The pathogenesis of HIV-1 infection

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Epidemiologists have long established beyond all reasonable doubt that infection by the human immunodeficiency virus type 1 (HIV-1) leads to the acquired immune deficiency syndrome (AIDS). Natural history cohorts have demonstrated that the median time from infection to development of AIDS is approximately 12 years, and that this long duration is broadly similar in all populations infected by HIV-1, in all risk groups, in all ethnic groups and in all geographical areas. These epidemiological observations suggest that HIV-1 causes AIDS largely independently of human major histocompatibility complex (MHC) and HIV-1 sequence polymorphisms, as great diversity of both these factors exist world-wide. This is not to say that HLA and HIV diversity do not affect the natural history of HIV disease, but these observations support a common mechanism of HIV-1 pathogenesis which is largely independent of human and viral diversity.

The genome of HIV-1 is small, less than 10 kb, and hundreds of full-length HIV-1 sequences have been studied. All nine HIV-1 genes and their products are characterised, mostly in great detail. The molecular basis of viral entry and tropism is known, and the humoral and cellular immune responses to infection characterised at the level of the individual epitopes. Given that there is greater understanding of the biology of HIV-1 than for any other pathogen, it is frustrating that the pathogenesis of HIV disease is still so difficult to fully define at a molecular level.

The essence of HIV-1 infection is a slow decline in CD4+ T-cells over time, such that once a threshold of approximately 200 x 10^9 CD4 cells/l is passed, immune deficiency and virally-induced tumours are increasingly liable to occur. It has been known for 17 years that the primary receptor for HIV-1 is the CD4 molecule, expressed on the surface of mature T-helper lymphocytes in peripheral blood and lymph node, and also on macrophages and dendritic cells. More recently, the HIV-1 co-receptors have been defined as the 7-transmembrane spanning chemokine receptors, principally CCR5 and CXCR4. The distribution of these co-receptors on primary, activated CD4+ T-lymphocytes defines the tropism of HIV in vitro, and almost certainly in vivo. Only
CD4+/CCR5+ T-lymphocytes are infectable by primary HIV-1 isolates taken directly from patients.

The central question of HIV-1 pathogenesis is through what mechanism does HIV-1 destroy CD4+ T-cells? Is the virus directly lytic for these cells through infection and viral replication, or is the mechanism indirect? For example, are HIV+/CD4+ T-cells killed through the action of HIV-specific cytotoxic T-lymphocytes (CTLs), or through the action of toxic soluble viral products such as gp120, or even through induction of apoptosis leading to the death of virally infected cells?

The loss of CD4+ cells begins during primary HIV-1 infection, and continues, not necessarily at a constant rate, throughout the course of infection. In late HIV disease, after the decline of CD4+ T-cells to below 200 x 10⁹/l, there is some evidence that CD4+ cells decline more rapidly. In this review, I shall focus in turn firstly on primary HIV-1 infection, secondly the ‘chronic’ phase of asymptomatic HIV infection and finally on late HIV disease, and review the data on the mechanisms affecting the loss of CD4+ cells at these periods. The natural history of HIV-1 infection is shown diagrammatically in Figure 1.

**Primary HIV infection**

The early events of HIV infection are likely to be important to the later course of the disease, and are, therefore, the most appropriate starting point to consider pathogenesis. The very earliest events in the entry of HIV-1 *in vivo* are impossible to study in humans. Our knowledge of the mechanism of infection is thus mostly inferred from experimental infections of rhesus macaques with SIVmac251. In this model, animals can be sacrificed at intervals after mucosal exposure in order to...
determine the localisation of the virus over time. As SIV shares a very similar genetic structure to HIV and also uses CD4 as the primary receptor, it is probably a legitimate model for the study of viral entry \textit{in vivo}. However, there is little evidence that mucosal transmission is the dominant route of transmission for primate lentiviruses in natural infection. As with any animal model, therefore, caveats as to exact applicability to human disease must be borne in mind.

After mucosal exposure to SIV\textsubscript{mac}251, for example across the vagina of the rhesus macaque, HIV-1 appears to first infect mucosal Langerhans’ cells (mLc)$^{6,7}$. These cells are migratory within mucosa, and possess long processes which interdigitate among the mucosal epithelial cells, and thus sub-mucosal Lc may be present on the surface of epithelia. Langerhans’ cells are a modified macrophage, are fundamental to antigen presentation and attract CD4$^+$ T-cells to their dendritic processes. Mucosal Lc weakly express CD4 and CCR5, and hence are infectable by HIV-1$^8$; however, these Langerhans’ cells also express a cell surface lectin, DC-SIGN, which is capable of binding the HIV-1 gp120 with a high affinity$^9$. The most plausible mechanism for infection across a mucosal surface is that free infectious virions are bound to DC-SIGN on the surface of mLc, which interdigitate and migrate within the vaginal mucosa. Virus bound to mLc is then moved away from the mucosal surface, and is brought in close proximity to CD4$^+$ T-cells. The DC-SIGN bound virus is then able to infect CD4$^+$/CCR5$^+$ T-cells, and these infected T-cells then migrate to their regional lymph nodes (reviewed by Mascola$^{10}$).

