AIDS IN THE TWENTY-FIRST CENTURY:
A Challenge for Biosciences

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Introduction

Among viruses the human retroviruses may be of special interest to immunologists, because they target cells of the immune system, particularly mature CD4+ T cells, impair their function and cause them to grow abnormally (HTLV) or to die (HIV). Human retroviruses cause disease ranging from neurological disorders and leukemias (HTLV-1) to AIDS (HIV) and promote development of several types of malignancies (HIV). They share many common features, but their contrasts are greater, especially the far greater replication and variation of HIV associated with its greater genomic complexity. Both have evolved striking redundancy for mechanisms which promote their survival. Thus, HTLV has redundant mechanisms for promoting growth of provirus containing T cells needed for virus continuity, because it is chiefly through its cellular DNA provirus that HTLV replicates and not through production of virions. Conversely, HIV has redundancy in its mechanisms for promoting virion replication and escape from the host immune system. It is via these redundant mechanisms that they produce disease: leukemias from mechanisms promoting T-cell proliferation (HTLV-1) and AIDS from mechanisms promoting virus replication and T-cell death (HIV). The practical challenges for the future are clear. For HTLV-1, education and control of breastfeeding. For HIV, the formidable tasks now ahead in part demand new kinds of talent, talents that will foster greater insights into the development of therapy for the developing countries, new forms of less toxic therapies for all infected persons, a continued and expanded commitment to education, and a persistent "never say die" commitment to the development of a truly preventive vaccine with all the scientific and non-scientific challenges that these objectives face. In this report I will summarize my perspective on HIV and especially its mechanisms of inducing AIDS.

Clinical investigators first identified a new disease in the U.S. in 1981 that they defined as a syndrome with reduction in CD4+ T cells (reviewed in 1). By 1982 after additional case clustering, CDC epidemiologists suggested it was infectious in nature. Many theories for its cause were proposed, surprisingly including some non-infectious ones (2). In 1982, Max Essex and I first suggested AIDS was likely due to a new retrovirus, one presumably in the HTLV family. Sometimes this idea was misconstrued as a suggestion that AIDS was due to HTLV-1 itself. These ideas came from the recognition by Essex that variants of the leukemia causing retrovirus of cats (FeLV) could cause immune disorders and from our experiences with HTLV-1 and 2, namely their T-cell tropism, modes of transmission, capability of producing mild immune impairment *in vitro* (3, 4) and *in viv*o (2, 5-8). I speculated in more detail in early 1983 that the causative agent would likely be a new recombinant retrovirus with the 5' genomic region acquired from an HTLV and the 3' region unique (2). This speculation was made when we found several samples from AIDS patients which were retrovirus positive, as indicated by extracellular positive RT assays that were also positive by immune-fluorescent assays for

antigens related to HTLV-1 core structural proteins, known to be encoded by the gag gene of the 5' region of the HTLV genome (2) and occasionally HTLV sequences (9). Only in mid-1983 would we realize that these patients were doubly infected with HTLV-1 (or HTLV-2) as well as with the new retrovirus, which was later defined as HIV. The idea of a retrovirus as a causative agent in AIDS prompted our work and also that of Luc Montagnier and Jean-Claude Chermann and their colleagues in France (8, 10). The first *bona fide* isolate of the new retrovirus was described in the seminal report in May 1983 by the French group. However, they did not link the virus to AIDS and cautiously did not claim to do so (10). They first referred to the virus as a new human T-lymphotropic retrovirus (10). Later in the same year at scientific meetings they described a few additional isolates and coined the term LAV for the first one, because it came from a patient with lymphadenopathy (but not frank AIDS) and their more recent isolates as IDAV-1 and 2 (immune deficiency associated viruses) because they came from patients with AIDS. These were published in late 1984 under the generic designation of human T lymphotropic retroviruses (11). The variety of names was due to their caution; one new retrovirus might cause only lymphadenopathy and the other AIDS. Later it would be clear that lymphadenopathy was simply an early state of infection leading to AIDS. By mid-1983 we began to obtain a number of isolates that did not exhibit immunological cross-reactions with HTLVs, and by late 1983, 2 of these were growing in continuous cell line cultures using CD4+ T cell lines as targets (2). This would be one of the prerequisite breakthroughs for the development of the blood test. By the spring of 1984, several additional virus isolates were successfully adapted to cell line culture, and we made the first publications since the 1983 report by the French group. We described isolates from 48 different patients of the new virus, which we termed HTVL-III (12), methods for the continuous production of several in CD4+ T cell lines (13), characterization of their major structural proteins (14, 15), development of the blood test (15, 16), and collectively, evidence that the new retrovirus, later to be called HIV, was the single cause of the new epidemic, AIDS. Until these reports were published in May 1984 the cause of AIDS remained hotly debated. Indeed, in February 1984, almost one year after the original paper by the French group, some NIH investigators led by the late K. Sell announced that a new fungus was the cause of AIDS, and others that a mycoplasma was the cause. Later in 1984, J. Levy and co-workers added independent isolates of HIV and also achieved continuous cell line culture of one (17).

The continuous cell line cultures also allowed for drug testing, and the first partially successful anti-HIV drug, AZT, was tested in these systems (18). The pace of scientific research was remarkable in 1984-85, particularly related to the molecular biology: genes defined (19-25), extra regulatory genes discovered (19-30), genomes of several isolates sequenced (19-22), virus heterogeneity discovered (31-33), CD4 receptor discovered (34), presence of virus in central nervous system (35) and semen (36, 37) described, evidence provided that macrophages and not only CD4+ T cells were targets (38), and related simian retroviruses discovered (39, 40). These are some of the notable advances in those 2 years.

Progress in how HIV causes AIDS (Pathogenesis)

The following were established in the very early years of HIV/AIDS researchconcerning HIV infection: (1) it is often associated with an acute viral syndrome with viremia, (2) that CD4+ T cells and macrophages are the main target cells, (3) that astrong primary immune response occurs, (4) that nonetheless infection takes hold, (5) that infection is followed by a slow but generally progressive decline in CD4+ T cell numbers associated with a hyperactivated immune state, and (6) ultimately the development of opportunistic infections and often neoplasias occurs. It was also almost immediately discovered that in most cases HIV variation was striking (41-44), even within a population of virions from one individual. Later we learned from the work of James Mullins and Jaap Goudsmit and their co-workers what at first glance appeared paradoxical, namely that less variation was associated with more rapid progression (45), apparently signaling the failure of the immune system to eliminate the dominant quasi-species of the initial infection.

