MINIREVIEW

New insights into the immunology and evolution of HIV

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ABSTRACT

Fewer than one million HIV infected individuals are currently receiving anti-retroviral therapy. The limitations of such treatment have underscored the need to develop more effective strategies to control the spread and pathogenesis of HIV. Typically, naturally occurring protective immune responses provide the paradigm for such development. It is now clear however that HIV can utilise the millieu of an activated immune system to its own replicative advantage. Mobilisation of the immune response, intended to thwart the virus, may instead fuel its dissemination, 'immune escape' and spread. The immense genetic variation of HIV contributes to lack of immune control and the development of progressive disease in the majority of infected, untreated individuals. Further delineation of the intimate interactions between the HIV and the immune system will be critical and recent advances in this direction are discussed.

Key words: *HIV, immune response, CTL, evolution, vaccine.*

The dissemination of HIV-1 in humans over the last 4 decades represents a catastrophic example of genome transmission and expansion. Over 60 million individuals have been infected leading to nearly 20 million deaths and 20 million orphaned children [1-4]. As most of the 16,000 individuals newly infected each day live in developing countries where anti-retrovirals are not readily available, the phrase 'living with AIDS' can be replaced by the statement 'dying from treatable HIV infection' characterised by the relentless decline in both the number and function of HIV specific CD4+ T helper cells which are preferentially infected[5]. In those for whom it is available, highly active antiretroviral therapy (HAAR

T) has reduced short-term mortality and markedly increased quality of life by preventingopportunistic diseases. Despite the initial optimism concerning this selective targeting of the HIV reverse transcriptase and protease[6](see front cover), HAART is associated with clinical side effects, drug resistance[7], and does not appear able to target the latent reservoir of the virus. Fig 1 illustrates the life cycle of HIV-1 with reference to current and future therapeutic targets one of which is shown on the cover.

The interaction between hosts and their infecting pathogens has been likened to a race. Here, the opponent appears able to run very fast indeed[8,9]. Misincorporation, insertion, deletion, or duplication of nucleotides occurs during reverse transcription with a frequency of 10^{-4} to 10^{-5}, owing to a lack of $3' \rightarrow 5'$ exonuclease 'proof reading' activity. This error frequency, the virion production of in excess of 10^9 virions per day in vivo, the large number of