The HIV-infected T-cells remain sequestered in regional lymph nodes until a threshold of replication is reached within 2–6 weeks, following which a burst of plasma viraemia occurs. This is termed primary HIV infection (PHI). Following PHI, virus is disseminated within days throughout the body and seeds local, peripheral and distal reservoir sites. PHI is associated with a very high plasma viral burden, and levels of viral RNA detected by quantitative RT-PCR may exceed $5 \times 10^6$ copies RNA/ml. PHI may be accompanied by either symptoms of a ‘seroconversion illness’, comprising rash, painful lymphadenopathy, arthropathy and fever, or more often this period is clinically asymptomatic. The viraemic peak resolves spontaneously after 2–4 weeks, associated with a primary immune response to HIV. Although plasma viraemia is suppressed after seroconversion, HIV-1 is never eliminated and the HIV genome can be found in T-cells in all subjects at all stages of disease, and in varying quantities as virion-associated RNA in the plasma.

The viraemic peak of PHI is invariably associated with a transient reduction in CD4$^+$ T-cells in peripheral blood. This is generally modest and short-lived, but may occasionally lead to a reduction of CD4$^+$ T-cells below $200 \times 10^9$/l, which in turn may lead to overt clinical immunosuppression. Indeed, cases of opportunistic infections such as \textit{Pneumocystis carinii}
pneumonia and oesophageal candidiasis have been reported from this period of acute, reversible immunosuppression. Once the viraemic peak has been resolved, CD4+ cell levels return towards baseline levels, but remain lower than that seen pre-infection.

This observation of an acute loss of CD4+ T-cells associated temporally with the rapid appearance of plasma viraemia, and the recovery of CD4 count once the plasma viraemia is reduced, would appear to support the capacity of HIV-1 to directly kill CD4+ T-cells through lysis. Certainly, high levels of primary virus replication in activated peripheral blood mononuclear cells (PBMCs) in vitro can lead to syncytial formation (multinucleated giant cells) and consequent cell death, and HIV-1 is ultimately lytic in primary cell culture, even if syncytia are not observed. However, viraemic levels are higher in PHI than at any other time in the course of HIV-1 infection, and there is no immune response against the virus at PHI. It is possible, therefore, that the transient reduction in CD4+ T-cells at PHI represents the unopposed effect of high level HIV replication, which is curtailed by the immune response. Thus, the direct viral cause of CD4 T-cell destruction at PHI may not represent a common mechanism throughout the rest of the course of HIV-1 infection, when an immune response is always present.

The initial viraemic peak falls to a set ‘steady state’ within several weeks or months of infection, the level of which varies considerably between individuals and is predictive of prognosis11. Coincidently with the peak of viraemia there is a vigorous HIV-specific immune response involving cell mediated immunity, CD8+ CTL and CD4+ T-helper HIV-specific responses, in addition to antibody production, all of which are believed to play an important role in controlling the initial plasma viraemia. The study of neutralising antibodies to the autologous primary isolates at PHI suggest that these are relatively slow to develop, and are rarely detectable until 6 months after infection12. By contrast, HIV-specific, CD8+, HLA-restricted CTLs do appear to be related temporally to the reduction in viraemia13. Furthermore, a number of groups have documented the emergence of escape mutations within CTL epitopes following the immune response in PHI14,15. These observations have been taken as evidence that CTLs are the major effector of the immune containment of HIV-1 following PHI. Certainly, it would be attractive to consider that the slow progression of HIV-1 disease is related to the balance between viral replication and the cellular immune response mediated through CD8+ CTL. However, other hypotheses exist for the control of viraemia at PHI. Following the appearance of the plasma viraemia, non-neutralising antibodies appear at the same time as CTL, principally to the core (p24), matrix (p17) and envelope (gp120) proteins16. These non-neutralising antibodies may bind to virions to generate circulating immune complexes, which are subsequently cleared.
through Fc-receptor binding in the spleen, a mechanism of viral clearance thought to be relevant to enterovirus viraemia. Furthermore, availability of activated CD4+ T-cells may become limiting during the viraemia of PHI; exhaustion of the CD4+ substrate for HIV replication may lead to reduction of viraemia without an immune mechanism being involved. This hypothesis has some experimental support from the observation that treatment with low dose cyclosporin-A at PHI reduces T-cell activation and leads to reduction in viraemia and preservation of CD4 cells.