There has been considerable discussion about the failure of the immune response to stop HIV after the initial infection. However, this should not be surprising because the same is true with almost all retroviruses. To date, no matter how early, long or "hard" anti-HIV chemotherapy is employed, HIV eradication does not occur, despite predictions for such success made to that effect in 1996. HIV proviruses as well as unintegrated linear and circular forms of viral DNA can be found in some cells, and virus production almost returns when therapy is discontinued. Generally, persistence of HIV, even after vigorous anti-HIV therapy, is attributed

to "reservoirs" containing "latently" infected cells (46-49). Such latently infected cells were found *in vitro* in the earliest years of HIV research, but were first proven in patients by Siliciano and his co-workers (47), and then shown to be established in the first days of HIV-1 infection by Fauci and co-workers (48). The number of such cells in asymptomatic patients was estimated at about 0.05% of the total resting CD4+ T cell population (approximately 10^6 cells) (46).

It is sometimes inferred that a reservoir implies a distinct tissue site, but this is unlikely or at least an unnecessary hypothesis because the response of a given cell to infection is variable. Some cells die quickly upon infection. Others only intermittently release virus or fail completely to express virus and escape the immune response. It seems likely that the "reservoir" is simply a fraction of the infected cells in any compartment. Supporting this line of reasoning are recent results of Eric Verdin and colleagues. Their experiments provide us a molecular basis for the variable effects of HIV on an infected cell, particularly the amount of virus it produces - if any. They find that the consequences of the multiplicity of different integration sites of the HIV-1 provirus are highly different levels of virus expression. Proviruses integrated within heterochomatin express little or no virus (50). Greene and Peterlin also highlight this molecular mechanism for latency, but they note that a given infected cell will usually have more than one site of integration, so it is unlikely that all would be in heterochromatic segments. They propose variation in the environment, which affect the levels of various activators and transcription factors, as an additional determinant of virus expression (51). However, a specific tissue locus of latently infected cells has not been discovered, and it is more likely that cells exhibit a variety of phenotypes within the same tissue. This then would favor the notion that cellular differences alone are sufficient to account for the variation. Thus, it seems to me that the site of provirus integration could be the single determinant. Local levels of cytokines do, of course, effect expression of HIV genes, but most cells in a given tissue locus will face the same cytokine environment. Since latently infected cells are relatively rare, I think it is likely that some cells would only contain provirus in silenced regions of the genome. We have already considered another possibility, namely that some cells are inherently predisposed to repress expression even with proviruses integrated in non-silenced parts of the cellular genome. This might be the case with NK cells. About 10 years ago reports from our group and by Chehimi et al. suggested that NK cells might also be HIV-1 targets of infection *in vitro* (52, 53). Recent studies by G. Pavlakis and his colleagues verify these results but with more rigorous evidence, and more

importantly, they show that many NK cells are infected *in vivo*. Moreover, they demonstrate that HIV-1 infected NK cells can be found in patients even after extensive anti-HIV chemotherapy (54). These cells are CD56+, CD3- and express CD4 as well as co-receptors CCR5 and CXCR4. Thus, these findings may have significance in terms of a cell-type specific reservoir of HIV infected cells. They may also be relevant to the cellular mechanisms underlying pathogenesis, because NK cells may be important contributors to innate immunity against HIV.

As already noted, HIV is not unique with respect to its persistence in the face of an immune response. Indeed, retrovirus infections in man (HTLV-1, HTLV-2) and most of those in animals persist despite "healthy" immune responses to them. Some do not even develop the variation characteristic of HIV. Indeed, the exceptions are some of the other animal *lenti* retroviruses, such as the equine infectious anemia virus (EIAV), which apparently can be eradicated by its host's immune response. Oddly, it is HIV's status as a lenti-retrovirus with 2 striking and common features: greater variation and the capacity to infect macrophages, as distinct from the oncorna retroviruses, (e.g., avian, feline, murine, and gibbon ape leukemia viruses), and HTLV and bovine leukemia virus, that is often discussed as the key traits that allow HIV to escape from the host immune response. However, as noted above, almost all retroviruses persist, and some, like the HTLVs, show very little variation. EIAV, which can be eradicated, has more variation and infects macrophages. Thus, it seems that persistence has little to do with either of these traits. EIAV is the exception not HIV.

Clinical observations in the early years of HIV research showed that after the initial viremic burst and the sharp decline in viremia, presumably from an adaptive immune response and likely also from innate immunity such as NK cell attack and release of HIV suppressive β-chemokines (see below), the outcome of disease could be estimated by the viral peak and the subsequent "set point" of HIV in the plasma, the long-term basal and slightly fluctuating HIV level (55, 56). The higher the peak and set point, the faster the progression to AIDS. This is usually interpreted as indicative of the loss of a critical mass of some irreplaceable cells during early infection, or as an inherent lack of an individual's immune capacity to better control HIV in a person with a high set point. Thus, in the absence of intervention, one's fate is sealed by, for example, destruction of a number of important thymic cells and/or bone marrow hematopoietic progenitor cells needed for recovery and/or by a predictably inherently very poor capacity to

control HIV. Not mutually exclusive to this idea is the possibility that one given HIV is more pathogenic than another, but in most cases that seems less likely, since epidemiological results indicate major differences in the clinical outcome among several people infected with the same strain of HIV (57).

By the late 1980s it became clear that some individuals were less likely to become infected than others, and that among infected persons, some progressed much slower than others. Coupled with the considerable variation in the rates of progression among infected persons, this finding triggered a search for host genetic factors. One hopeful claim came from Levy and co-workers 15 years ago. They reported "a factor" was present in the extracellular fluid of cultured CD8+ T cells that inhibited HIV infection (58). HIV-1 replication was suppressed greatest with factor derived from long-term non-progressors. After this period, "the factor" has remained unidentified by Levy and colleagues, but it stimulated work in many laboratories. The first identification of such naturally occurring HIV suppressive factors to emerge was our report on the β-chemokines RANTES, MIP-1α, and MIP-1β, which were shown to be potent suppressors (nM range) of viruses of the more macrophage tropic variants (59). The reason for this specificity was subsequently explained by the CCR5 usage of these HIV types (60). Later, we identified a weaker inhibitor of viruses, which inhibit both the more macrophage tropic as well as the more CD4 T-cell tropic variants. This was another β-chemokine, MDC (61), but its mechanism of inhibition is still unsettled. Our unpublished results show that it does not account for all the activity released by activated T cells that suppress these viruses; one or more additional factors remains to be defined.

Especially exciting in terms of understanding resistance to infection have been molecular-genetic studies on the HIV co-receptors (particularly CCR5) (reviewed in 60, 62). A naturally occurring mutant of CCR5 lacks 32 nucleotides in its coding region and is not functional. This △32 allele occurs in about 20% of Caucasians and about 1% are homozygous. The △32/△32 homozygotes are protected against infection except in unusual cases. This result verified earlier evidence that HIV-1 variants which use CCR5 for entry are markedly favored for infection, but the reason for this is not yet understood. One △32 allele does not generally provide protection, but it is correlated with slower progression to AIDS (reviewed in 60, 62). More recently, interesting results have been reported on the levels of production *in vitro* of the

anti-HIV suppressive β-chemokines (63-66) and on HLA patterns (67), which correlates with protection against infection or in progression to AIDS once infected. Difference in chemokine receptors CCR2 and CCR3 may also influence HIV infection, but the reasons for this are unclear though it has been suggested that CCR3 may be an epithelial cell receptor for HIV which could be important for mucosal infection (reviewed in 62).

