thus constitute an "environment of evolution", whereby differences among individuals or inter-species, lead to viral variants adapted to different host partner proteins. Eventually, escape mutants with potential fitness deficits or increase pathogenicity may contribute to shape the epidemic. One of the main focuses of the research in my laboratory has been to understand interindividual differences in

susceptibility to HIV-1 by developing *in vitro* systems for the standardized assessment of the contribution of genetic variants of selected host genes, and that would allow the genomic mapping of determinants of susceptibility to HIV-1.

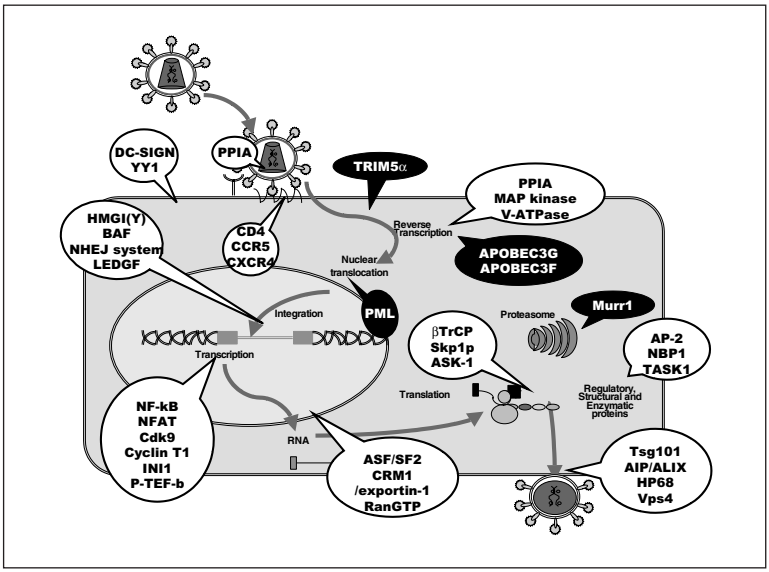

Figure 1:
The HIV-1 life cycle. Indicated are host proteins that participate at specific steps of the replication cycle (white), and proteins acting as antiretroviral host factors (black).

The first goal was approached by defining the steps in the viral life cycle that exhibit the most variation between individuals (Ciuffi, 2004). We used replicating HIV-1 and single cycle lentiviral vectors in a population approach to identify polymorphic steps during viral replication. We found that stimulated CD4 T cells (the main cellular target of HIV-1) exhibited up to 5 log unit differences in virus production. We were able to attribute up to 42% of the total variance in virus production to entry factors, and 48% to post-entry steps. Efficacy at key intracellular steps of the replicative cycle (reverse transcription, integration, trans-

cription and splicing, translation, and budding and release) varied among individuals. However, differences in transcription efficiency alone explained up to 80% of the total variance in viral production that was attributable to post-entry factors. The observation that transcription polymorphism has a weight comparable to that of entry variation should trigger a detailed analysis of genes participating in the activation, transcriptional status, and transcriptional machinery of the cell. This observation is set in the context of current awareness of the role of variation in human gene expression underlying complex traits (Cox, 2004, Morley, 2004).

The development of this well-characterized in-vitro system allowed us to use it as screening tool for HIV-1 restriction alleles (Bleiber, 2004). Susceptibility to HVI-1 is a complex trait compounded by unknown environmental and genetic factors pertaining to both the human host and the virus. To circumvent some of these difficulties we proposed a two-step strategy, in which distinct alleles in potential candidate genes are first assessed *in vitro* for their impact on viral replication, and subsequently validated *in vivo* for their role in restricting HIV-1 infection. As a benchmark test we first tested 12 previously reported HIV-1 restriction alleles as well as single nucleotide polymorphisms in 10 additional candidate genes. This led to the identification of 3 new alleles associated with differences in progression of HIV-1 disease in the Swiss HIV Cohort Study. We estimate that the genetic effects of these markers might be responsible for lengthening or shortening the latency period to AIDS by up to 4.6 years. It is to facilitate this type of studies that we proposed the creation of the Genetics Project of the Swiss HIV Cohort Study (Telenti, 2004, Furrer, 2004).

To complement the approach using CD4 T cells from blood donors, we are now using immortalized B lymphocytes from the Centre d'Etude du Polymorphisme Humain (CEPH) Utah pedigrees (Dausset, 1990). Here, quantitative traits can be mapped to chromosomal locations by genome scans (Schork, 2002, Jen, 2003, Cheung, 2003, Pastinen, 2004, Morley, 2004, Watters, 2004). While susceptibility to HIV-1 cannot be approached *in vivo* by linkage analyses, but only through association studies, the CEPH resource allows conventional linkage analysis for quan-

titative traits. These cells also allow the re-assessment of haplotypes that are possibly associated with differences in gene expression, by the measurement of allele differential expression (Yan, 2002, Pastinen, 2004).