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affected individuals and the persistence of infection, affords HIV-1 tremendous scope for the generation of viral diversity at 6 orders of magnitude greater than that observed for mammalian gene families. Developing more effective prophylactic and therapeutic strategies against a highly mutable virus that is integrated into the genome of a crucially important population of cells poses the major medical challenge of this century[10-12]. Increasing attention is being given to developing regimens that not only control replication but actually restore immune competence in those infected[13]. This would undoubtedly be enhanced by a better understanding of HIV pathogenesis.
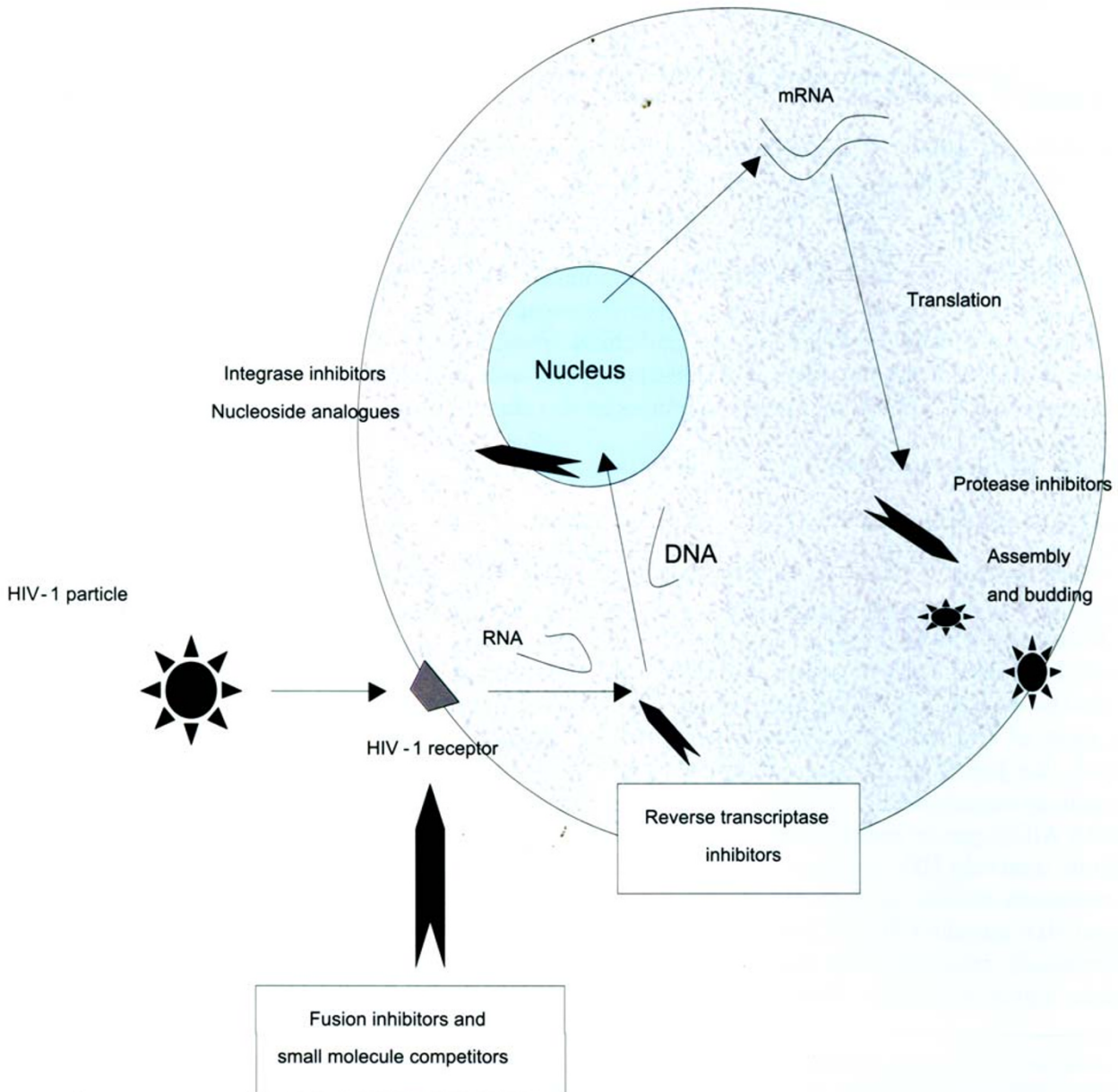

Fig 1. The life cycle of HIV-1 and sites of action of anti-retroviral drugs. New therapies include fusion and integrase inhibitors and small molecules that compete with receptor binding of HIV-1.

The immune response to HIV-1

One major issue hindering progress toward an effective vaccine to prevent or modulate HIV-1 infection is that the critical features needed for a protective immune response are not fully understood [13]. Although potent neutralising antibodies can protect against experimentally acquired infection in animal models, they are rarely generated in vivo in the infected person, and neutralisation-resistant viral variants develop rapidly in chronic infection. It is generally agreed that cellular immune responses, particularly specific cytotoxic T lymphocytes (CTLs), are important in the host response to HIV-1. This was shown by a rapid upsurge in viral replication in macaques depleted of CD8+ T cells, in both acute [14] and chronic SIV infection. CTLs develop very early in acute HIV-1 infection, coincident with a rapid fall in plasma viraemia, and in chronic infection, their levels are inversely related to viral load. Their weapons against HIV-1 include the ability to kill infected cells before new virus progeny are produced and to secrete potent antiviral factors, including chemokines, that compete with HIV-1 for the CCR5 receptor. These properties are not CTL specific, but the ability to recognize infected T cells through the presentation of viral peptides complexed with class I HLA molecules allows CTLs to home in on sites of active viral replication.