Perhaps the most important indicator of disease progression in infected persons is the state of activation of the immune system. This concept was partially prompted by *in vitro* experiments showing greater HIV infection of activated cells (68) and production of inflammatory cytokines and ultimately T-cell apoptosis (68, reviewed in 69). Clinical correlative studies, such as the studies by Bentwich and colleagues of Ethiopian Jews (70) and activation marker studies of several investigators, and more recently by studies of SIV infection of Sooty mangabeys (71) also explored this concept. Indeed, the extent of hyperactivation may be the most important determinant for the clinical outcome and may explain the discordance sometimes seen between rising virus titer but stable CD4+ T cell numbers in patients in whom HAART has been deliberately interrupted (72). Bentwich and his colleagues showed that this activation state was associated with higher expression of HIV co-receptors (73). However, we do not have any clues as to the host genetic (or viral) basis for the variation.

Cellular Pathogenesis

Presumably, HIV infection may begin by targeting of CD4+ T cells when there is direct virus entry into the blood, for example from contaminated needles. This mode of transmission could also occur by sex in the presence of mucosal tears, possibly even by T-cells traversing intercellular epithelial junctions, or by direct entry of HIV into mucosal epithelial cells. Sexual transmission may also occur via dendritic cells (DC) of the mucosa, commonly referred to as Langerhan cells, which trap HIV within their cytoplasmic projections by binding to polysaccharide surface molecules referred to as DC-sign (74), followed by temporary entry of HIV into DC vesicles but without replication (possibly another HIV immune escape mechanism) and then resurfacing within the lymph node (75). In lymph nodes the DC "feed" HIV to CD4+ T cells. HIV also infects macrophages, usually without killing them but influencing their production of cytokines such as augmenting their release of INF-α and TGF-β (see below).

However, there are other results which demonstrate that virus can rapidly spread from mucosa to draining lymph nodes, suggesting that initial infection is local and is followed by hematogenous spread to distant sites. In this scenario, the DC play an insignificant role (76). Thus, several possible pathways for sexual transmission and viral dissemination have been suggested, but it is uncertain which are more important. Related to macrophages are the microglial cells of the brain, which can also be targets of HIV, and, thereby initiate CNS disorders (77). This could be another example of the use of a minor HIV-1 receptor, CCR3 (reviewed in 62). Other cells within the brain have also been described as targets of HIV-1 infection. These are less certain and in any event less abundant than the microglial cells.

Above we have noted that latency at the cellular level among some cells has been undisputed from the earliest days of HIV research, but what about at the clinical level? Because of the long period that may occur from the onset of infection to the time of visible evidence for disease, it appears as if there may be a generalized clinical-virological latency. However, this is not true. Again, from the very earliest studies we obtained evidence that HIV-1 expression occurs in some cells at various stages after infection (78). This means that from day one there is some small number of latently infected cells and also cells expressing virus. This conclusion was later verified by more extensive and thorough clinical studies of others (79-81), which also revealed much more HIV production *in vivo* than previously suspected.

We now turn to the pathological effects of HIV infection and the mechanisms that produce them. The model given the most attention in the past was based on the direct killing effect on CD4 T cells by HIV, and it was mainly derived from the widely discussed viral "dynamics" and CD4+ T cell turnover data in the mid-1990s (82). These results gave very useful insights for following anti-HIV chemotherapy, but they promoted the notion that such therapy would eradicate HIV infection in a relatively short period of time and prompted thinking behind the "faucet-drain" model of HIV-1 pathogenesis (*Fig. 1*), articulated by D. Ho and co-workers. This model depicts HIV-1 killing its target CD4+ T cell, followed by a compensatory proliferative response, and ultimately failure of the response because of "immune exhaustion", thereby leading to AIDS (82). The problem with the model is that it is a vastly simplified version of the events that lead to immune deficiency and dysregulation. Firstly, it does not account for the important variable of the contribution of T cell migration to and from tissues.

Secondly, it is certain from both *in vitro* and *in vivo* results that some but not all infected CD4+ T cells die. Thirdly, and most importantly, many uninfected cells become anergic, and these do die mediated by apoptosis. Indeed, the evidence suggests that impairment or death of "bystander cells" may be the most important part of HIV pathogenesis. These last points deserve more discussion.

General assessments of the state of affairs of an HIV-1 infected PBMC culture show a diversity of problems. These problems include the following: impairment of proliferation of a majority of cells, a major disruption of cytokine regulation, death of some infected cells by non-apoptotic mechanisms, death of infected cells by TNF-α -TNF-R and Fas-L - CD95 (Fas) interactions leading to apoptosis, and death by apoptosis that bypasses the TNF-α and FasL caspase 8 system and instead is mediated by mitochondrial disruption and a separate caspase pathway. All of these have been reported. The major death mechanism is via apoptosis, as originally described by Montagnier and his co-workers (83) and by Terai et al. (84), and shortly after by other groups, notably Pahwa and her co-workers (85, 86). However, death of some infected cells is only part of the problem. *In vitro* experiments as far back as the 1984-85 period showed that HIV-1 infection of PBMCs markedly impair T-cell proliferation compared to controls, yet only a small fraction of the T cells are infected (87). Ultimately, many of these uninfected cells die by apoptosis, as shown by the Montagnier group (83) and by Oyaizu and Pahwa (86). Thus, from the very beginning it was necessary to take into account the infected cells which do not die, but much more importantly, the deadly effects on so-called bystander uninfected cells for any understanding of pathogenesis. Clinical support for these observations has come both from studies of SIV infected monkeys and in HIV infected humans. For example, the early results of T. Finkel and her co-workers convincingly demonstrated that apoptosis in lymph nodes occurs predominantly among bystander cells rather than in SIV or HIV infected cells (88, 89). Herbein et al have also shown apoptosis of bystander cells (90). Many other reports have come to the same conclusion, notably from the work of Paya and co-workers (91-93). These studies are also supported by the evidence that uninfected cells also decline during disease progression, e.g., CD8+ naïve T-cells, as shown by Roederer et al. (94).

