AIDS Rev 2001; 3: 133-144

Biological and Molecular Mechanisms in Progression and non-Progression of HIV Disease

Nitin K. Saksena[1], Bin Wang[1] and Wayne B. Dyer[2]

[1]*Retroviral Genetics Laboratory, Centre for Virus Research, Westmead Millennium Institute, Westmead, Australia*
[2]*Viral Immunology Laboratory, Tissue Typing Department, Australian Red Cross Blood Service, Sydney. Australia*

Abstract

The natural history of HIV-1 infection varies considerably from one individual to another, with some individuals progressing to AIDS rapidly after primary infection, while others remain clinically asymptomatic with no evidence of immune dysfunction. Recent studies have shown that reasons for progression and non-progression are multifactorial and may involve genetic, virological and immunological factors that influence HIV disease progression in various ways. However, it remains unclear whether a relatively benign course of HIV infection is due to viral or host factors or a combination of both. Thus, a clear understanding of host and viral factors, that determine the likelihood of infection or the rate of disease progression, could unveil the key factors that are involved in either progression or non-progression of HIV disease. Here, we have reviewed various genetic, viral and immunological factors that may cause progression and/or non-progression of HIV disease. In addition, this review provides in detail some current hypotheses and perspectives on true non-progressive HIV disease which is a subject of intense investigation, as these individuals may provide relevant information for the development of future HIV-1 vaccines and treatments.

Key words

HIV. Disease progression. CD8 factors. Helper T cells. Resistance. AIDS.

Introduction

The human immunodeficiency virus (HIV) which causes AIDS has brought about a global epidemic far more extensive than what was predicted even a decade ago. UNAIDS and WHO now estimate that the number of people living with HIV and AIDS at the end of year 2000 stands at 36.1 million.

Correspondence to:
Nitin K. Saksena
Retroviral Genetics Laboratory
Centre for Virus Research
Westmead Millennium Institute
Westmead NSW 2145
Australia

The pathogenic mechanisms that underlie HIV-1 infection are complex and highly variable and depend on the interplay between numerous viral and host factors[1]. The median time from infection to the development of AIDS is about 10 years, although AIDS can develop in as little as 3-6 months[2,3]. There is considerable clinical variability following infection with HIV from one individual to another resulting in variable severity of HIV disease. The typical course of HIV infection includes acute clinical syndrome, a prolonged period of clinical latency, and then a stage of clinically apparent disease which is commonly characterized by opportunistic infections and neoplasm[1]. The establishment of chronic infection occurs despite vigorous HIV-specific cell-mediated and humoral immune responses

that are usually present early in primary infection[1,4-6]. Although early immune responses can reduce the plasma viral load considerably, they fail to eliminate the virus from the body[4,7]. However, these immune responses can influence the period of clinical latency of the virus, with progression to AIDS occurring over a median period of 8-10 years[8,9]. In addition, a range of viral and host factors operate in tandem to determine the course of HIV disease. Thus, due to enormous variability in host factors and differences in infecting HIV-1 strains, the time to the development of AIDS varies from one individual to another and can be categorized as rapid, slow or long-term nonprogressive causes of disease progression.

These different progression rates are primarily defined on the basis of CD4+ T cell counts and viral load because progressive loss of CD4+T cell counts and increasing plasma viremia are two most important biological features of progression to AIDS. The Kaplan Meier survival curves clearly showed that individuals with a viral load higher than 36,270 HIV-RNA copies per ml progressed to AIDS within five years, whereas only 8% of individuals with a viral load less than 4,530 RNA copies per ml progressed to AIDS. In contrast, the plasma levels of HIV-1 RNA in the subjects with non-progressive HIV infection were usually 20 times lower than those with progressive disease[10]. A range of viral and host genetic and immunological factors have been shown to play an important role in disease progression.

Resistance to HIV infection has been identified in Kenyan[11], Gambian[12], and Thai prostitutes, who despite frequent exposure to HIV-1 have remained persistently seronegative. So far, no single mechanism or factor conferring resistance to HIV infection in these highly exposed uninfected individuals has stood out.

Taking some of these issues into consideration, this review critically examines various perspectives and current hypotheses on each of the host and viral factors that may play a significant role in determining the course of HIV disease, and also the factors that may be responsible for inducing a state of "truly non-progressive HIV infection".

Viral load-induced architectural differences in lymphoid organs and disease progression

The clinically latent phase was once believed to represent a lack of viral replication and activity, based on observations of the peripheral blood. However, it is now known that viral replication can occur unabated in lymphoid compartments, and they are considered to be a major reservoir of infectious virus[13]. As a result of this viral activity, the disruption of lymph node architecture in HIV infected individuals worsens through the different clinical stages toward AIDS. The use of antiretroviral drugs in human and non-human primates with progressive disease has shown that lymph node architecture can be restored, and treatment initiated even at advance stages of HIV disease could slowly reverse pathological changes in the follicular dendritic cell network[10,14]. It has been shown that lymph nodes from long-term non-progressive subjects were palpable with intact germinal centres and mantle zones, and with very little evidence of follicular lysis. In contrast, lymph nodes from subjects with progressive HIV disease showed histologic features typically seen in HIV-associated lymphadenopathy, such as large, irregular, fusing germinal centres, follicle lysis, loss of the mantle zone, hypervascularity, plasma cell hyperplasia, focal fibrosis and lymphocyte depletion[10]. Thus, such architectural integrity of lymph nodes can define the different stages of HIV disease progression. Host immune factors that control viral replication in LN without damaging LN structure may be crucial determinant of delayed progression.

Viral genetic diversity and disease progression

The extent of genetic variation present in the HIV population of a host depends on the size of the viral population, extent of viral replication, retroviral mutation rate, recombination rate, and the selective pressure exerted upon the viral population. HIV quasispecies emergence is a direct consequence of the influence of host selective pressures. Viral strains that can escape immune control and which carry advantageous mutations for viral fitness eventually dominate *in vivo*.

There is now ample evidence to suggest that viral factors play a key role in non-progressive HIV disease[15-21]. Despite an overwhelming evidence of the role of viral factors, there is extensive variability in viral gene defects from patient to patient. A rapid CD4+ T cell decline is observed when viral populations are relatively homogeneous[22,23]. Supporting these hypotheses is a detailed comparison of patients from the Atlanta (USA) cohort, examining both rapid and slow progressors as well as non-progressors. Both genetic and phylogenetic analyses revealed a striking restriction in *env* gene-based viral quasispecies diversity in rapid progressors, compared with the accumulation of increasing diversity in slow progressors and LTNP (Fig. 1). This could be attributed to the fact that the infecting viral strain undergoes drastic molecular divergence after infection is established as a result of host immune pressure, and that a lack of immune selective pressure during early disease stages equates with failure to contain high-level viral replication and disease progression. Several other studies have supported these data[23-25]. Taken together, these studies strongly suggest that accumulation of higher genetic diversity over time in individuals is indicative of immunocompetence, which is associated with long-term survival. In addition to genetic diversity in structural or enzymatic genes, simple amino acid changes or additions can play an important role in prolonging non-progressive infection. The addition of a string of asparagine residues resulting in an extension of the V2 region in *env* has been shown to be prevalent in HIV-1 strains from slow progressors