However, the HIV-specific CTL response ultimately fails to control HIV-1 replication. One reason could be the emergence of viral variants that escape CTL recognition. Viral variants with CTL escape mutations in the Simian immunodeficiency virus (SIV) tat gene were detected at high frequency in macaques shortly after infection with cloned SIV [15]. Escape variants also accumulate in HIV-1-infected humans[16](see below). Recent attention has focused on the quality of the HIV-specific CTL response, following observations that LCMV-infected mice deficient in T cell help generated virus-specific CD8+ T cells that neither killed nor produced antiviral factors. Since most HIV-1-infected people lack T-helper (T_h) responses to the virus, CD8 T cell function could be similarly impaired. In less immunocompromised patients, HIV-specific CD8+ T cells are sometimes able to produce a full range of antiviral cytokines and chemokines on encounter with antigen, but are characterised by low levels of perforins, a key mediator of cytolysis. This is associated with inefficient target cell killing and a cell-surface phenotye previously thought to indicate immature T cells[17].

In the case of HIV-1 infection, a small proportion of individuals termed long-term non-progressors (LTNPs) are able to maintain their CTL responses. In contrast to progressors, LTNPs also maintain CD4 related HIV responses[18]. Recent research has shown that they do in fact have increased and maintained expression of perforins associated with their CD8+ HIV-specific T cells[19]. Furthermore, since the first description of the antiviral factor secreted by CD8 T cells (CD8 antiviral factor, CAF), it has been recognised that soluble factors can inhibit viral replication. Previous studies had indicated that β-chemokines (CCL-3, -4, -5) may account for CAF's antiviral properties by competing with HIV-1 for binding to CCR5. However, such factors could only inhibit R5 viruses, and not X4 strains which utilise CXCR4 as their entry receptor. Using protein- chips and comparisons of mass spectra from patients with known different rates of progression, it has recntly been elegantly demonstrated that the a-defensins enable much of the anti-HIV activity of soluble factors from stimulated CD8+ T cells that is not attributable to β-chemokines[20]. Depletion of these molecules from culture supernatants eliminated activity against both X4 and R5 viruses and synthetic a-defensins were shown to reduce HIV-1 replication in vitro. Further work has shown high levels of the heat shock protein receptor (part of the innate arm of the immune system) in LTNPs (Stebbing et al, In press), and the recent demonstration that sub-physiological concentrations of a-defensins binds the heat shock protein receptor in a specific, saturable and dose-dependent manner[21] appears to provide a unifying theory regarding maintenance of the CTL response in some individuals. Accordingly, the viral phenomenology of apparent unlimited complexity with its multiple strategies and systems of pathogenic progression and immune evasion can be simplified.

The evolutionary response of HIV-1

Due to the dynamic nature of its replication, HIV-1 has evolved into multiple sub-types or clades which are partly shaped by evolutionary selective forces in

the host (mainly the immune response)[22]. Intra-subtype diversity may be as large as 20% and inter-subtype genetic distances as high as 35%. Subtypes A, C and D have spread throughout Africa whereas subtype B is dominant in the US and Europe. The distribution of clade C viruses is now most widespread (responsible for most new infections worldwide including those in Asia) and vaccine developments require consideration of this[23].