It is possible to think about the loss of uninfected cells as (a) mediated by cell-cell contact (infected cells with infected cells), or (b) by a viral or host humoral factor, or (c) by more

"upstream" generative abnormalities of the bone marrow an/or thymus, which could also be exogenous and due to cell-cell contact or humoral factors unless the progenitors themselves are infected. In the latter case, diminished regeneration could be due to direct killing of precursors of T cells. Indeed, generative abnormalities of the bone marrow of HIV infected persons have been conclusively demonstrated. Clinical studies have demonstrated that cytopenias of several blood cell lineages occur frequently. These cannot be explained by opportunistic infections or by therapy, because they can develop in the absence of both. The clinical observations are substantiated by several corroborative studies showing incontrovertible evidence of bone marrow hematopoiesis defects, as determined by colony formation assays. The controversies begin with various attempts to delineate the mechanism for impaired hematopoiesis. Some results favor an indirect effect, e.g., HIV infection of non-progenitor cells such as stromal fibroblasts (95) or macrophages (96) of the bone marrow which can be induced by Tat to release factors that may inhibit hematopoiesis, such as TGF-β production (96). These observations take on added importance if bone marrow hematopoietic progenitor cells (HPC) are not infected as indicated by Scadden and colleagues (95). However, a few other groups report different results. Beginning with the early studies of Lunardi-Iskander et al. (97), some investigators have suggested that a subset of CD34+ HPC are infected *in vitro* and even i*n vivo*. The most extensive of these studies has been by Fauci and colleagues (98, 99), but in the latter reports from the Fauci group there were no experiments showing functional progenitor capacity of these cells. Thus, they could be CD34 + but not HPC, such as endothelial cells or their precursors or a subset of committed partially differentiated CD34+ HPC which are tightly lineage restricted. The results of Chelucci et al., however, do prove that a small subset of CD34+ cells with hematopoietic progenitor capacity can be infected *in vitro* (100).

The difficulty with interpreting these results as relevant to pathogenesis is that no lineage of blood cells, other than the CD4+ T cells and macrophages, contain HIV proviral sequences. Consequently, if HPC are infected *in vivo* then it is a very small subset, one which is not detectable by analyses for HIV nucleotide sequences in their descendents. Alternatively, a small number of infected HPC could die prematurely, in which case no HIV proviral sequences would be found in the mature leukocytes of the peripheral blood. But, that seems unlikely. A “take-home” message from all these studies is that hematopoiesis is impaired in many HIV-1 infected persons, and the impairment may be explained by infection of a small subset of HPC but perhaps

more significantly by indirect effects of HIV infection of other cells, which is the interpretation favored by Scadden and co-workers. Similarly, diminished thymopoiesis has been suggested by several groups (101-103). It is possible that double positive (CD4+ and CD8+) thymic T cells may be infected and contribute to thymic abnormalities, but here again it appears that "bystander cells" may also be impaired.

In conclusion, like the impairment and/or killing of infected cells there are several distinct mechanisms of disturbance to bystander cells which can be demonstrated *in vitro*, and there is no reason to believe that they are all not operative *in vivo*. From the point of view of a persistently infecting virus, it makes sense not to rapidly kill off its target cell, since the provirus containing target cell is the source of more viral progeny. Rather, it makes sense to impair and/or kill the uninfected immune cells responding to the virus. HIV has evolved overlapping multiple mechanisms to do so. Nonetheless, it is clear that some infected CD4+ T cells do die after infection and that this is a separate component of HIV pathogenesis. Overall it appears that HIV “desires” to kill its target cell, but it is not in a hurry to do so.

Progression

Upon first infection and after initial binding of gp120 of HIV to the cell surface CD4 molecule, an envelope conformational change occurs which fosters binding to the co-receptor CCR5 as discussed above (*Figs. 2 and 3*). This interaction involves specific portions of gp120 which include the V3 region. Such viruses (called R5) are less cytopathic *in vitro* as evidenced by less replication, killing, and syncytia induction compared to those that use another chemokine receptor, CXCR4 (called X4 viruses). This greater *in vitro* virulence, coupled with observations that X4 viruses are often generated as disease progresses, gave rise to the notion that pathogenesis depends upon the evolution of X4 viruses. However, this notion is marred by the fact that AIDS develops with R5 viruses alone, and in these cases progression is often just as rapid as with emergence of X4 viruses. Thus, in southern regions of Africa most infections from beginning to end are only with R5 viruses (104). Consequently, it is just as plausible that X4 viruses are not major factors promoting progression but arise secondarily to advanced progression toward AIDS. What then can we say about progression? The amount of virus is clearly a major factor during the disease. This is determined by the type of HIV, innate immunity such as NK cells and anti-HIV suppressor factor production, the magnitude of co-

receptor expression, and the caliber of the immune response. Many of these responses are determined by genetics, e.g., number of cell surface co-receptors and MHC patterns. Others may be environmentally influenced, such as the levels of HIV-1 suppressor β-chemokines influenced by non-specific stimulation of cells, which produce them. Hyperactivation of the immune system, from chronic parasitic exposure is a detrimental factor.

Molecular pathogenesis

From the above discussion it is clear that any attempt to describe the important molecular events leading to AIDS ultimately will have to take the following into account: (a) mechanisms (viral and host) for the fate of the infected cell, (b) mechanisms (cellular or humoral) for anergy and/or apoptosis of "bystander" cells, (c) mechanisms for the generation of the hyperactive state, and (d) mechanisms for impaired hemopoiesis and thymopoiesis.

The long lived infected cell

Though few in number, it is clear that HIV-1 infected macrophages may have impaired function and aberrant cytokine production, and the latter especially could have patho-physiological significance. However, we have little information on the molecular mechanisms involved. Infected macrophages usually harbor viral genes without dying and without high levels of HIV production. As such, they may form a small reservoir for HIV. The infected CD4+ T cell is another matter. When infected, some CD4+ T cells produce large amounts of viral gene products and may die. At the other extreme, a smaller fraction may make little or no viral proteins and become a major source of the long-lasting HIV reservoir, while hardly expressing any virus during most of that period. As noted, a small fraction (0.05%) of "resting" CD4+ T cells, mainly memory cells, harbor DNA proviruses but may not express the viral genes. This finding leads to speculation that such cells escape from immune detection, and thereby they become the main reason for failure to eradicate HIV by chemotherapy. However, in order to ever become the cell source for virus production, the quiescent T cell must ultimately produce virus. Why then is it long-lived? Why after expression of viral gene products is it not killed by viral proteins or by CTLs? It is possible that a distinct sub-subset of these cells is relatively resistant to apoptotic death, which could be analogous to the results of Varadhachary et al. showing considerable variation in the capacity of various T-cells to undergo activation-induced

death (105). It is also possible that upon expression these latent cells do die, but before doing so they seed new cells, a subset of which will be like themselves acting as reservoirs.

The viral DNA in cells not making virus can take many forms. A major fraction has unintegrated DNA as single or double LTR circles as well as non-circular forms (47, 106-107). Apparently, most of the circular forms cannot express virus as tested *in vitro* (47). However, that does not exclude *in vivo* environmental conditions, which might allow expression by some of these cells. If so, even if it is a small portion of the large number of infected cells containing unintegrated DNA, those with this capacity could be sufficient as HIV "reservoirs". Additionally, there is no question about the very small fraction that contain integrated proviruses and which can indeed be induced to express virus *in vitro*. HIV-1 infected cells synthesize small fragments of viral DNA and harbor these sequences in the cytoplasm (106-108) (*Fig. 4*). Until that cell reaches a metabolic state for sufficient deoxynucleoside triphosphate biosynthesis, the pools of these DNA precursors may remain too low for completing viral DNA synthesis. Like the quiescent DNA provirus positive CD4 T cells and those with unintegrated circles and full length DNA forms, these cells will not be detected by CTLs. When fully active, they could make virus and seed infection of new cells and at that point be sacrificed, similar to what I have suggested for the latently infected cells containing the integrated full length proviruses.