When the immune system first responds to HIV-1, the outcome is thought to determine much of the subsequent natural history of the disease. The titre of the antibody response to p24 at seroconversion is associated with disease outcome. Acute primary infection activates the HIV-1 specific CD4+ T-helper response. However, this HIV-specific CD4+ T-helper response is generally lost during the course of untreated HIV-1 infection. In untreated patients with high levels of plasma viraemia after PHI, the HIV-specific T-helper response is undetectable within the first year of infection. When anti-retroviral drugs are given 6 months or later after seroconversion, HIV-specific T-helper responses do not return if already lost, and cytotoxic T-lymphocytes directed against HIV continue to decline. However, early anti-retroviral therapy, during PHI, is associated with preservation of both CD8+ CTL and CD4+ T-helper lymphocyte responses to HIV. Most studies have failed to detect HIV specific CD4+ T-helper responses in untreated patients except for those with very slow progression and low viral loads.

**Summary**

Primary HIV-1 infection is manifest by a viraemic peak associated with a temporally related decline in CD4+ T-cells; the viraemia is probably curtailed by an HIV-specific CD8+ CTL response. There is some evidence that the reduction in CD4+ T-cells in PHI may be a direct and unopposed effect of HIV-1 replication which is curtailed and then controlled by cellular immunity.

**Chronic asymptomatic HIV-1 infection**

As shown diagrammatically in Figure 2, following PHI the CD4 count returns towards baseline but does not regain pre-infection levels. CD4 counts decline slowly, and in a linear manner, during the chronic asymptomatic stage of HIV-1 infection. During this period, HIV-1 RNA levels as determined by RT-PCR are highly variable between individuals, and may
HIV-1 proviral DNA is always detectable in PBMCs, even if plasma viraemia is undetectable. The small number of infected cells in peripheral blood, generally 1:50,000 PBMCs, has suggested that direct viral lysis is an unlikely mechanism for the decline of CD4 cells in this period of infection.
The breakthrough in the investigation of the pathogenesis of CD4+ T-cell loss in this period of HIV infection came from careful multi-disciplinary observations of the effect of potent, combination anti-retroviral chemotherapy. Use of anti-retroviral drugs leads to suppression of viral replication, a reduction of plasma viraemia and an increase in CD4 count. Two landmark papers from Ho and Shaw revealed that starting anti-retroviral therapy altered the steady state of HIV-1 replication, where viral replication and clearance were in balance21,22. The drug therapy strongly suppressed viral replication and lead to an exponential decline in plasma viraemia over 1–2 weeks, followed by a slower second phase decline after 2–4 weeks. Mathematical modelling of the plasma viraemia decay slope enabled the production rate of HIV-1 virions to be determined, as $10^7$–$10^8$ virions/day. The rapid replication of virions is from within the peripheral CD4+ T-cell compartment, and leads to a greatly reduced T-cell life expectancy of approximately 24–36 h, against an expected life-time in the absence of HIV infection of 100 days. The slower second phase decline represents HIV-1 replication in long-lived cells such as macrophages and dendritic cells. The model also accounted for the loss of CD4+ T-cells by assessing the rate of CD4 turnover as 70-fold over baseline, caused by the viral replication within this compartment leading to premature cell death.

These models of HIV dynamics and pathogenesis have been termed the ‘bath-tub’ analogy. The loss of CD4+ T-cells is the result of greatly increased turnover through HIV-1 driven CD4 death; new CD4+ T-cell production fails to match the increased CD4 cell loss, and hence a slow, continuous reduction in CD4 cells is seen. As with a bath-tub, the level of water can be maintained if the taps are on full, even if the plug is out. However, over time the taps will fail to keep up with the rate of water going down the plug, and a gradual loss of water level will be observed, until the bath is nearly empty.

There have been a number of objections to this simple model of HIV pathogenesis. Firstly, Miedema’s group studied the telomere length in CD4 and CD8 lymphocytes from HIV-infected subjects23. Telomeres are repetitive DNA sequences at the end of all chromosomes which are cut by about 50 bp with each cell division. Although there is an enzyme which can re-extend telomeres (telomerase), the enzyme is only expressed in germ cells and tumours. Hence, telomere length should be an estimate of the number of times a cell has divided. Miedema showed that there was telomere shortening in HIV infection, but that it occurred in CD8+ cells, and not in CD4+ cells. He concluded that there was evidence for increased CD8+ cell turnover in HIV infection, but no evidence for increased CD4+ turnover.