Innate cellular defense. While certain host proteins are necessary for infection and for sustaining viral replication, others represent antiviral factors *(Figure 1)*. Some cellular antiviral factors can be selectively suppressed by viral proteins, as shown by the interaction between human APOBEC3G, a cytidine deaminase, and the HIV-1 Vif protein (Sheehy, 2002, Mariani, 2003, Harris, 2003, Mangeat, 2003, Lecossier, 2003). An early interplay between incoming retroviral preintegration complexes and the nuclear proteins INI1 (integrase interactor 1) and PML (promyelocytic leukemia protein) creates an antiviral state that interferes with the immediate-early steps of HIV-1 infection (Turelli, 2001). ZAP, a zinc finger protein, inhibits production of retroviral RNA (Gao, 2002). Other antiretroviral factors target the capsid protein, imposing a post-entry block (Hatziioannou, 2003, Stremlau, 2004). Murr1, a gene product known previously for its involvement in copper regulation, acts as a genetic restriction factor that inhibits HIV-1 growth in unstimulated CD4+ T cells through its effects on the proteasome (Ganesh, 2003).This growing list of specific antiretroviral factors adds to the current knowledge on antiviral innate defense mechanisms that implicate interferon responses mediated by double stranded RNA-dependent protein kinase, the MX proteins, and RNase L-mediated degradation of viral RNAs (Goff, 2003).

Two antiviral proteins, APOBEC3G and TRIM5α, underscore the concept that the biological barrier preventing the entry of simian immunodeficiency viruses into the human population as zoonotic infections is potentially quite fragile. A single APOBEC3G residue, aspartic 128 in human, lysine in African green monkeys controls the ability of the HIV-1 Vif protein to bind and inactivate this host defense factor (Schrofelbauer, 2004, Mangeat, 2004). HIV-1 efficiently enters the cells of Old World monkeys but encounters a block before reverse transcription by modulating the uncoating of a retroviral capsid (Stremlau, 2004). The cytoplasmic body component TRIM5α restricts HIV-1 infection in Old World monkeys while the simian immuno-

deficiency virus, which naturally infects Old World monkeys, is less susceptible to the TRIM5α-mediated block. Host cell barriers may largely explain the current distribution of these viruses among human and non-human primate species (Lee, 2004).

We have investigated variation in these antiviral proteins (Bleiber, 2004, An, 2004) and identified a significant polymorphism, including the occurrence of nonsynonymous amino acid substitutions in defined protein motifs. This would indicate that these innate defense elements are under significant selective pressure (Sawyer, 2004, Zhang, 2004). A histidine 186 to arginine change in APOBEC3G, frequently identified in individuals of African ancestry, may be associated with more rapid progression of HIV-1 disease (An, 2004). TRIM5α variants are, in the *in vitro* assay used in our laboratory, associated with differences in the degree of permissiveness of CD4 T cells to HIV-1 infection. Exploring differences in expression or function of these antiviral proteins may shed light on the basis of inter-individual or inter-species differences in susceptibility to retroviruses, and possibly other pathogens (Turelli, 2004).

Perspectives

The various processes described above lead us to the last issue, whether we are witnessing HIV-1-host co-evolution. The best evidence for the influence of human pathogens on natural selection is exemplified by Malaria (Winkler, 2004). This disease became endemic 6000–10 000 years ago, coincident with the rise of agriculture (Tishkoff, 2001, Joy, 2003). The selective pressure over 300–500 generations has resulted in adaptive shifts in the allele frequency of several genes with a role in malaria resistance: the X-linked glucose-6-phosphate dehydrogenase *(G6PD)*, the Duffy antigen receptor for chemokines *(DARC)*, and the α and β-globin genes (Fortin, 2002). Another suggestive example of population shaping constitutes the *FUT2* – fucosyltransferase 2 and resistance to Norovirus infection. *FUT2* encodes α1,2 fucosyltransferase that produces H type 1 carbohydrate found in the surface of the intestinal epithelium, required for Norovirus binding (Lindesmith,

2003). As regards to HIV-1, the only well characterized human population escape mutants are individuals who are homozygous for CCR5 Δ32. The resulting truncated chemokine receptor provides resistance to HIV-1 infection and is present in approximately 2% of Caucasian individuals. However, *CCR5 Δ32* was selected approximately 700 years ago, not by HIV, but possibly by another pathogen (Stephens, 1998, Elvin, 2004, Mecsas, 2004). If no effective control of the HIV-1 pandemic is achieved, the continuing pressure may give rise to a formal selection of a HIV-resistant human population.

For years researchers have been studying the determinants of acquired immunity to HIV-1; and with the same intensity and funding we should now define the cellular determinants of susceptibility to HIV-1. Our work will continue to probe the genetic basis of interindividual differences in retroviral restriction. This information is to be placed in the context of the primate phylogeny, through detailed comparative genomics.

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