What is the molecular basis for the variation of HIV-1 expression from one extreme to another among the infected CD4+ T cells? Perhaps not only is resistance to death upon production of virus inherent to a particular subset of the cells, but also the control of virus expression that may be an inherent property of a given subset. Alternatively, it may be an event dictated by the virus or both. Until recently we had few clues to explain this important phenomena. As summarized above, Jordan et al. (50) have shown that the site of provirus integration may provide an explanation. It is well known that the HIV proviral integration (*Fig. 4*) sites are numerous and not selective but not completely random, and that many infected cells harbor more than provirus. Utilizing cell clones and an HIV-1 based retroviral vector in which the green fluorescent protein was under control of the HIV promoter, the Verdin group found wide differences in transcription and in Tat responsiveness. Some clones showed basal levels of expression that were so low as to be insufficient to generate sufficient Tat protein at levels that could foster Tat-dependent expression. These clones (about l.5%) were reproducibly found

during infection, and they could be induced by various activators, e.g., TNF-α and phorbol esters. Integration sites of these latently infected cells were within or close to repeat alphoid elements of heterochromatin (50 and E. Verdin, personal communication). Therefore, it appears that by random chance, HIV infected CD4+ T-cell populations may always have a small subset of competent provirus containing cells which exhibit latency.

The infected and killed CD4+ T cell

As noted, many acutely infected cells die and both apoptotic and non-apoptotic pathways have been described. What is the molecular basis for rapid death of infected CD4+ T cells? The earliest attempts to explain HIV-1 killing of infected cells showed that killing was associated with cells that express abundant viral gene products associated with the activated state of the cell (68), and this is still an accepted general view today. The earliest models for a specific molecular mechanism were proposed by Haseltine, Sodroski, Walker and co-workers, who correlated killing with the amount of CD4 and envelope glycoprotein expressed in the infected cell (109). The latter, as also suggested by others, would foster syncytia formation and could potentially lead to a rapid killing of many CD4+ T cells, as evident in SIV/monkey models. There are no results which refute this mechanism, but syncytia are not abundantly observed *in vivo* in HIV-1 infected persons. More recently, Sodroski and colleagues have also provided evidence for single cell killing via the envelope (110, 111). They demonstrated that *in vitro* killing by HIV-1 was associated with a fusion competent envelope expressed concommitantly with CD4 and the appropriate chemokine receptor (110). These studies utilized Jurkat cells and transduced primary human T-cells (111). Sodroski and collaborators also provided *in vivo* results to support the likely relevance of the
in vitro studies. They showed that the degree of loss of CD4+ T cells in SHIV infected macaques was correlated with the membrane fusing capacity of the SHIV envelope glycoprotein (112, 113). However, no evidence was obtained to indicate that these mechanisms were operative in the killing (via fusion) of bystander cells. In some respects, their mechanism for killing of infected cells is supported by results of Westerndorp et al. showing that when CD4 of T cells is cross linked with gp120 in the presence of Tat, the T cells undergo apoptosis (114). However, the main experiments in this report were carried out with exogenous Tat and imply a role for its extracellular presence. We and others have hypothesized that Tat is one of several key contributors to pathogenesis related specifically to bystander cells. It is based chiefly on

in vitro experiments (115) (see section below on humoral factors and anergy and death of bystander cells), but as yet a role for extracellular Tat in HIV pathogenesis is not a widely accepted concept.

The activated state of the cell may be induced by HIV infection itself, particularly from the HIV Nef protein (116). The HIV Nef protein influences cell structure and promotes more HIV production by effecting cell signaling, but its detrimental effects on the infected cell, including pro-apoptotic signals, may be countered by anti-apoptotic effects (117). Consequently, its role in promoting the killing of infected cells *in vivo* is questionable. HIV infection leads to an abundance of inflammatory cytokines, which can contribute to a more generalized immune activation and another mechanism for cell death, i.e., a pro-apoptotic state of the cell (69). This mechanism could be applicable to both infected and bystander cells. Recent results of Warner Greene and his co-workers may provide still another mechanism (see below).

Following entry, formation of the reverse transcription complex and reverse transcription, HIV infection involves another special feature: the formation of the pre-integration complex (PIC), consisting of the newly synthesized viral DNA and several HIV proteins - the matrix protein (p17), integrase, RT, and viral protein R (Vpr) (*Fig. 4*). The HIV proteins in the complex, including Vpr, are carried into the cell within HIV virions. The complex allows HIV proviral DNA to initiate infection of quiescent resting T cells and macrophages. The case is stronger for macrophages, since the bulk of T cell infection requires DNA synthesis and cell division, which eliminates the need for nuclear transport. In the context of HIV pathogenesis the capacity to infect non-dividing cells is important, because it enhances the number and types of HIV infected cells. Vpr is a major promoter for facilitation of macrophage infections (118), and more recently, results have been reported which suggest that Vpr may be important for infection of non-dividing cells by allowing entry into the nucleus of the pre-integration complex (119). A mechanism to explain nuclear entry in these cells has been sought, because the nuclear pore size is about half the size estimated for the complex (120). Vpr (a protein of about 12kd) was shown to enter the nucleus of these cells, become widely distributed, and somehow cause nuclear envelope herniations and a repairable rupture, apparently by weakening of the nuclear lamina (the filamentous proteins attached to the inner nuclear membrane by membrane proteins) through an unknown mechanism (119). However, as the authors note, this cannot be the sole (and maybe

not the main) mechanism for PIC nuclear entry, since HIV vectors lacking Vpr still replicate in some non-dividing cells. These results, however, clearly shed light on pathogenesis because of the consequences of these micro-herniations. Following proviral integration (*Fig. 4*) the next stage of the replication cycle, expression of the viral genes, involves the cellular "machinery" and the Tat and Rev proteins of HIV for full length RNA synthesis and nucleus export of the larger viral RNA transcripts respectively. *Fig. 5* summarizes the steps in these processes, which are reviewed with varying emphasis by Wong-Staal and colleagues (121), Pavlakis, Felber and co-workers (122), and Greene and Peterlin (51). Proviral expression occurs chiefly in the short window of the G_2 phase of the cell cycle. A strategy to increase HIV expression in the infected cell by prolonging or arresting cells in G_2-M checkpoint was evolved by HIV, and this structure depends upon Vpr (123-127). deNoronha et al. used time-lapse video fluorescence microscopy to study of synchronized HeLa cells expressing G_2 cell-cycle regulators (the kinase Wee1, the phosphatase CdC 25C, and cyclin B1) fused to fluorescent proteins. They found that transfected wild type Vpr induced disruptions of nuclear lamina, whereas mutants of Vpr that fail to arrest cells in G_2 did not. Similar herniations were found in HIV-1 infected cell cultures (119). The cell cycle regulators, which control cells at the G_2-M checkpoint, repeatedly entered the cytoplasm, providing a mechanism for G_2-M arrest and the promotion of more HIV expression. Though not emphasized by deNoronha et al., the results they describe also may provide another mechanism for death of infected cells. Thus, in addition to its role in integration and in augmentation of virus production by G_2-M prolongation, Vpr may have a more direct role in pathogenesis by providing another means of inducing death of an infected cell. Indeed, that may be a reason why cell line adapted HIV are often Vpr negative. The herniations may sometimes be fatal to the cell, depending on the amount of Vpr expressed. Perhaps these micro-herniations are direct causes of the apoptotic effect reported for Vpr (128) rather than the apoptosis being the consequences of the G_2M arrest itself.