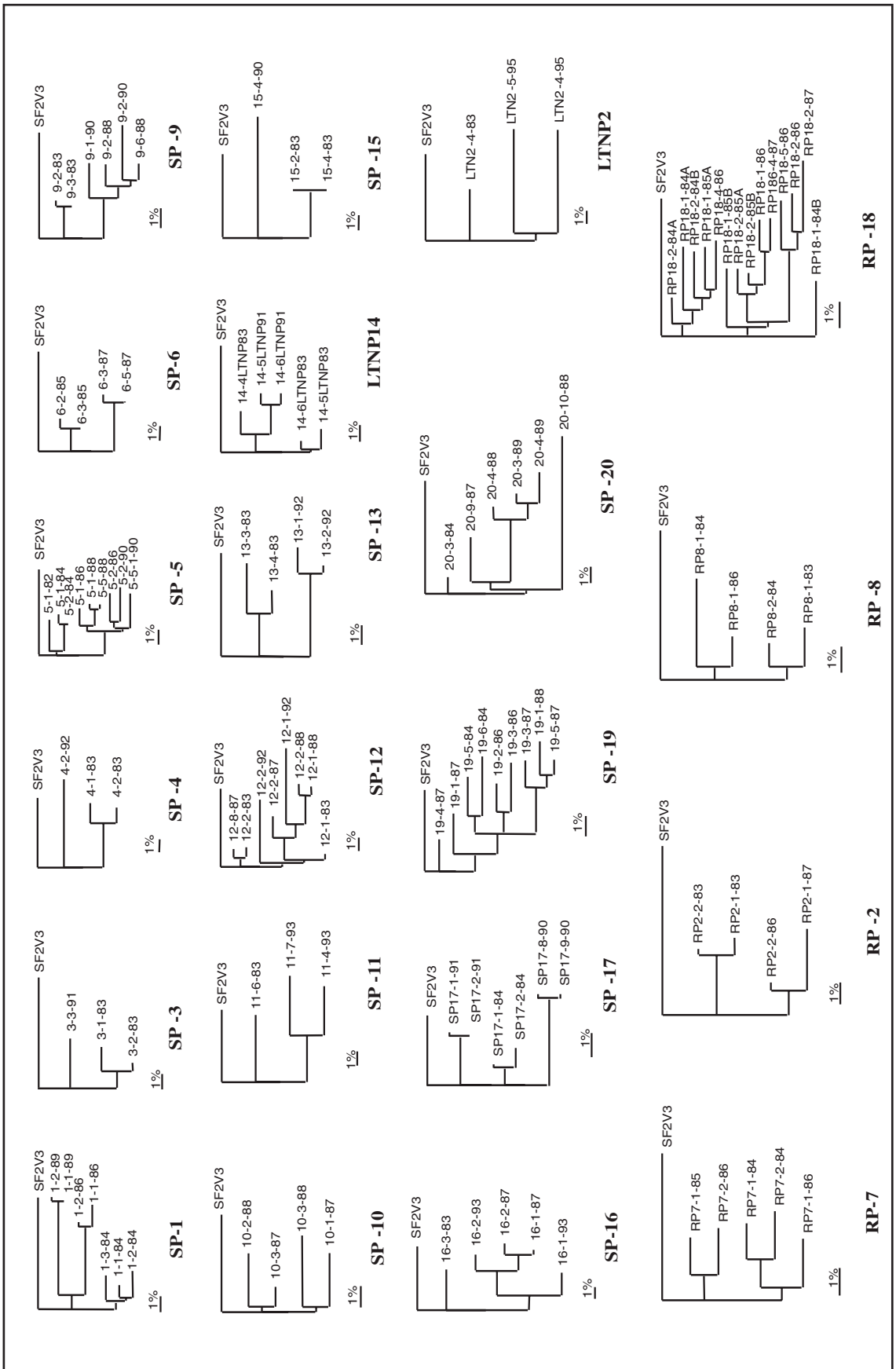

Figure 1. *Neighbor joining distance trees of PBMC-derived env gene (312 bp) clones from individual patients from the Atlanta, CDC cohort, SP (slow progressor), RP (rapid progressor) and LTNP (long-term non-progressor) showing viral evolution and phylogenetic relationships over time in vivo. Longer branch lengths over time in case of SP and LTNP suggest accumulation of higher genetic diversity over time and is related to their immunocompetent period. In contrast, the branch lengths in RP (RP 7, 2, 8 and 18) do not vary much over time.*

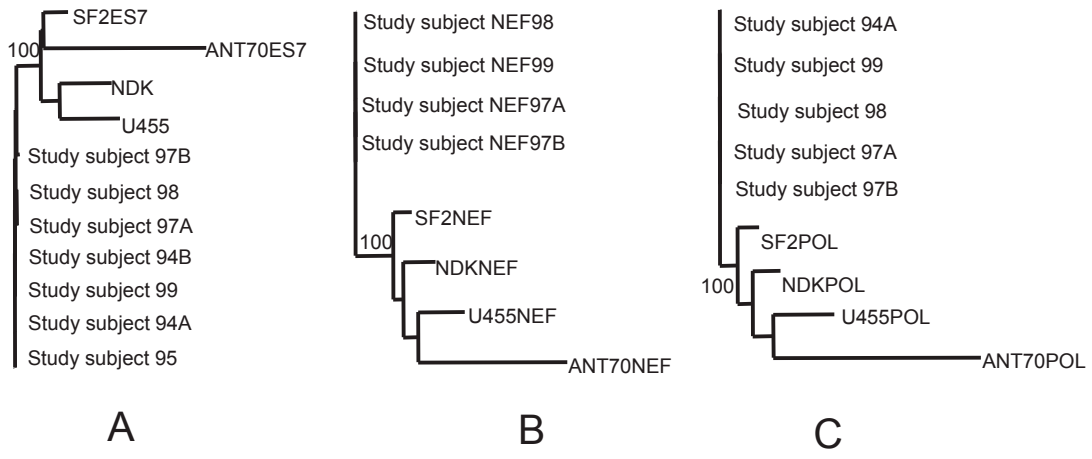

Figure 2. *Neighbor joining trees showing phylogenetic relationships between sequences obtained from the peripheral blood mononuclear cells (collected between 1994-1999) of a true non-progressor. Tight clustering (supported by 100 bootstrap replications in each case) of viral strains from the patient over time based on* env *gene (a),* nef *gene (b), and* pol *gene (c) clearly suggest no evolution of viral strains* in vivo*. In addition, the sequences recovered in each gene were completely identical between 1994 and 1999. Reference sequences (SF2: subtype B, NDK: subtype D, ANT70 subtype O, U455: subtype A) were retrieved from the HIV database, Los Alamos and were used as prototypes for comparisons.*

and non-progressors[26,27]. Studies on *vpr* gene quasispecies from an LTNP mother-child pair (infected for 17 years) have also shown that the defects in the *vpr* gene may also be related to slow or non-progressive HIV disease. It should be cautioned that these changes in *vpr* or any other gene may be patient and/or strain dependent[20]. Further, a detailed functional study of defects in *vpr* gene identified from sequential viral isolates from this mother-child pair showed reduced Vpr localization to the nucleus and no cell killing, which must have played a crucial role in delaying the course of disease progression.

Other studies[18] have also shown that defects in other regulatory and accessory genes such as *vif, vpu, rev, tat* and *nef* may also contribute to slow or non-progressive HIV disease. Based on the role of these accessory genes in the HIV life cycle, any defect in these genes may be attenuating and may have the potential to change the disease outcome. Although examples of mutant accessory genes resulting in a lack of disease progression are rare, these show that no single pathogenic determinant can uniformly explain the lack of disease progression, and that efforts to counteract the biological function of more than one of these genes may provide a greater effect on viral replication and disease progression.