The remarkable genomic plasticity of HIV-1 and mutation away from an immune response has been shown in a recent study using automated DNA sequencing of the reverse transcriptase gene in 473 HIV-1 infected individuals[24]. Certain polymorphisms were associated with particular HLA class I alleles, and in some cases they clustered around known sequences for epitopes recognised by cytotoxic T lymphocytes and their flanking regions involved in processing antigenic peptides. One viral epitope was mutated in all but one of the HIV-infected subjects whose T lymphocytes had the potential to recognise it in its original form. In that single instructive case where no mutation had occurred, treatment with antiretroviral therapy has been started within days after exposure to HIV. This finding suggests that early treatment may limit escape mutations by blocking viral replication. Indirect evidence that HIV-1 has evolved within humans to avoid recognition by T lymphocytes in the context of the most prevalent HLA class I allele, HLA-A2, has also been found. Infected HLA-A2 -positive subjects were less likely than subjects without this allele to harbour HIV-1 with escape mutations. This had been previously suggested by the finding that the most prevalent HLA class I alleles worldwide (HLA-A1, A2, and A3) rarely present the dominant epitopes in the HIV envelope protein to CTLs. When a dominant epitope in HIV mutates in a way that allows it to evade recognition by CTLs, a further population of T lymphocytes recognising subdominant, type-specific epitopes may arise. Since these T lymphocytes recognise infected targets poorly, and are often generated after the function of CD4 T cells has been compromised, their ability to control the infection may be impaired. Indeed, during chronic infection most HIV-specific CTLs are incapable of lysing HIV-infected cells when tested directly ex vivo (unlike those obtained from LTNPs)[25,26].

HIV is an evolving pathogen that has only recently (on an evolutionary scale) infected humans [27]. Mutations that enable the virus to evade immune surveillance do not necessarily mean that it is evolving towards greater pathogenicity. For example, AIDS does not usually develop in the presumed original host (the chimpanzee), although recent evidence suggests that SIV had long since caused a selective reduction in intron orthologues of HLA class I alleles[28],[29]. However, the ability of the virus to evade recognition by CTLs appears likely to cause harm in humans by rendering the host's immune system unable to control the infection[30],[31]. Indeed, individuals harbouring HIV with more escape mutations have been shown to have a level of viraemia one log higher than that of patients who were infected with HIV that lacked such mutations. These findings suggest that an important clinical benefit of early treatment of HIV infection may be to limit escape mutations, which occur only when the virus has a chance to replicate.

Future work

The impact of HIV-1 diversity on drug and vaccine development remains uncertain[22], [32]. The race appears to be against a whole team of fast runners as opposed to one single fast opponent, and our chances of fulfilling Clinton's 1997 declaration of a vaccine within 10 years may decrease in proportion to the number of competitors. The major biological obstacles to the development of a preventative vaccine are (i) the fact that HIV incorporates its DNA into active sites of the hosts? chromosomes, leading to persistent infection for the life of that cell, its progeny and often the destruction of those cells; (ii) the lack of a small, readily available animal model; (iii) the genetic variability of HIV meaning that an effective vaccine must be broadly active to prevent emergence of resistant viruses; and, (iv) the fact that HIV utilises the same immune activation pathways required for successful vaccination, for its own replication.

However, the development of all successful vaccines has relied on the fact that some individuals become infected and do not develop symptoms of disease and our understanding of these cases in HIV-1 has increased enormously. In addition, some indi-

Tab 1. Some of the phase I and phase I/II HIV preventative vaccine studies that are currently ongoing or awaiting recruitment (Adapted from http://chi.ucsf.edu/vaccines and http://www.iavi.org/trials/basicsearchform.asp).