A fifth mechanism for the death of infected cells, which some have proposed as the most important, is by classical CTL attack on cells expressing viral proteins. However, to do so the CTL has to overcome several effects of Nef and avoid its own death from the specialized "bullets" HIV has in store for it (see below). Finally, Greene and Peterlin suspect that free ends of viral DNA, which imitate double-stranded chromosomal breaks, also contribute to direct cell

killing by HIV (51), and D. Pauza and his co-workers have shown an accumulation of unintegrated DNA in re-infected T cells is associated with death of the cell (129).

The mechanisms for direct killing of infected cells by HIV are all countered to some extent by Nef. Like Tat and Rev, Nef is an early protein in the replication cycle of HIV. Along with the envelope, Nef may have undergone more analyses than any other HIV protein, prompted by reports of its diverse biological effects and the striking role it plays in promoting HIV replication *in vivo* (130). Nef may be the most important HIV-1 gene product that distinguishes HIV from other retroviruses, such as HTLVs. This distinction is suggested because of the multiple activities of NEF. Nef contributes to diminished immune function of the infected cell by down-regulating CD4 (131) and thereby contributing to overall immune deficiency, while concomitantly promoting more HIV production by removing the CD4 impediment to envelope budding (132). It also contributes to the prevention of super infections, which lead to rapid death of the infected cell. Nef also fosters escape of the infected cells from CTLs by down-regulating MHC class I expression (133). Recently, Geleziunas et al demonstrated that Nef associates with and inhibits apoptosis signal regulating kinase-1 (ASK1) (117), which is a critical intermediate in death signaling mediated by both FasL and TNF-α. Whereas Nef enhances expression of FasL on infected cells and thereby fosters bystander cell apoptosis (see below), it appears to protect to some extent against the many mechanisms for killing the infected cell by mimicking the action of cellular negative regulators of apoptosis, such as p21 (CIP1/WAF1), in this case by interacting with p21 associated kinase (PAK-2) (134). The final outcome for the infected cell would appear then to depend on the relative contribution of all these factors at any given time (*Fig. 6*). As discussed below, Nef may also play a central role in the induction of bystander cell death by cell-cell contact.

The final stages in the HIV replication cell include viral protein synthesis, maturation, modification (*Fig.7*), assembly at cholesterol-gylcolipid rich segments of the cell membrane with 2 copies of the viral RNA genome and Vpr, followed by budding from the cell surface and release (*Fig.8*). With the exception of studies on the possible role of the envelope glycoprotein in the process of viral budding suggested by Sodroski, these final stages of viral production have not been a focus of studies of the mechanisms of HIV killing of the infected cell. When broadly viewing reports on the molecular events of killing of the infected cell, it becomes reminiscent of

what we have seen concerning HTLV-1 induction of cell proliferation; the events leading to pathogenesis at the cellular level described in the above sections, and for the molecular mechanisms of impairment and killing of bystander cells upon HIV infection described below, namely, a redundancy of mechanisms are reported. Among the studies of infected cell killing by HIV, however, the studies of Sodroski and colleagues on envelope-mediated killing clearly stand out, and they offer the one mechanism where the *in vitro* mechanism is directly supported by *in vivo* studies in monkeys. However, it is not possible for me to determine envelope-mediated cell killing is of greater importance than other indicated mechanisms or whether this conclusion is instead influenced by the greater depth and quality of the investigations.

The killed uninfected bystander cell: Background

As discussed above, in considering pathogenesis at the cellular level, there are many reports which suggest that anergy and/or death of uninfected cells are significant aspects of HIV pathogenesis, and just as with the impairment or killing of infected cells, the mechanisms are many. These observations begin with the earliest and most simple demonstration, namely, that HIV-1 infected PBMCs either taken directly from patients or obtained by infection of normal PBMCs *in vitro* show marked impairment of proliferative responses, even though only a small fraction of T cells are infected (68, 83, 85). This is true for antigen-induced proliferation at any stage after infection, but loss of mitogen stimulated proliferation is only reduced in later stages. It was subsequently shown that many uninfected T cells die by apoptosis (83, 85) and had increased expression of Fas and FasL (76-78). These *in vitro* studies were extended to experiments in SIV infected monkeys and HIV infected humans with similar conclusions (88, 89, 135). More recently, several groups have attempted to define the molecular basis for bystander cell death.

Cell-cell contact in promoting anergy and apoptosis of bystander cells

Although there are some conflicting results, I have attempted to synthesize many studies that point to mechanism of probable significance for the growth inhibition or death of bystander cells. A summary of the major ones induced by cell-cell contact is shown in *Figs. 9-11*: (1) R5 HIV-1 infection of macrophages up-regulates FasL on these cells, which in turn can kill bystander T cells, but inexplicably only of the CD4+ population (91, 93). No explanation has been provided or suggested in these studies for the sparing of CD8+ T cells. (2) Nef up-regulates

FasL in SIV infected macaques (136). Since Nef also activates T-cell signaling and since TCR ligation of antigens gives rise both to activation and FasL up-regulation, Xu et al. sought to determine whether Nef interacts with the TCR. They found a specific interaction of SIV and HIV-1 Nef with the Z subunit of the TCR, forming a signaling complex which bypasses the requirements of TCR ligation by antigen (137). Their observations indicate that the CTL brought to the infected cell in order to kill it may instead be killed by the infected cell through FasL-Fas interaction. (3) The gp120 of X4 infectious virus, or of inactive virus particles, as well as free gp120, appears to be equally dangerous to bystander cells, but the mechanism differs. Interaction of CXCR4 with Gp120 of X4 viruses causes up-regulation of membrane bound TNF-α of macrophages. In the environment of HIV infection, uninfected T cells have increased TNF-R. Consequently, a cell-cell contact fosters apoptosis of the bystander T-cell mediated by TNF-α-TNF-R interaction, and in this case it may chiefly effect CD8+ cells (138). If those mechanisms are relevant *in vivo,* they might contribute to the later decline in CD8+ T cells in HIV-1 infected people (53) by correlating with the later arrival of the X4 variants of HIV.