In total contrast to most studies of viral divergence, some "true non-progressors" also display a rare phenomenon of absence of any viral evolution *in vivo* over an extended time[28] (Fig. 2). This lack of viral evolution in our study was associated with undetectable viral loads, high CD4+ and CD8+ T cell counts, below 10 copies of integrated provirus, no recovery of any culturable virus *in vitro* and potent HIV-specific CD4+ and CD8+ T cell responses. Thus, lack of viral evolution over time may be an indicator of the strength of the host's immune system in completely containing HIV replication and this may underlie "true non-progression" in a very small group (0.8% of HIV-infected population) of rare individuals.

Role of *nef* deletions in HIV disease progression: lessons from human and non-human primates

Nef is essential for viral pathogenicity *in vivo,* and may be involved in binding to CD4 and the down-regulation of its cell surface expression, enhancement of HIV-1 replication in primary T cells, binding to several cellular protein kinases[29].

Studies on SIV suggest that *nef* is essential for viral replication *in vivo,* and induction of high viral loads and progression to fatal AIDS (SAIDS)[30]. SIV with *nef* gene deletions failed to produce AIDS in most primates[29], but a recent study[31] demonstrated that continuing deletions in the *nef* gene eventually contributed to disease progression. Similarly, some Sydney Blood Bank cohort patients (who acquired a *nef*-deleted virus from the same source) have shown that additional *nef* deletions and rearrangements coincided with the turning point toward disease progression[32], not non-progression as previously expected[15]. Data showing positive selection at the *nef* gene during disease progression[33] further suggest that there are certain domains within the Nef protein that the virus needs for the maintenance of virulence. Further functional characterization of such domains may define the regions that regulate virulence of infecting strains. Overall, these data on macaques and humans with natural *nef*-defective infections have raised concerns about the use and safety of live attenuated HIV vaccines. At the present time, mechanisms by which Nef enhances viral replication and

how Nef expression modulates the progression and non-progression of HIV and SIV disease requires further characterization before genuinely attenuated constructs can be tested as vaccines.

Role of viral phenotype and co-receptor usage in HIV disease progression

The majority of HIV-1 strains that are involved in acute HIV infection and which persist through the early phase of infection are primarily M-tropic or NSI viruses, whereas at later stages SI strains may predominate, leading to rapid progression characterized by accelerated CD4+ T cell decline. Changes in the V3 region determine the syncytium inducing phenotype[34]. There is no clear demarcation observed between NSI and SI HIV strains in their ability to cause AIDS, as both phenotypes can lead to HIV disease progression and either one can persist at later stages. In >50% of HIV infected individuals NSI strains persist till the late stages of AIDS. One major difference between NSI and SI strains is the consistency of SI strains in infecting CD4+ T cells[35]. These findings indicate that SI phenotypes may have some relevance in the rapid loss of CD4+ lymphocytes in individuals progressing rapidly to AIDS.

Recent studies have also shown that the differences in viral phenotypes also correlate with their ability to use different co-receptor molecules, with T-tropic strains using CD4 and CXCR4, and M-tropic strains CD4 and CCR5 for cell entry[36-38]. Additionally, there are other members of the seven transmembrane chemokine receptor family (CCR1-CCR4, BOB and BONZO) which are also known to be used as coreceptors by HIV for entry[39]. As far as CCR5 usage is concerned, mutations in the envelope *gp*120 (especially the V3 loop) have been found to effect co-receptor usage[40]. Interestingly, it has also been suggested that infection with viral variants capable of utilizing a broad range of co-receptors correlate with increased disease progression rates[39]. In contrast, LTNP maintain exclusive usage of CCR5 and generally produce high levels of β-chemokines. A comparison of virus isolated from rapid and slow progressors and LTNP has shown that NSI strains with slow replicative capacity may contribute to slow progression or non-progression of HIV disease[40]. Also, both viral and host determinants leading to the emergence of viral variants capable of using an expanded range of co-receptors may increase the rate of disease progression.

Possible influence of polymorphisms in co-receptor genes in HIV disease progression

Because these co-receptors are crucial for viral entry, natural mutations in co-receptors genes have been thought to be significant in influencing HIV pathogenesis and disease progression. A number of host gene polymorphisms have been assessed for their possible role in HIV disease progression.

CCR5 Δ32: This polymorphism in the CCR5 gene, a deletion of 32 bases in the open reading frame (ORF-Δ32), homozygous in 1% of the Caucasian population, encodes a non-functional CCR5 protein and is strongly associated with resistance against HIV-1 infection[41-46]. While this genotype protects against infection with CCR5-tropic strains of HIV-1 *in vitro,* PBMC from CCR5 Δ32 homozygotes are infectable with CXCR4 using strains, and this appears to have been the cause of infection in some CCR5 homozygotes[47]. Heterozygosity for the CCR5 Δ32 allele has also been attributed to cases of resistance against infection[42,43]. However, while heterozygousity may not necessarily protect against infection in all cases, it has been associated with decreased rates of progression to AIDS. Some studies have shown the progression to AIDS is delayed by an average of 2 years in CCR5 Δ32 heterozygotes compared to people lacking this allele[42,43,46,48]. Therefore, since this host genetic factor appears to predispose an individual to significant levels of resistance against HIV infection and/or disease progression, therapeutic interventions to block or reduce the expression of CCR5 may have an impact on disease, and is currently sought.

CCR5 59029G/A, CCR2 64I and SDF-13'A are single nucleotide polymorphisms in the CCR5 promoter, CCR2 ORF and SDF-1 3' untranslated region, respectively. Another allelic variant in CCR2 has been shown to be responsible for delaying the progression to AIDS in heterozygotes[49,50]. CCR2b has a single base change that causes a conservative substitution in a transmembrane region and linked to a base change in the CCR5 promoter[49], however, the functional importance of this remains unknown. CCR2-64I has been associated with significantly lower viral loads at 9-12 months after seroconversion[49]; the early viral load is a "set point" highly predictive of progression rate[51]. Since CCR2 co-receptor is used by very few known HIV strains[52], there is substantial doubt on the role of this polymorphism in delaying HIV disease progression.

Haplotype analysis has shown that the 59029A allele is in complete linkage disequilibrium with both CCR5 Δ32 and CCR2-64I, that is all chromosomes bearing CCR5 Δ32, 64I also have 59029A. The Multicenter AIDS Cohort study (MACS) has shown that men having the 59029G/G allele had survival times augmented by an average of 3.8 years over individuals with the 59029A/A allele ($p = 0.004$)[53].

Recently, some more evidence appears to suggest that the genetic polymorphism in the CCR5 promoter region may affect the rate of progression to AIDS. The promoter region of CCR5 has been characterized, and transcription is regulated by two domains[54,55]. Screening of several thousand AIDS patients have shown four common allelic variants (CCR5P1-P4) and six rare alleles (CCR5P5-P10)[54-56]. These CCR5-promoter polymorphisms appear to affect progression of established HIV-1 infection, but not transmissibility, by regulating CCR5 gene transcription[49,57]. CCR5, CCR5-promoter, and CCR2 polymorphisms affect disease progression by regulating CCR5 co-receptor expression to interfere

virus interaction with the CCR5 co-receptor. Replication of CXCR4 using HIV can also be affected by a single base change in the 3'-untranslated region of SDF-1 mRNA (SDF1-3'A)[58]. SDF-1 is a ligand for the HIV-1 co-receptor CXCR4. Homozygotes show an increased production of SDF-1 leading to blockage of CXCR4, and therefore exhibit reduced infectability with T-tropic viral strains. Other mutations in the CCR5 gene, such as M303, encodes a truncated receptor and protects against HIV-1 infection[59].