Subsequently, a number of techniques to label lymphocytes in vivo have been developed, using bromodeoxyuridine (BrdU), [6-2H]-glucose or [13C]-
glucose. A summary of all these studies supports increased turnover (2–6-fold) of both CD4+ and CD8+ T-cells in HIV infection, with a reduction in half-life of about 60% compared to HIV-negative subjects (reviewed by Johnson24). The mechanism for the effect of anti-retroviral therapy on raising CD4+ lymphocyte counts could, therefore, be either through reducing the rate of cell death (as in the bath-tub model), or through increasing CD4+ cell production. Unfortunately, there are conflicting data supporting both these hypotheses. Interestingly, studies from sooty mangabeys, an old-world monkey species naturally infected with SIVsm, a virus which is non-pathogenic in this host, show that T-cell turnover is normal in this model despite high levels of plasma viraemia25. This observation, if reproduced, would suggest that indirect mechanisms for T-cell loss are more likely to account for the observations in HIV-infected subjects.

The unexpected, but reproducible, observations that CD8+ lymphocytes have a higher turnover and shorter half-life in HIV-infected subjects re-focuses attention on the role of CTL in HIV infection. Potent drug treatment in chronic asymptomatic infection produces impressive restoration of immunity as judged by rise in CD4 count and loss of disease progression. However, distortions in the CD4+ repertoire are not corrected and the HIV-specific CD8+ CTL populations decline. Although this decline has been attributed to a loss of antigenic drive, because potent therapy is so effective at suppressing viral replication, very early use of anti-retroviral drugs preserves CD8 populations at a low but easily detectable level (reviewed by Siliciano26). Since it is becoming clearer that there is on-going viral turnover even in patients on continuous efficient treatment, the decline in CD8+ numbers may be attributable, at least in part, to loss of HIV-1 specific T helper function19. There is good evidence that the preservation of CD8+ CTL function and numbers in animal models is intimately dependent on T helper function.

A synthesis of these data supports HIV replication leading to loss of CD4+ T-lymphocytes both by direct (or indirect) cell killing, and through the action of HIV-specific CTL killing of CD4+/HIV+ T-cells. This would also account for the observed increase in CD8+ T-cell turnover in HIV infection, as the action of CTL killing also increases the killing of the effector cells. The relative roles of alterations in T-cell production induced by HIV-infection and anti-retroviral therapy require further experimental study.

**Summary**

Chronic asymptomatic HIV infection is associated with highly dynamic, persistent viral replication, with the production of approximately $10^8$
Virions/day. Viral replication leads to loss of CD4+ T-cells, which could be due either to increased cell death, or to reduced production, or both. The increased turnover of both CD4+ and CD8+ T-cells in HIV-1 infected subjects compared to controls supports the killing of virally infected cells by HIV-specific CTL as a leading hypothesis for CD4+ T-cell decline in HIV infection. However, the direct relationship between plasma viral load and rate of CD4 decline suggests that viral replication also contributes, directly or indirectly, to CD4 loss.

**Late stage HIV-1 infection**

The decline in CD4 count during the course of HIV-1 infection is not constant over time. There is a very rapid, transient decline in CD4+ T-cells at primary HIV infection, as noted above. The decline in CD4 count in the chronic asymptomatic phase of HIV-1 infection is variable, and related principally to the ‘steady state’ level of plasma viraemia. However, the decline in this phase appears to be approximately linear, and hence constant over time. However, in late stage HIV disease, when the CD4 count is < 200 x 10^9/l, there is evidence of an increase in the rate of CD4 decline.

It was observed early in the HIV epidemic that the phenotype of HIV-1 isolates grown from patients at different stages of HIV infection were different. Asjo and Levy showed independently that viral isolates taken early in the course of infection were slow growing, producing low titres of reverse transcriptase in culture (slow, low). These isolates could grow in fresh primary peripheral blood mononuclear cells (PBMCs), but were not able to infect transformed, immortalised T-cell lines such as H9, CEM or MT2. By contrast, isolates made from patients with advanced HIV disease were able to grow rapidly to high titre in PBMCs and a wide range of T-cell lines (fast, high). Subsequently, Tersmette showed that the viral phenotype could be defined by the ability to produce syncytia (multinucleated giant cells) in the MT-2 cell line; this allowed viral isolates to be characterised as non-syncytial (NSI slow/low) or syncytial (SI fast/high). Fouchier then showed that the viral phenotype NSI/SI could be defined genetically through the charge of the V3 loop in the gp120 envelope. Since the discovery of the chemokine receptors as HIV co-receptors, it has been possible to understand these phenomena at a molecular level.