Humoral factors in promoting anergy and apoptosis of bystander cells

Several examples of humoral factors which affect bystander cells have also been described. The most prominently discussed are the cytokines soluble TNF-α and IFN-α, and the viral proteins gp120 and Tat, and the following discussion is limited to them.

INF-α and other inflammatory cytokines, such as IL-1, IL-6 and TNF-α, are elevated in HIV infection and may remain so throughout the course of infection and disease (69). This may chiefly reflect the response to HIV antigens, but specific induction of TNF-α has been described. As noted above, CXCR4 ligation of gp120 on macrophages may induce expression of membrane bound TNF-α (138)(*Fig. 12*), but this might also be a mechanism for increasing local soluble TNF-α. A second specific mechanism is mediated by Tat (137). Tat's pleiotropic cytokine/chemokine-like effects are mediated by a cyclic phosphodiesterase-4 pathway (139). Tat also interacts with CXCR4 (140), and might cause similar pro-apoptotic effects on macrophages as described for gp120, but this has not been reported. Extracellular Tat may also be detrimental to the immune system via its role in binding CD26 (141), IL-10 induction (142), suppression of IL-12 (143), and promotion of IFN-α production. Since CD26 is involved in T-cell proliferation, it is tempting to propose that this is the most important mechanism to explain

the role of Tat impairing T-cell proliferation. But, I will focus more on IFN-α, because it is often vastly overproduced after HIV infection.

INF-α was featured prominently in the early history of AIDS research, because it was noted that INF-α was excessively produced (144). With few exceptions, it has not been a recent focus of discussions on HIV pathogenesis. Perhaps this is because of the paradox that IFN-α has sometimes been used therapeutically in AIDS due to its anti-viral effects. However, stimulated by experiments initiated by Daniel Zagury (145), we showed that IFN-α at elevated levels is immunosuppressive, markedly inhibiting T-cell proliferation and inducing expression of Fas (CD95), and reducing IL-2 production and IL-2 receptor function (146, 147). We suggest that the over-produced IFN-α may significantly contribute to disease progression. One mechanism for INF-α over-production demonstrable *in vitro* is mediated by Tat activation of macrophages (148). Still other pathological effects of Tat have been reported. In experiments which showed that gp120 cross-linked to CD4 causes T-cell death in the presence of Tat, the experiments also employed exogenous Tat, and the level of Tat in the extracellular fluid was comparable to the level needed for the pro-apoptotic effect of Tat on bystander cells (114).
In the context of the significance of extracellular Tat to pathogenesis, it is interesting to re-emphasize that T-cell proliferation stimulated by antigens is impaired early after HIV-1 infection, but the proliferation in response to mitogen develops much later. Tat's effect on T-cell proliferation is much more evident with antigen stimulation.

Tat also promotes more HIV replication, which may be due to its capacity to up-regulate HIV-1 co-receptors (149, 150) and to promote hyperactivation (114, 115). However, all these effects of Tat require its presence in the extracellular fluid. In this regard it has been shown that Tat, in fact, can be released by acutely infected cells and taken up by bystander cells (151). An argument put forward against a role of extracellular Tat in HIV pathogenesis is that the serum concentrations are inadequate compared to the concentrations needed for the *in vitro* effects. However, we have already noted the results by Krammer's group on serum Tat, which would be readily compatible with adequate local concentrations adequate for these effects (114), and other results suggest the presence of Tat in uninfected cells *in vivo* (151). Moreover, the negative argument is more than a little flawed in that like cytokines/chemokines, Tat is rapidly taken up by cells, acts locally, and may have a very short half-life. Indeed, if we argued that cytokines,

chemokines, hormones, and other growth factors would need to be at substantial levels in serum for them to be biologically relevant, many of them would not qualify to be of any biological use. In short, we cannot conclude anything by finding low serum levels of a regulator. Tat, of course, also plays an essential role in infected cells as a virtually indispensable nuclear protein for HIV gene expression, mainly involving RNA chain elongation mediated by the Tat - CDK9/cyclin T1 phosphorylation of SPT5 transcriptional regulator and RNA polymerase II (152) (see Legend to *Fig. 5* for more detail of the role of Tat in the infected cell). A surprising additional role for Tat in HIV replication was discovered by Gaynor and his co-workers. They found that Tat also potently stimulated HIV-1 reverse transcription by an as yet undefined mechanism, but one distinct from its promotion of "forward" transcription (153, 154). Thus, Tat has numerous possible roles in HIV pathogenesis, likely at least in part attributed to its promiscuous binding properties. It would not be surprising if its role in stimulating reverse transcription were mediated by Tat binding RT.

Generative abnormalities which affect uninfected cells

Unfortunately, very few attempts have been made to understand the molecular basis for the diminished thympoiesis and hematopoiesis short of determining whether progenitors are infected or not. Results of Re et al. indicate apoptosis of bone marrow cells of AIDS patients studied *in vitro* (155), and one study did suggest that TGF-β production may be promoted by extracellular Tat effects on bone marrow macrophages (96). One might also anticipate that TNF-α levels may be high in the local environment, and each could have anti-proliferative or pro-apoptotic effects. IFN-α production has also been suggested as a putative inhibitor of thympoiesis (M. McCune, personal communication). A list of some of the major mechanisms involved in bystander cell impairment on death is given in *Fig.12*.

In conclusion, it is not contestable that bystander cell impairment and/or death is a major contributor to HIV pathogenesis. As noted above, the mechanisms involved are multiple and the cells effected diverse. Most recent and perhaps most important are the results of M. Feinberg and co-workers, which provide further evidence of the importance of the effect of viral proteins on uninfected cells to pathogenesis. They have shown that in the SIV infected mangabey monkey, the animals have an attenuated immune response and tolerate high levels of viremia with a cytopathic virus. They have shown new evidence for a high level of immune activation for

HIV infected humans and for SIV infected monkeys that do progress to AIDS, thus demonstrating the clear importance to disease determined by the behavior of the infected cell and the indirect effects of the virus (M. Feinberg personal communication) (71).

HIV-1 and neoplasias

HIV-1 is not viewed by viral oncologists as a tumor virus because the infected cells do not become neoplastic, as do classical tumor viruses. Nonetheless, HIV-1 infection causes an internal environment that fosters neoplastic transformation of some uninfected cells. In untreated patients, HIV-1 can act as one of the most potent of all co-carcinogens. It has been suggested that these neoplasias occur due to failure of immune surveillance. However, there are several arguments against this including the following: (a) development of some tumors prior to immune deficiency, (b) the selective types of tumor which develop (i.e., why would the incidence of all kinds of neoplasias not increase, if the concept of immune surveillance was correct?), (c) the increased incidence of only a few of these tumor types in some other immune deficiencies, and (d) the fact that the tumors which are increased in HIV-1 infected persons are all associated with a known tumor virus, Burkitt lymphoma (EBV), childhood leiomyosarcoma (EBV), genital-cervical and anal carcinomas (HPV), Kaposi sarcoma (HHV-8), body cavity lymphoma (HHV-8), and liver carcinomas (HBV and HCV). In the case of Kaposi sarcoma, it has been suggested that the increase in these tumors is the result of the chronic increased levels of inflammatory cytokines caused by the hyperactive immune state and the enhancing effect of the extracellular Tat protein (156, 157). This case might be the same for all these tumors. These conditions favor replication of *bona fide* tumor viruses thereby enhancing the probability of neoplastic transformation. At the moment, there are 2 exceptions that need explanation: an increase in some forms of B-cell lymphomas, which are negative for any known virus (notably EBV and HHV-8 negative), and the lack of increase in adult T cell leukemia in HTLV-1 infected AIDS patients. I suggest that the former will be explained by another B-tropic virus yet to be discovered or possibly by the persistent polyclonal B-cell activation from HIV proteins, or both, and the latter to the failure of HTLV-1 to replicate in response to the same cytokines.