Therefore, there are numerous combinations of host co-receptor and viral envelope and promoter polymorphisms which, from the onset of infection, combine to determine the level of infectivity and rate of viral replication, which thereby influences the course of disease progression. While these viral and host genetic factors may remain fixed, disease progression is also influenced by host immune responses, which may change in response to ongoing viral evolution. Effect of HLA on disease progression.

Associations between host genetics and the rate of disease progression and susceptibility to various opportunistic diseases have demonstrated either a positive or negative benefit of certain HLA genotypes[60]. Rapid disease progression has been associated with and single alleles, eg. HLA-B35[61], or HLA haplotypes, eg. HLA-A1/B8/DR3[62,63], and a weak association between risk of seroconversion upon exposure has been linked to the HLA-A1/B8/DR3 haplotype[62]. There has been some contradictory conclusions made on the effect of some HLA types on disease progression, however a combined analysis of these HLA associations supports a role for particular HLA polymorphisms in delaying disease progression[60,64]. It has been suggested that differences in HLA affect antigen presentation or recognition of HIV epitopes by T cells[64]. HLA diversity is important for providing a wider range of epitopes recognized by CTL[65]. The number of HLA-specific CTL epitopes potentially recognized by an individual has been associated with the rate of disease progression[65]. This shows that HLA does have an effect on HIV disease, and vaccine candidates must be designed with HLA diversity in view.

Cytotoxic T lymphocyte control of viral replication

Initial efforts to contain HIV focussed on a search for humoral immune correlates of viral clearance, but it eventually became clear that CTL were more important in controlling viraemia, as large numbers of these virus-specific cells were expanded in infected individuals[66]. The extent to which CTL provide protection as opposed to being a casual marker of viral replication, or even causing immunopathogenesis, has been controversial[67]. There now exists substantial evidence supporting the association between CTL and the control of viraemia during both primary[6,68], as well as chronic infection[69,70], and in delaying disease progression[71-74]. But it is increasingly apparent that not all CTL can be associated with the control of viral replication. High frequencies of CTL have been detected in both LTNP as well as progressors[75]. It is possible to distinguish between inappropriately activated CTL and CTL induced to efficiently kill HIV-infected cells *in vivo*. While CTL responses to some immunodominant Gag epitopes can control viral replication *in vitro*, CTL specific for these epitopes do not necessarily exert strong selective pressure against HIV replication *in vivo*[76]. The presence of p24-specific proliferative responses in some patients is associated with CTL that exert strong selective pressure against HIV, based on viral evolution at the specific CTL epitope as well as control of viraemia in the host[76]. It was concluded that the priming of protective or 'efficient' *gag*-specific CTL activity as opposed to 'ineffective' CTL required *gag*-specific helper T cell responses[77]. The concept of inefficient CTL was supported further with the description of phenotypically silent CTL, defined as cells that bind MHC-peptide tetramers but do not produce IFNγ, indicating a lack of functional CD8+ T cell response to a cognate epitope and no effector activity[78]. An IFNγ-deficient 'stunned phenotype' was also demonstrated during acute HCV infection, with a reversion to IFNγ production occurring only after resolution of peak viraemia[79]. Likewise, the preferential targeting of activated HIV-specific helper T cells during acute infection may contribute to the establishment of chronic infection. This prompts the question as to whether defective antigen presentation and T cell help may be a common underlying factor behind the malfunction of CTL, as well as other CD8-mediated antiviral responses. Understanding what constitutes effective CTL activity with respect to the role of HIV-specific helper responses can contribute to the elucidation of what constitutes protective cell-mediated immunity (CMI), which may prevent or delay disease progression.

HIV-specific helper T cell responses are associated with non-progression

It is now understood that a deficiency in HIV-specific helper T cell function is associated with the failure of CTL to control viraemia, and that the association between CTL and the control of viraemia[70] may be qualified in the context of T cell help. A correlation between strong p24 proliferative responses in untreated patients with low or undetectable viral loads has been observed predominantly in LTNP [80]. This suggests that the association between helper T cell response and low viraemia is a functional immune response to HIV infection, which can result in a non-progressive disease course. This study also showed that cytokines produced by these protective helper T cells were primarily type-1 (mainly IFNγ). However, other studies have shown that the excessive production of gp120 may induce a type-2 cytokine response by helper T cells. The so-called type-1 cytokines, including IFNγ, IL-2, and IL-12, are generally involved in cell-mediated immune (CMI) responses, whereas Type-2 cytokines (IL-4, IL-5, IL-10, IL-13) are thought to promote humoral

immune responses[81]. The maintenance of a type-1 response to HIV antigens has been associated with the delay of disease progression, whereas a type-2 response, as a result of high viral replication, down-regulates CMI responses and accelerates disease progression[82]. It has even been suggested that the accelerated progression to AIDS seen in some African nations is a result of increased type-2 cytokine levels induced by a high prevalence of parasitic infections[83]. However, the cytokine network is far more complex than the type-1/2 models suggest, and due to conflicting observations about multiple cytokine responses, the relevance of these models have been somewhat discounted[84,85]. More recent studies on helper T cell function suggest that vigorous proliferation and IFNγ release[86], and other effector functions including appropriate costimulation of CD8+ T cells[87], may be more important in priming an effective antiviral CMI response capable of containing viral replication.

Our studies of LTNP have failed to show a consistent relationship between CTL, viraemia and disease progression status[88], whereas HIV-specific helper T cell responses have independently defined the difference between individuals with detectable and undetectable viral loads, and progression and non-progression in long-term infected patients. The clear-cut division of LTNP with or without detectable viral loads (Fig. 3) was based on detectable p24 proliferative responses that were sustained over the long term. This association held true for an attenuated HIV-infected cohort as well as a cross-section of LTNP with unrelated infections. We have also shown that detectable p24 responses were associated with delayed progression to AIDS in a slow progressor cohort (Fig. 3E), whereas p24 responses were not detected at