NSI viruses are associated with primary HIV infection and early chronic disease. These viruses use CCR5 as their co-receptor. In late disease, viral isolates use CXCR4 as their co-receptor, or have dual tropism for both CCR5 and CXCR4. CCR5 is expressed principally on activated T-lymphocytes and macrophages, and is not highly...
expressed on resting T-cells. This is part explains the association between T-cell activation and susceptibility to HIV-1 infection *in vitro*. By contrast, CXCR4 is more widely expressed on resting and activated immune cells. With the switch in viral phenotype from NSI to SI, and from CCR5 to CXCR4 usage, the capacity exists for HIV-1 infection of a broader range of target cells. Furthermore, SI/CXCR4 viruses are more cytopathic *in vitro*, and replicate to higher levels than NSI/CCR5 viruses, which may lead in turn to more efficient T-cell killing.

The difference between NSI/CCR5 usage and SI/CXCR4 usage can be shown to reside in 2 amino acid substitutions in the V3 loop of gp120. The mutation rate of HIV-1 is high, at 1:10,000 bases substituted per replication, and as the HIV genome is approximately 10,000 bases, and 10^8 virions are produced/day, the capacity to generate the two SI/CXCR4 mutations in V3 must occur on a daily basis. Yet, throughout the course of early and chronic HIV infection, it is only possible to isolate NSI viruses. Genetic studies of HIV+ subjects who have died from unrelated causes during this period show no evidence of SI/CXCR4 mutations. If these SI/CXCR4 mutations are being generated, then they are strongly selected against in favour of NSI/CCR5 using envelopes.

One hypothesis for the continued selection of NSI/CCR5 viruses is that the SI/CXCR4 mutations are under strong cellular immune control. Thus, whenever these mutations appear, the dominant immune response against the mutant form leads to maintenance of the NSI/CCR5 form. In late HIV disease, the loss of cellular immune regulation which leads to AIDS also suppresses the regulation of the SI/CXCR4 variants. Thus, late stage HIV-1 infection may see an increased rate of CD4 loss through broadening of the viral tropism, mediated by a switch in co-receptor usage from CCR5 to CXCR4. Presumably, CD4+ T-cell loss is this period of infection is entirely mediated through the direct (or indirect) effects of viral replication, as there is no remaining cellular immune response.

The critical role of the CCR5 co-receptor in defining the entry of HIV-1 into activated T-cells can be demonstrated through the impact of CCR5 polymorphisms. A deletion mutation of 32 base pairs of CCR5 has been described, Δ-32, where the co-receptor is synthesised but is unable to be expressed on the cell surface. Subjects who are homozygous for the Δ-32 mutation appear to be immunologically normal, yet are resistant to HIV-1 infection, at least by NSI/CCR5 using primary isolates. Approximately 1% of Caucasian populations are homozygous for this mutation, and 17% heterozygous. Heterozygosity for Δ-32 does not prevent HIV-1 infection, but is associated with a slower rate of CD4 decline, and hence a better prognosis in HIV-1 infection. Other polymorphisms, such as in the SDF-1 promotor region, also impact on the rate of CD4 decline. Presumably, these polymorphisms affect the relative infectability of activated CD4+ T-cells,
and further support the central role of viral replication in the destruction of CD4+ lymphocytes.

**Summary**

The natural history of HIV-1 infection is marked by a prolonged asymptomatic period with a continuous slow decline in CD4+ T-lymphocytes. While this period is clinically quiet, the virus is highly dynamic, with large numbers of virions produced every day. The rate of CD4 decline is directly related to the quantity of virus detected in plasma, suggesting a direct relationship between viral replication and CD4+ T-cell destruction. A direct viral T-cell killing mechanism may indeed be dominant in the absence of an effective cellular immune response to HIV-1, as seen prior to seroconversion (primary HIV infection) and at late stage disease when HIV-specific cellular immunity is exhausted. However, during the chronic asymptomatic phase of HIV-1 infection, which is characterised by an active HIV-specific humoral and cellular immune response, turnover of both CD4+ and CD8+ T-lymphocytes is elevated. The most plausible explanation for this is that HIV-specific CD8+ CTL are the main effectors of HIV+/CD4+ T-cell destruction.

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