Elite controllers: understanding natural suppressive control of HIV-1 infection

A characteristic of HIV is the incredible variability in the course of the disease.

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HIV-1 infection: broad variability in disease course

Worldwide, an estimated 34 million people are infected with the human immunodeficiency virus (HIV) and approximately 3 million new infections occur every year. Sub-Saharan Africa remains the region most heavily affected by the HIV pandemic, accounting for over two-thirds of all people living with HIV and for nearly three-quarters of AIDS-related deaths. South Africa has the highest number of HIV-infected individuals in the world, estimated at 5.6 million in 2009.

The prognosis for untreated HIV infection is almost always universally poor with gradual but inexorable decline in immune function. In the absence of suppressive treatment with an appropriate highly active antiretroviral (HAART) regimen HIV-infected individuals will, after a period of 8 - 10 years, experience severe opportunistic infections and AIDS-related illnesses, followed approximately 2 years later by death. A rare group of individuals, however, are able, over the long term, to inherently limit the impact of HIV infection on their immune system, or even control replication of HIV without antiretrovirals.

Approximately 5 - 15% of HIV-infected individuals are able to maintain stable CD4 T cell counts of over 500 cells/µl for at least 5 and even 10 years in the absence of antiretroviral treatment; they are termed long-term nonprogressors (LTNPs). Within this group some individuals maintain good immunological control even in the presence of very high viral load, a situation very similar to that found in some nonhuman primates that are natural hosts for chronic infection with SIV (simian immunodeficiency virus) that do not develop AIDS (e.g. sooty mangabeys).

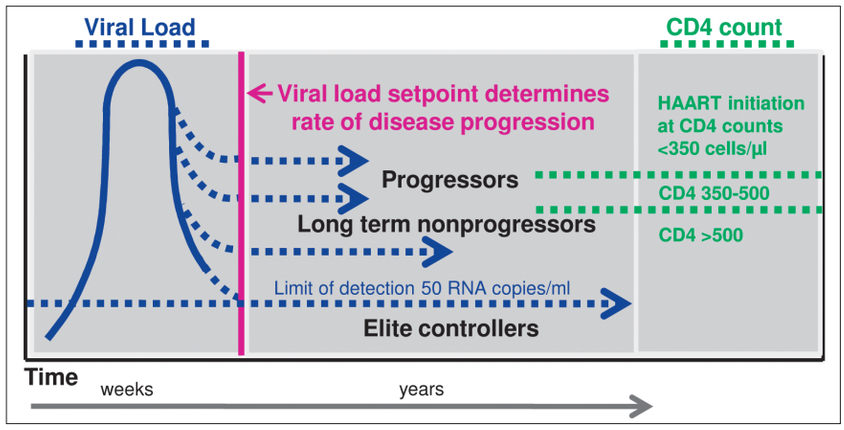

Fig. 1. Schematic representation illustrating the various categories of HIV-infected individuals with differing rates of disease progression, based on viral load (RNA copies/ml) and/or CD4 T cell count (cells/µl).

Another even less frequent but distinct group is called elite controllers (ECs) or elite suppressors.[1-2] They are somehow able to retain their viral load without HAART, at less than 50 RNA copies/ml for at least 1 year.[3] This low viral 'setpoint' (defined as the peripheral blood level of virus copies/ml reached shortly after seroconversion) would be present early in infection and reflects untreated suppression of HIV-1 replication to undetectable levels (Fig. 1).

Understanding the mechanisms responsible for this untreated, spontaneous and exceptional control of HIV-1 infection may be crucial to the development of HIV vaccines that provide protection from

disease progression, and for the development of novel anti-HIV treatments. Although there are scanty data describing possible mechanisms for this apparent resistance to the deleterious effects of HIV, the limited research, mainly from developed world settings, has provided some valuable clues.

Factors that may be involved in suppressive control of HIV-1 infection in ECs:

- viral
- intrinsic antiviral
- host genetic
- immune.

Viral factors

Attenuated strains of HIV-1 may contribute to host control of disease progression. Viral subtype has been shown to have an influence and 'defective' viral genes have been described in long-term surviving adults and South African children older than 7 years.[4-5] The classic example is the Sydney Blood Bank Cohort, where a group of 8 recipients of blood products were infected with a nef/LTR-deleted strain of HIV from a long-term nonprogressor (reviewed in Zaunders *et al.*[6]). Six individuals had sufficient time of follow-up, 3 were LTNPs and 3 were ECs. It was originally postulated that these individuals were infected with a less fit virus unable to replicate efficiently. However, recent data implicate host factors in addition. In virtually all other instances replication-competent viruses with normal replication kinetics have been isolated from ECs without common viral genetic variants or polymorphisms. This suggests that underlying host-mediated control in viral suppression, rather than acquisition of replication-incompetent virus, is the primary mechanism of inherent HIV replication control. HIV mutations thought to be selected by ongoing immune pressure have been shown in virus from ECs, highlighting the possible role of cytotoxic T-cells and selection of mutant viruses able to partially escape even under conditions of such restricted viral replication when viral load is virtually undetectable.