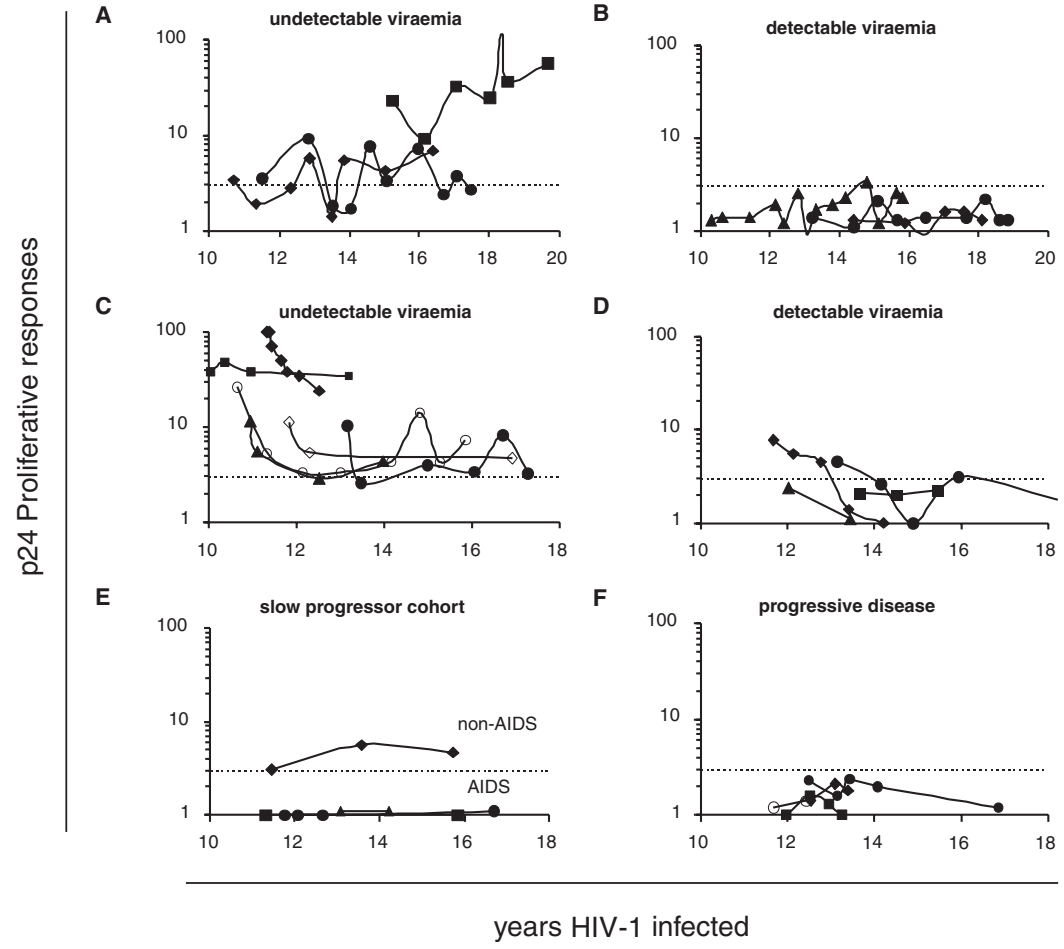

Figure 3. *HIV-specific T cell proliferative responses are associated with the control of viremia and the delay of disease progression in LTNP and slow progressors. A detectable response to HIV-1 p24 (stimulation index > 3; indicated by broken line) strictly divided a cohort of individuals, with HIV-1 infection from a common source (the Sydney Blood Bank Cohort), with undetectable viraemia (A) from those with detectable viremia (B), suggesting this immune response may play a crucial role in delaying disease progression despite infection with an attenuated virus. Positive p24 responses in LTNP with non-attenuated HIV-1 infection were also associated with the control viral replication (C), whereas individuals whose p24 responses declined below the limit of detection (D) had detectable and increasing viremia. A slow progressor cohort of individuals with a common infection source (E) demonstrated an association between p24 responses and lack of progression to AIDS, in the one patient who did not progress to AIDS during the study, even though this individual had low-level viremia. Another group of patients with progressive disease and increasing viremia failed to respond to p24 at any time during the study (F).*

any time point in patients who progressed to AIDS. Similar results have been demonstrated in studies of other LTNP, showing various levels of viral suppression associated with the strength of the p24 response (Fig. 4). An undetectable p24 response was typically associated with the loss of non-progressor status upon follow-up of these patients. The rarity of detectable HIV-specific helper T cell responses in untreated individuals, with the exception of genuine LTNP with low or undetectable viral loads, shows that this is a crucial component of the protective CMI response against HIV infection and in delaying disease progression.

CD8+ cell antiviral factors and inhibitory chemokines

The two main types of CD8+ T cell-mediated antiviral activity described in HIV infection consist of either the classic antigen-specific, HLA-restricted lysis of infected cells, discussed earlier, or the inhibition of viral replication *via* soluble factors in the absence of cell killing[89]. A CD8+ T cell-derived antiviral factor (CAF) has been shown to inhibit HIV replication[90,91]. CAF is a small cellular protein that is heat and pH stable, and is produced by activated CD8+ T cells. Based on functional features and blocking assays, CAF is not one of the currently identified cytokines or chemokines[92,93]. Although the presence of this antiviral activity against infected CD4+ T cells can be shown in CD8+ T cell culture supernatants, cell-to-cell contact results in maximum viral suppression[94,95]. This suppression of viral replication takes place before RNA transcription[96], resulting in no viral pathogenic effect on CD4+ T lymphocyte proliferation and cell morphology[28].

In general, the levels of antiviral factor(s) produced by CD8+ T cells can be correlated with the clinical disease stage in HIV-infected patients[94], and it is one of the most important mechanisms controlling HIV replication in non-progressors[28]. One study showed that the CD8+ T cell-derived antiviral response was more closely associated with viral control than was CTL activity[97]. However, the involvement of HIV-specific helper T cell responses was not determined. Preliminary data from our laboratories suggests that strong HIV-specific proliferative responses are associated with potent CD8+ T cell antiviral responses. We hypothesise that strong helper T cell responses with the provision of appropriate costimulation in the context of minimal viral replication and associated immune activation, may preferentially induce this antiviral activity instead of a CTL dominated response which is typically associated with chronic immune activation. This may also explain how helper T cell responses are involved in viral control independent of CTL activity.

Various CD8+ T cell-derived chemokines, including MIP-1α, MIP-1β, and RANTES, block the entry of HIV strains that utilize the CCR5 co-receptor by competing for binding[39,98,99]. Unlike CAF, which down-regulates post entry viral transcription, these β-chemokines mediate antiviral activity by blocking the entry of only M-tropic strains into CD4+ T cells and macrophages. Other non-CD8-derived chemokines may play some role in reducing viral infection. The ligand for CXCR4, SDF-1, can inhibit entry of T-tropic strains but not M-tropic or dual tropic strains[100]. A 801G-A transition in the 3' untranslated region in SDF-1 is thought to upregulate this effect. However, conflicting studies on the effect of this mutation have shown either delayed[58] or accelerated[101] rates of disease progression. Overall, the data does not suggest any significant role of these chemokines in preventing HIV disease progression[102-104]. In contrast, CAF is largely associated with non-progressive HIV disease, and its response is targeted at both cytopathic and non-cytopathic HIV-1 strains. In patients who subsequently progress to AIDS, this activity declines over time, the precise reasons for which remain unknown. However, based on our studies of LTNP, we hypothesise that T cell help may be required for the maintenance of the CD8 antiviral factor response.

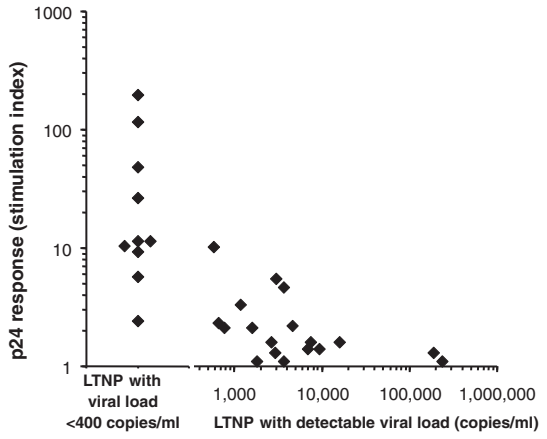

Figure 4. *The proliferative response to p24 in LTNP is associated with the control of viraemia and non-progression. All individuals with detectable virus either progressed to symptomatic HIV disease upon follow-up, or showed some signs of disease progression.*

Despite the obvious potential for CD8+ T cells to provide immunity against viruses, these cells may not always be the dominant effectors of antiviral immunity. So far, the infection of CD8+ T cells has remained controversial, but recent data[28,105] has shown that HIV can infect CD8 cells without using CD4. It has been hypothesised that under selective pressure due to a declining pool of CD4+ T cells in the body, the virus changes and targets CD8+ cells, as a final phase in the immunological decline to AIDS. These data are new and are based on a single patient and need further investigation. It remains to be determined whether CD8 tropic HIV can also promote increased rates of disease progression if transmitted.