Protocol and number of individuals for recruitment	Status	Class
Phase I trials		
HIV vaccine trials network026 HVTN (n=160)	Immunisations complete	Canarypox vector (Clade B Env, Gag, Pro)
HVTN039 (n=110)	Immunisations complete	Canarypox vector (Clade B Env, Gag, Pro, RT, Nef)
HVTN041 (n=87)	Ongoing	Protein (Clade B Nef-Tat fusion protein + Env sub-unit); AS02A adjuvant
HVTN045 (n=30)	Imminent	DNA plasmid (Clade B Env, Gag, Pro, RT, Tat, Vpu, Rev)
HVTN048 (n=42)	Imminent	DNA plasmid (poly-epitope: Gag, Pol, Vpr, Nef, Rev and Env)
HVTN049 (n=168)	2003	DNA plasmids (Clade B Gag, Env); PLG adjuvant
HVTN040 (n=96)	2003	VEE vector (Clade C Gag)
HVTN044 (n=60)	2003	DNA plasmids (Clade B Gag-Pol-Nef, Clade A, B, C Env); DNA plasmid cytokine adjuvant (IL-1g)
VRC4302	Ongoing	DNA plasmid (gag-pol in frame fusion expressing protease, RT and integrase)
ALVAC vCP205	Ongoing	Canarypox containing (gp120-gp41 and pol fusion genes)
HVTN046 (n=100)	2003	DNA plasmid (Clade B Env, Gag, Pro, RT, Tat, Vpu, Rev)
To be announced		MVA vectors (Env, Gag, Tat, Rev, Nef, Pol)
		DNA plasmid (Gag); DNA plasmid cytokine adjuvant (IL-12)
		DNA plasmid (Gag) and peptides (poly-epitopic: Clade B Env, Gag, Nef)
		Non-replicating adenovirus vectors (Clade B Gag-Pol-Nef; Clade A, B, C Env)
		DNA plasmids (Clade C Gag, Env)
Phase I/II and Phase II trials		
HVTN203 (n=330)	Immunisations Complete	Canarypox vector (protein subunit Clade B Env); Aluminium hydroxide gel as adjuvant
HVTN047/Merck015 (n=400)	Imminent	HIV-1 Gag DNA in non-replicating adenoviral vector
HVTN042/ANRSVAC19 (n=180)	2003	Canarypox vector (Env, Gag, Pro, RT, Nef) and lipopeptides (poly-epitopic Gag, Pol, Nef)
THO23/LAI-DID	Ongoing	Recombinant envelope glycoprotein containing the gp160 from HIV-1 E (primary strain)
AIDSVAXB/E and B/B	Ongoing	Bivalent formulation of gp120 protein (Clade B)
AIDSVAXB	Ongoing	gp120 protein subunit (Clade B) produced in CO cells
To be announced		DNA plasmids (Clade B Gag-Pol-Nef; Clade A, B, C Env)

Fig 4. Illustration of angiotensin II signal transduction pathways involving heterotrimeric G-proteins and tyrosine kinase cascades.

viduals (termed exposed seronegatives, ESN) 'see the virus' but do not develop infection and study of the unmatched monogomous partners of HIV-1 positive individuals is already yielding valuable insights. The table (below) describes some of the current phase I and phase I/II HIV prophylactic vaccine trials in progress (adapted from the websites http://chi.ucsf.edu/vaccines and http://www.iavi.org/trials/basicsearchform.asp). Many others including those under the auspices of the International AIDS Vaccine Inititative (IAVI) are also underway.

The host has many mechanisms of preventing infection, both remembering it has been previously infected and an evolutionary ancient system of innate immunity, which recognises danger signals indicating that the invader is potentially harmful or is causing tissue damage. It has only recently been recognised that this ancient system, the first line of defence, is preserved in complex organisms. Research endeavours in this area have unveiled new functions and a deeper understanding of the HIV-1 armamentarium. For example, cell fusion and complementary DNA subtraction experiments have shown that a non-permissive phenotype (ie resistance to HIV-1 infection) may be conferred by the cytidine deaminase HIV-vif binding protein CEM-15, that appears to be responsible for the innate antiviral phenotype of certain T cells[33]. As for many of the other factors mentioned (and not mentioned[34]) above, such entities may become targets for the development of new drugs to alter the course of infectivity and infection[35]. Genetic influences on the outcome of infection are increasingly recognised and studies investigating these are valuable because the devastating immunodeficiency observed is often out of proportion to the level of Th cell destruction; here, the precise mechanisms governing cell death still remain unknown. An understanding of the immune responses that control HIV-1 in the context of individual host genetics and ever increasing viral diversity represents our best hope of a vaccine.

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