Intrinsic antiviral factors

Intrinsic antiviral factors are constitutively expressed molecules that can directly limit virus replication within cells, through recognition of specific virus components (for review see Yan and Chen[7]). Such factors have been considered in the spontaneous control of HIV-1 replication. CD4 T-cells from ECs can support HIV-1 replication when activated *in vitro* and have been found to be permissive to exogenous infection with X4 and R5 HIV-1 strains and APOBEC3G/F activity (one of the best characterised restriction factors) could not explain the virus restriction. However, in a recent study the selective upregulation of a cyclin-dependent kinase inhibitor p21 (cip-1, waf-1) has been shown to account for the ability of CD4 T-cells from ECs to resist HIV-1 infection.[8]

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Host genetic factors

Genome-wide association studies (GWAS) conducted on HIV-1 infected individuals have identified the *HLA* locus as a major region involved in control of HIV-1 infection,[9] in particular *HCP5*, *HLA-B* and *HLA-C* loci (using viral load set point). GWAS of rapid progressors and LTNPs have also identified the same target genes as for viral set point as well as the zinc ribbon domain containing 1 (ZNRD1). Additional genes of importance include CXCR6, prospero homeobox 1 (PROX1), an intergenic SNP between ribosomal protein S6 kinase alpha-6 (RPS6KA6) and cylicin-1 (CYLC1) genes (X chromosome). Therefore, only a few new targets of significant effect have been identified using this approach. Of all the GWAS studies conducted on HIV-1 infected individuals to date, the majority were on male individuals of European descent.[9]

GWAS do not detect minor gene variants that may, with other gene variants, singly or collectively be involved in control of HIV-1 infection. This underscores the importance of continuing to source well-defined groupings of HIV-1-infected individuals described by a particular phenotype (e.g. such as ECs) and in large numbers, and apply single gene or system biology approaches in the search for appropriate targets for detailed study. These studies should be extended to include individuals of African descent and include women and also children. Frequencies of *HLA* alleles and *KIR* genes can vary dramatically between different populations. The existence of gene copy number variation (CNV) which contributes to differences in gene expression at the transcript and/or protein level also needs to be considered in genetic studies.

Overall, CNV occurs in ~12% of the human genome and is recognised as an important indicator of inter-individual differences.[10]

It is remarkable that even in a group defined by such stringent characteristics of viral control as ECs, there is such heterogeneity in immune and genetic characteristics between individuals.

Several studies have associated specific *HLA* allotypes with differential rates of HIV-1 disease progression, with the *HLA-B* locus, the most polymorphic of *HLA* loci, showing the strongest effects.[11] Specific *HLA-B35* alleles have repeatedly been associated with rapid disease progression, while *HLA-B27* and *HLA-B57* alleles have been shown to be associated with delayed disease progression. The majority of protective *HLA* alleles have Bw4 serospecificities and it has further been demonstrated that individuals who possess two such *HLA* alleles (*Bw4/Bw4*) progress to AIDS at a slower rate than individuals with two *HLA* alleles with Bw6 serospecificities (*Bw6/Bw6*).

Human leukocyte antigen (HLA) class I molecules present endogenously acquired antigenic peptides on the surface of infected cells to adaptive CD8 T-cells, so leading to targeted cell killing by these T cells. Natural killer (NK) cells, highly versatile cells that contribute to both innate and adaptive immunity, display receptors (killer-cell immunoglobulin-like receptors or KIRs) that bind HLA class I molecules, an interaction crucial to killing of infected cells and other functions. *HLA* and *KIR* loci represent the most polymorphic and second-most polymorphic regions of the human genome, respectively. All studies of *HLA* and *KIR* genes to date have included mainly populations in the United States and Europe and have not as extensively interrogated associations among sub-Saharan African populations, the populations most affected by the HIV epidemic. The role of NK cells in the control of HIV-1 infection is being investigated with many genetic association studies of the role of select KIR receptors in isolation, or in combination with their HLA ligands, on HIV-1 disease progression and to a more limited extent HIV-1 adult transmission. However, the *KIR* locus has not been intensively studied in ECs. The importance of HLA-B has been demonstrated in multiple studies and HLA-C is increasingly emerging as a contender for influencing disease progression, most probably through its role as a ligand for KIR receptors.[12]

Immune factors

The immune system has classically been divided into two compartments: (*i*) adaptive immune responses (T- and B-cells), which provide long-lived immunity to specific antigens, and are boosted through specific re-exposure to antigen, and (*ii*) innate immune responses (dendritic cells, phagocytes, natural killer cells), which are first to act upon exposure to a foreign antigen, and have been considered as 'nonspecific' and lacking the potential for memory of prior antigenic exposures (these features have been challenged by recent findings of NK cells in mouse models [13]).