Possible role of CD8-derived factors in resistance against HIV infection in highly exposed uninfected individuals

Some individuals who remain uninfected despite repeated exposures to HIV may have been naturally vaccinated against HIV. A detailed study of 87 individuals (56 adults and 31 children) has shown that despite repeated exposure to HIV these individuals have remained uninfected. No co-receptor or other host gene polymorphisms were seen which could be attributed to their uninfected status. The only shared immunological feature was high production of a CD8+ T cell-derived antiviral CAF-like factor in about 50% of individuals. Since soluble CD8-derived antiviral factors are present in asymptomatic individuals, but are not detected in uninfected individuals, the only plausible explanation for the priming of CD8 cells to produce these factors is exposure to sub-infectious doses of virus, or perhaps abortive infection. A recent report about Thai prostitutes (J McNicholls, unpublished) who have also been repeatedly exposed to HIV-1 show that they may be primarily protected by elevated antiviral factor(s) produced by CD8+ T cells.

More recent studies of highly exposed Kenyan prostitutes are discouraging. Out of 43 highly exposed prostitutes who discontinued their profession and were involved in HIV vaccine trials, six have become infected with HIV. Their CD8+ T cells appear to have lost the ability to protect against infection. These data suggest that in order to maintain sustained protective immunity against HIV infection, continued exposure to HIV-1 antigen may be imperative. Thus, because of continued antigenic exposure, the ability to induce CD8-derived antiviral factors is maintained and provides long term protection, particularly in genuine LTNP. These observations need to be considered in future vaccine design.

Protective mechanisms in CD4+ T cells: clues from superinfection studies

CD8+ T cell-derived β-chemokines may be able to block HIV entry by occupying all available co-receptor sites, while CAF-like factors may inhibit viral replication within infected CD4+ T cells. Results from our study of highly purified CD4+ T cells from true non-progressors also reveal that, although these cells can be superinfected by both mono-and dual-tropic HIV-1 strains, there are post-entry protective mechanisms against syncytia induction, apoptosis and development of cytopathic effect[28]. No ultrastructural abnormalities were found in CD4+ T cells following challenge with mono-and dual tropic HIV-1 strains, whereas cytopathology was pronounced in uninfected donor cells. Further, this protection was beta-chemokine independent. The mechanisms of such post entry protection of CD4+ T cells in the absence of CD8+ T cells is novel, and may be coincident with antiviral factors in contributing to true non-progressive HIV disease. These data further suggest that in order to achieve strong antiviral responses, and CD4+ T cell protective mechanisms, presence of strong helper T cell responses may be crucial.

Concluding remarks

While prospective observational studies have documented a role for helper T cell and CD8+ T cell responses in containing viral replication and prolonging non-progression, the loss of these CMI responses, and associated increases in viral replication and disease progression, raises questions as to what truly is protective immunity. Although evidence presented in this review suggests that CMI is associated with viral suppression and non-progression, and that this is no mere casual association, there have been cases of symptomatic disease progression despite strong p24 proliferative responses. A former LTNP infected with a *nef*-defective viral strain eventually experienced declining CD4 counts, although his declining p24 responses remained relatively high and viral load remained below detection[106]. This is an extreme case, but it suggests that HIV-specific helper T cell responses acting alone do not protect against disease progression, and that a combination of host, and probably in this case viral evolutionary factors, combine to determine the disease outcome. The reason for the initial decline in CMI responses that leads to the loss of viral control, after years of non-progressive infection, is a critical issue that remains to be addressed.

Clearly, there are many factors associated with disease progression, and it is unlikely that only one of these is responsible. Whichever factor(s) are involved, an upsetting of the fine balance that enables host control over viral replication and pathogenesis, gives the virus the upper hand to overcome these host factors. There is evidence that specific viral evolutionary events may directly lead to disease progression. Our recent studies of the Sydney Blood Bank Cohort (manuscript in preparation) have shown that an accumulation of mutations have led to the emergence of variants with increased replicative fitness, as well as potential immune escape sequences, that contributed to disease progression in two cases. But these two individuals did not have detectable p24 responses prior to the emergence of these new strains. Therefore, the effect of this kind of viral evolution on disease

progression remains to be determined in true LTNP, who have protective CMI. However, viral evolution has been attributed to disease progression in other studies, based on immune escape[107,108], change to a syncytium-inducing phenotype[109], increasing virulence, and other determinants of replicative fitness. Therefore, a detailed prospective study documenting the effect of any of these viral evolutionary events in LTNP, based on a stringent definition of non-progression, who have protective CMI as discussed above, could provide an indication of the likelihood of host immune factors in maintaining a non-progressive course in the face of these viral changes. It may determine what triggers the change from a non-progressive disease course to that of progression to AIDS. Since knowledge of protective host immune correlates against HIV may be used to evaluate candidate vaccines and therapeutic immunogens, subsequent studies are needed to confirm the true immunological nature of non-progressive disease.

Acknowledgements

The authors are thankful for the NHMRC for funding NKS (grant ID 96895), and to Dr. Renu Lal and Dr. Tom Spira for their collaborative support with the Atlanta Cohort.

References

1. Pantaleo G, Graziosi C, Fauci A. The immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993; 328:327-35.
2. Lifson A, Rutherford G, Jaffe J. The natural history of human immunodeficiency virus infection. J Infect Dis 1988;158:1360-7.
3. Jason J, Lui K-J, Ragni M, Hessol N, W D. Risk of developing AIDS in HIV-infected cohorts of haemophiliac and homosexual men. JAMA 1989;261:725-7.
4. Clark S, Saag M, Decker W, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med 1991;324:954-60.
5. Safrit J, Andrews C, Zhu T, Ho D, Koup R. Characterization of human immunodeficiency virus type 1-specific cytotoxic T lymphocyte clones isolated during acute seroconversion: recognition of autologous virus sequences within a conserved immunodominant epitope. J Exp Med 1994;179:463-72.
6. Borrow P, Lewicki H, Hahn B, Shaw G, Oldstone M. Virus-specific CD8+ cytotoxic T lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol 1994;68:6103-10.
7. Daar E, Moudgil T, Meyer R, Ho D. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med 1991;324:961-4.
8. Saksela K, Stevens C, Rubinstein P, Baltimore D. Human immunodeficiency virus type 1 mRNA expression in peripheral blood cells predicts disease progression independently of the numbers of CD4+ lymphocytes. Proc Natl Acad Sci USA 1994; 91:1104-8.
9. Jurriaans S, Van Gemen B, Weverling G, et al. The natural history of HIV-1 infection: virus load and virus phenotype independent determinants of clinical course? Virology 1994; 204:223-33.
10. Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. N Engl J Med 1995;332:209-16.
11. Rowland-Jones S, Dong T, Fowke K, et al. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. J Clin Invest 1998;102:1758-65.
12. Rowland-Jones S, Sutton J, Ariyoshi K, et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Med 1995;1:59-64.
13. Pantaleo G, Cohen O, Schacker T, et al. Evolutionary pattern of human immunodeficiency virus (HIV) replication and distribution in lymph nodes following primary infection: implications for antiviral therapy. Nature Med 1998;4:341-5.
14. Zhang Z, Schuler T, Cavert W, et al. Reversibility of the pathological changes in the follicular dendritic cell network with treatment of HIV-1 infection. Proc Natl Acad Sci USA 1999;96: 5169-72.
15. Deacon N, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science 1995;270:988-91.
16. Kirchhoff F, Greenough T, Brettler D, Sullivan J, Desrosiers R. Absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. N Eng J Med 1995;332:228-32.
17. Huang Y, Zhang L, Ho D. Biological characterization of nef in long-term survivors of human immunodeficiency virus type 1 infection. J Virol 1995;69:8142-6.
18. Michael N, Chang G, d'Arcy L, et al. Defective accessory genes in a human immunodeficiency virus type 1-infected long-term survivor lacking recoverable virus. J Virol 1995;69:4228-36.
19. Saksena N, Ge Y, Wang B, et al. RNA and DNA sequence analysis of the nef gene of HIV type 1 strains from the first HIV type 1-infected long-term nonprogressing mother-child pair. AIDS Res Hum Retroviruses 1997;13:729-32.
20. Wang B, Ge Y, Palasanthiran P, et al. Gene defects clustered at the C-terminus of the vpr gene of HIV-1 in long-term nonprogressing mother and child pair: in vivo evolution of vpr quasispecies in blood and plasma. Virology 1996;223:224-32.
21. Wang B, Ge Y, Jozwiak R, et al. Molecular analyses of human immunodeficiency virus type 1 V3 region quasispecies derived from plasma and peripheral blood mononuclear cells of the first long-term-nonprogressing mother and child pair. J Infect Dis 1997;175:1510-5.
22. Delwart E, Sheppard H, Walker B, Goudsmit J, Mullins J. Human immunodeficiency virus type 1 evolution in vivo tracked by DNA heteroduplex mobility assays. J Virol 1994;68:6672-83.
23. Wolinsky S, Korber B, Neumann A, et al. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. Science 1996;272:537-42.
24. Shankarappa R, Gupta P, Learn G, et al. Evolution of human immunodeficiency virus type 1 envelope sequences in infected individuals with differing disease progression profiles. Virology 1998;241:251-9.
25. Lukashov V, Kuiken C, Goudsmit J. Intrahost human immunodeficiency virus type 1 evolution is related to length of the immunocompetent period. J Virol 1995 1995;69:6911-6.
26. Wang B, Spira T, Owen S, Lal R, Saksena N. HIV-1 strains from a cohort of American subjects reveal the presence of a V2 region extension unique to slow progressors and non-progressors. AIDS 2000;14:213-23.
27. Shioda T, Oka S, Xin X, et al. In vivo sequence variability of human immunodeficiency virus type 1 envelope gp120: association of V2 extension with slow disease progression. J Virol 1997;71:4871-81.
28. Wang B, Dyer W, Zaunders J, et al. Comprehensive analyses of a unique HIV-1 infected non-progressor reveal novel biological, molecular and immunological mechanisms suppressing viral replication. J. Immunology, (in press).
29. Miller M, Warmerdam M, Gaston I, Greene W, Feinberg M. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. J Exp Med 1994;179:101-13.
30. Kestler H, Ringler D, Mori K, et al. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 1991;65:651-62.
31. Sawai E, Hamza M, Ye M, Shaw K, Luciw P. Pathogenic conversion of live attenuated simian immunodeficiency virus vaccines is associated with expression of truncated Nef. J Virol 2000;74:2038-45.
32. Learmont J, Geczy A, Mills J, et al. Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1. A report from the Sydney Blood Bank Cohort. N Engl J Med 1999;340:1715-22.

33. Kirchhoff F, Easterbrook P, Douglas N, et al. Sequence variations in human immunodeficiency virus type 1 Nef are associated with different stages of disease. J Virol 1999;73:5497-508.
34. Oka S, Ida S, Shioda T, et al. Genetic analysis of HIV-1 during rapid progression to AIDS in an apparently healthy man. AIDS Res Hum Retroviruses 1994;10:271-7.
35. Fouchier R, Simon J, Jaffe A, Malim M. Human immunodeficiency virus type 1 Vif does not influence expression or virion incorporation of gag-, pol-, and env-encoded proteins. J Virol 1996;70:8263-9.
36. Moore J, Trkola A, Dragic T. Co-receptors for HIV-1 entry. Curr Opin Immunol 1997;9:551-62.
37. Samson M, Libert F, Doranz B, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722-5.
38. Wu L, Gerard N, Wyatt R, et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 1996;384:179-83.
39. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996;381:661-6.
40. Xiao L, Rudolph D, Owen S, Spira T, Lal R. Adaptation to promiscuous usage of CC and CXC-chemokine coreceptors in vivo correlates with HIV-1 disease progression. AIDS 1998;12:F137-F43.
41. Pantaleo G, Graziosi C, Fauci A. Virologic and immunologic events in primary HIV infection. Springer Semin Immunopathol 1997;18:257-66.
42. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science 1996;273:1856-62.
43. Huang Y, Paxton W, Wolinsky S, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med 1996;2:1240-3.
44. Liu R, Paxton W, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86:367-77.
45. Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 1996;35:3362-7.
46. Zimmerman P, Buckler-White A, Alkhatib G, et al. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Mol Med 1997;3:23-36.
47 Biti R, French R, Young J, Bennetts B, Stewart G, Liang T. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. Nat Med 1997;3:252-3.
48. Eugen-Olsen J, Iversen A, Garred P, et al. Heterozygosity for a deletion in the CKR-5 gene leads to prolonged AIDS-free survival and slower CD4 T-cell decline in a cohort of HIV-seropositive individuals. AIDS 1997;11:305-10.
49. Kostrikis L, Huang Y, Moore J, et al. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. Nat Med 1998;4:350-3.
50. Martin M, Dean M, Smith M, et al. Genetic acceleration of AIDS progression by a promoter variant of CCR5. Science 1998;282:1907-11.
51. Mellors J, Rinaldo C, Gupta P, White R, Todd J, Kingsley L. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167-70.
52. Doranz B, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the b-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996;85:1149-58.
53. McDermott D, Zimmerman P, Guignard F, Kleeberger C, Leitman S, Murphy P. CCR5 promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). Lancet 1998;352:866-70.
54. Mummidi S, Ahuja S, McDaniel B, Ahuja S. The human CC chemokine receptor 5 (CCR5) gene. Multiple transcripts with 5'-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. J Biol Chem 1997;272:30662-71.
55. Moriuchi H, Moriuchi M, Fauci A. Cloning and analysis of the promoter region of CCR5, a coreceptor for HIV-1 entry. J Immunol 1997;159:5441-9.
56. Guignard F, Combadiere C, Tiffany H, Murphy P. Gene organization and promoter function for CC chemokine receptor 5 (CCR5). J Immunol 1998;160:985-92.
57. Smith M, Dean M, Carrington M, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Science 1997;277:959-65.
58. Winkler C, Modi W, Smith M, et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. Science 1998;279:389-93.
59. Quillent C, Oberlin E, Braun J, et al. HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene. Lancet 1998 1998;351:14-8.
60. Keet I, Klein M, Just J, Kaslow R. The role of host genetics in the natural history of HIV-1 infection:the needles in the haystack. AIDS 1996;10 (Suppl A):59-67.
61. Itescu S, Mathur-Wagh U, Skouron M, et al. HLA-B35 is associated with accelerated progression to AIDS. J AIDS 1991;5: 37-45.
62. Steel C, Ludlam C, Beatson D, et al. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. Lancet 1988;1:1185-8.
63. Kaslow R, Duquesnoy R, Van Raden M, et al. A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection. Lancet 1990;335:927-30.
64. Kaslow R, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat Med 1996;2:405-11.
65. Nelson G, Kaslow R, Mann D. Frequency of HLA allele-specific peptide motifs in HIV-1 proteins correlates with the allele's association with relative rates of disease progression after HIV-1 infection. Proc Natl Acad Sci USA 1997;94:9802-7.
66. Walker B, Chakrabarti S, Moss B, et al. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature 1987;328:345-8.
67. Klein M, Van der Burg S, Pontesilli O, Miedema F. Cytotoxic T lymphocytes in HIV-1 infection:a killing paradox? Immunol Today 1998;19:317-24.
68. Koup R, Safrit J, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994;68: 4650-5.
69. Musey L, Hughes J, Schacker T, Shea T, Corey L, McElrath M. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. N Engl J Med 1997;337:1267-74.
70. Ogg G, Jin X, Bonhoeffer S, et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 1998;279:2103-6.
71. Klein M, Van Baalen C, Holwerda A, et al. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long term asymptomatics. J Exp Med 1995; 181:1365-72.
72. Rinaldo C, Huang X-L, Fan Z, et al. High levels of anti-human immunodeficiency virus type 1 memory cytotoxic T lymphocyte activity and low viral load are associated with lack of disease in HIV-1 infected long term non-progressors. J Virol 1995; 69:5838-42.
73. Lederman M. Role of cytotoxic T lymphocytes in the control of HIV-1 infection and HIV-1 disease progression. Curr. Oppin. Infect Dis 1996;9:14-8.
74. Pontesilli O, Klein M, Kerkhof-Garde S, et al. Longitudinal analysis of Human Immunodeficiency Virus type 1-specific cytotoxic T lymphocyte responses: a predominant Gag-specific response is associated with nonprogressive infection. J Infect Dis 1998; 178:1008-18.
75. Gea-Banacloche J, Migueles S, Martino L, et al. Maintenance of large numbers of virus-specific CD8+ T cells in HIV-infected progressors and long-term nonprogressors. J Immunol 2000;165: 1082-92.
76. Yang O, Kalams S, Rosenzwieg M, et al. Efficient lysis of HIV-1 infected cells by cytotoxic T lymphocytes. J Virol 1996;70: 5799-806.