Adaptive immunity

Many studies have looked at adaptive immune capability in elite controllers. Overall, control of HIV-1 infection has been shown to be associated with a more functional T-cell adaptive immune response capability as evidenced by measures of proliferation, cytokine production, cytotoxic potential, and reduced markers of dysfunction. The preferential T-cell targeting of the HIV-1 Gag protein is associated with enhanced control. Interestingly, LTNPs with low viral loads do not possess strong neutralising antibodies, and elite controllers have lower levels of neutralising antibodies than viraemic controllers and progressors.

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Innate immunity

There are few reports on the role of innate immune responses in ECs. Myeloid dendritic cells of ECs have been shown to have enhanced antigen-presenting capability compared with progressors and healthy controls while having a very much reduced ability to secrete proinflammatory cytokines.

NK cell activation is controlled by a balance between inhibitory and activating signals mediated through NK cell receptors and their ligands on target cells. Genetic

diversity at the *KIR* locus is the product of both varied gene content – an individual may possess anywhere between 7 and 14 functional *KIR* genes – as well as extensive allelic polymorphism within these genes. Individuals who possess more *KIR* genes in general have more activating genes, and NK cells with more receptors govern interactions that favour activation over inhibition and will thus be more able to control infectious diseases (but are more prone to autoimmune disorders). Furthermore, NK cells can be activated directly through binding of specific antibody to particular Fc-receptors (CD16) on NK cells, a process known as antibody dependent cellular cytotoxicity (ADCC).

A study of NK cell function in a cohort of ECs demonstrated only limited and less effective HIV-1 inhibitory capacity *in vitro* compared with CD8 T-cells. Interestingly, ADCC activity was elevated in ECs versus viraemic patients. In SIV-infected rhesus macaques with rapidly progressive disease, the passive infusion of plasma from animals with more slowly progressive disease reduced plasma viraemia in a manner most consistent with ADCC.[14]

Our laboratory has described unusual NK cell responses to HIV-1 peptides, in a whole blood assay, while studying the role of HIV-specific T-cell responses in maternal-infant HIV-1 transmission. These responses, which mainly targeted Envelope (Env) and Regulatory (Reg) peptide pools, were unique to HIV-exposed or -infected patients and were strongly associated with reduced maternal-infant HIV-1 transmission.[15] Detected in 49% of the HIV-1 infected mothers, these responses were also associated with reduced viral loads and higher CD4 T-cell counts and stronger HIV-specific T-cell responses.[16] Responses could be mapped to individual responding peptides using a whole genome peptide mapping assay.[15-16] Of 6 patients mapped, 1 patient was infected for at least 10 years with undetectable viral load (<400 RNA copies/ml). Tested again 3.5 years later, similar specificities and robustness of responses were encountered, highlighting the durability of these NK responses[16] in an individual with suppressive control. Subsequent studies on a small group of LTNPs/ECs have shown the presence of such NK cell responses in 13/14 individuals compared with 1/10 in a group of progressors (C.Tiemessen, unpublished data).

Conclusion

Although new data have suggested that people with HIV infection be treated as early as possible in their infection, to control the disease in the individual and to prevent ongoing HIV transmission, the jury is still out on the impact of HAART on the long-term survival of elite controllers. It is remarkable that even in a group defined by such stringent characteristics of viral control as ECs, there is such heterogeneity in immune and genetic characteristics between individuals. This underscores that different factors and combinations of factors are likely to play a role in suppressive control in different individuals. It will require the combination of systems biology approaches (high throughput genetic, genomic and proteomic techniques) together with in-depth studies of specific molecules or pathways identified to be involved in natural control in the absence of antiretroviral treatment, which will collectively contribute to the development of new and novel therapeutics for the treatment of HIV-1 infection.

References available at www.cmej.org.za

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- There is substantial heterogeneity in the disease course among different individuals chronically infected with HIV-1.
- Approximately 1 in 300 individuals, known as elite controllers, have the ability to naturally suppress HIV-1 replication to levels undetectable by current viral load assays (<50 RNA copies/ml).
- Host genetic variability underlies host immune response capability and ability to control HIV-1 infection.
- The human leukocyte antigen (HLA) complex represents the genome region identified as having the greatest influence on control of HIV-1 infection, and is the most variable region of the human genome.
- Genome-wide association studies identify gene regions of significant effect but do not detect minor gene variants that may, singly or collectively with other gene variants, be involved in control of HIV-1 infection.
- Elite control cannot be explained by the host acquisition of attenuated strains of HIV-1.
- Elite controllers have very heterogeneous HIV-specific T cell responses, ranging from absent to high magnitude responses.
- Neutralising antibody responses are weaker in elite controllers compared with viraemic controllers or progressors.
- Antibody-dependent cellular cytotoxic (ADCC) activity is enhanced in elite controllers.