77. Brander C, Hartman K, Trocha A, et al. Lack of strong immune selection pressure by the immunodominant, HLA-A*0201-restricted cytotoxic T lymphocyte response in chronic human immunodeficiency virus-1 infection. J Clin Invest 1998;101: 2559-66.
78. Goulder P, Tang Y, Brander C, et al. Functionally inert HIV-specific cytotoxic T lymphocytes do not play a major role in chronically infected adults and children. J Exp Med 2000;192:1819-32.
79. Lechner F, Wong D, Dunbar P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000;191:1499-512.
80. Rosenberg E, Billingsley J, Caliendo A, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997;278:1447-50.
81. Mosmann T, Cherwinski H, Bond M, Giedlin M, Coffman R. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348-57.
82. Clerici M, Shearer G. Cellular immunity and a type 1 cytokine profile in protection against HIV infection and progression to AIDS. Res Immunol 1994;145:635-41.
83. Bentwich Z, Kalinkovich A, Weisman Z. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol Today 1995;16:187-91.
84. Graziosi C, Pantaleo G, Gantt K, et al. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. Science 1994;265:248-52.
85. Fakoya A, Matear P, Filley E, et al. HIV infection alters the production of both type 1 and 2 cytokines but does not induce a polarized type 1 or 2 state. AIDS 1997;11:1445-52.
86. Rosenberg E, Walker B. HIV type 1-specific helper T cells: a critical host defense. AIDS Res Hum Retroviruses 1998;14(Suppl 2): 143-7.
87. Bennett S, Carbone F, Karamalis F, Miller J, Heath W. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. J Exp Med 1997; 186:65-70.
88. Dyer W, Ogg G, Demoitie M, et al. Strong Human Immunodeficiency Virus (HIV)-specific cytotoxic T-lymphocyte activity in the Sydney Blood Bank Cohort patients infected with nef-defective HIV type 1. J Virol 1999;73:436-43.
89. Walker C, Moody D, Stites D, Levy J. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 1986;234:1563-6.
90. Brinchmann J, Gaudernack G, Vartdal F. In vitro replication of HIV-1 in naturally infected CD4+ T cells is inhibited by rIFN α 2 and by a soluble factor secreted by activated CD8+ T cells, but not by rIFN β, rIFN γ, or recombinant tumor necrosis factor-α. J Acquir Immune Defic Syndr 1991;4:480-8.
91. Walker C, Erickson A, Hsueh F, Levy J. Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. J Virol 1991;65:5921-7.
92. Mackewicz C, Levy J, Cruikshank W, Kornfeld H, Center D. Role of IL-16 in HIV replication. Nature 1996;383:488-9.
93. Mackewicz C, Ortega H, Levy J. Effect of cytokines on HIV replication in CD4+ lymphocytes: lack of identity with the CD8+ cell antiviral factor. Cell Immunol 1994;153:329-43.
94. Mackewicz C, Levy J. CD8+ cell anti-HIV activity:nonlytic suppression of virus replication. AIDS Res Hum Retrovir 1992; 8:1039-50.
95. Mackewicz C, Yang L, Lifson J, Levy J. Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection. Lancet 1994;2:344.
96. Mackewicz C, Blackbourn D, Levy J. CD8+ T cells suppress human immunodeficiency virus replication by inhibiting viral transcription. Proc Natl Acad Sci USA 1995;92:2308-16.
97. Chun T, Justement J, Moir S, et al. Suppression of HIV replication in the resting CD4+ T cell reservoir by autologous CD8+ T cells:Implications for the development of therapeutic strategies. Proc Natl Acad Sci USA 2001;98:253-8.
98. Alkhatib G, Combadiere C, Broder C, et al. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996;272:1955-8.
99. Choe H, Farzan M, Sun Y, et al. The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996;85:1135-48.
100. Bleul C, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 1996;382:829-33.
101. Van Rij R, Broersen S, Goudsmit J, Coutinho R, Schuitemaker H. The role of a stromal cell-derived factor-1 chemokine gene variant in the clinical course of HIV-1 infection. AIDS 1998;12:F85-F90.
102. Blazevic V, Heino M, Ranki A, Jussila T, Krohn K. RANTES, MIP and interleukin-16 in HIV infection. AIDS 1996;10: 1435-6.
103. Clerici M, Balotta C, Trabattoni D, et al. Chemokine production in HIV-seropositive long-term asymptomatic individuals. AIDS 1996;10:1432-3.
104. Mackewicz C, Barker E, Greco G, Reyes-Teran G, Levy J. Do beta-chemokines have clinical relevance in HIV infection? J Clin Invest 1997;100:921-30.
105. Zhang J, Gupta A, Dave R, Yimen M, Zerhouni B, Saha K. Isolation of primary HIV-1 that target CD8+ T Lymphocytes using CD8 as a receptor. Nat Med 2001;7:65-72.
106. Greenough T, Sullivan J, Desrosiers R. Declining CD4 T-cell counts in a person infected with nef-deleted HIV-1. N Engl J Med 1999;340:236-7.
107. Goulder P, Phillips R, Colbert R, et al. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nature Med 1997;3:212-7.
108. Koenig S, Conley A, Brewah Y, et al. Transfer of HIV-1 specific cytotoxic T lymphocytes to an AIDS patient leads to selection of mutant HIV variants and subsequent disease progression. Nature Med 1995;1:330-6.
109. Koot M, Van Leeuwen R, De Goede R, et al. Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis. J Infect Dis 1999;179:254-8.