Therapeutic considerations for the future

Current anti-HIV therapeutic strategies were developed by the early 1990s, and they consist of at least 3 different small molecule inhibitors of 1 or 2 HIV enzymes: reverse transcriptase and the HIV protease. Used properly, these compounds are highly active against HIV. Consequently, the acronym HAART (highly active anti-retrovirus treatment) was born. By the mid-1990s, the clinical results were so impressive that coupled with mathematical models of the time, they gave rise to estimates that HIV would be eradicated.

Little information existed at that time about cells which harbor HIV proviruses for long periods without death, and the potential for drug resistant mutants and for drug toxicity were not sufficiently known. The lesson has been learned. Eradication has not been achieved; toxicity is now relatively common as are multi-drug resistant mutants. Approximately 50% of infected patients fail therapy in less than 2 years (R. Redfield, personal communication). Moreover, most of the drugs are not widely available for infected people in the developing world. Nonetheless, the therapeutic advances made by the pharmaceutical industry coupled with the basic information rapidly generated on HIV were tremendous for the developed nations, revolutionizing the care of most HIV infected patients and converting a fatal illness with high morbidity to a chronic disease with generally much less morbidity, while essentially eliminating pediatric AIDS in the developed world by interference with mother-infant transmission.

What then are the questions and needs for the future? Importantly, it is completely uncertain as to when therapy should be initiated, as reflected by the changing NIH guidelines. Since chemotherapy can produce severe side effects, the original guidelines favored some delay. This changed when eradication was believed to be feasible. The guidelines then advised treating as early as possible and with maximum tolerable amounts. Now that eradication is seen as out of the question and patient compliance is more difficult due to drug side effects coupled with the knowledge that escape mutants may occur, there has been a turn to periodic interruptions of therapy in those already treated, and a *deja vu* guideline recommendation now advises waiting for some pathological consequences, such as falling numbers of CD4+ T cells, before beginning therapy. This approach may also have been fostered by recent evidence that the levels of virus can increase while CD4+ T cell numbers stay the same or continue to improve as occurs with the

development of protease inhibitor resistant mutants (158). However, this case is special. Since these mutants replicate less than wild type, they might just as well be inherently less pathogenic (159), as already suggested by the studies of infected human thymus tissue (160). The caution in using drugs at the earliest time point (at diagnosis of HIV infection) because of the potential toxicity is theoretically offset by the irreparable damage early HIV infection may have on later regenerative prowess of both bone marrow and thymus gland progenitors. Thus, when to initiate therapy is still one of the most important of all unanswered clinical research questions in HIV/AIDS research. It is likely that its answer will only come from empirical results over the next few years or by the use of anti-HIV agents with little or no toxicity.

New therapeutic approaches are, of course, still needed. They are needed for the developed world as alternative therapies when drug resistance or serious toxicity occurs. If they can be made less toxic, inexpensive, and logistically feasible, they might also be available for the undeveloped nations. Four promising ideas are discussed next, but it must be acknowledged that these are viewpoints biased by personal experiences.

1. More judicial use of the HIV enzyme inhibitors currently available. The current practice of using HIV protease inhibitors with RT inhibitors has proven effective for many patients, but there is a 50% failure rate in about 2 years due to toxicity and resistance. Much of this toxicity stems from the protease inhibitors, and some clinicians believe the protease inhibitors will not have major use in the future (Robert Redfield, personal communication). Instead, a combination of 3 to 4 RT inhibitors may be a better choice.

It would be preferable if these inhibitors would not be cell cycle dependent in order that they may also be effective for HIV infected resting T-cells, e.g., such as some of the non-nucleoside RT inhibitors like nevirapine (161). Another approach is to use an anti-T-cell proliferation agent, such as rapamycin which prolongs late G1 of the cell cycle, thereby inducing high levels of endogenous HIV suppressive β-chemokines produced marginally during the G1 phase of T-cell growth (A. Heredia & R. Redfield, personal communication)

2. Entry inhibitors. Entry inhibitors can be divided into those that inhibit attachment, co-receptor interaction, and fusion – the 3 sequential events that occur in the first stage of HIV

infection (*Figs 2 & 3*). From the early results of identification of specific β-chemokines, RANTES, MIP-1α, and MIP-1β as potent inhibitors of HIV-1 infection (59), elucidation of their mechanism of action by the identification of their receptor, CCR5, as the key HIV-1 co-receptor (reviewed in 60), to the more recent clinical results showing higher endogenous levels of these specific chemokines are correlated with some protection against infection as well as a longer time to AIDS in infected persons (66, 162, 163). Dramatic results showing that individuals lacking CCR5 (homozygous △32 mutation) are virtually uninfectable (reviewed in 60), it has been clear that that development of entry inhibitors at the level of co-receptor had the potential to be one of the next major therapeutic advances. Even before this information was available, other workers had already targeted entry based on the theoretical consideration alone that blockage of entry should be the best possible approach for reducing HIV. Information was already available on HIV fusion and the earlier event of HIV attachment, the last and first stages of entry respectively. Thus, T. Matthews, D. Bolognesi and co-workers carried out pioneering work that led to specific peptide inhibitors of the gp41 mediated mechanism of fusion of the viral and cellular membrane (*Fig. 3*) (164, 165). The fusion event follows a conformational change in gp41 induced by gp120 co-receptor interaction. This conformational change in part involves the interaction of 2 regions of gp41. The inhibitors are peptide homologues that compete in this coil-coil interactions which is required for the gp41 penetration of the cell membrane that initiates membrane fusion (166). These peptides, beginning with one called T20, are already in phase 3 clinical trials with exciting anti-HIV effects (167, 168), and others are on the way.

In between attachment and the fusion event is the step of gp120 interaction with the co-receptor (*Figs. 3 A & B*). Therapeutic targeting of the gp120 interaction with CCR5 is also now a reality (*Fig. 3*). One approach is to use those β-chemokines which serve as CCR5 ligands themselves, modified so as to avoid signaling. However, small molecule agonists or preferably antagonists are usually better approaches for bioavailability, and at least one such compound developed by B. Baroudy, S. McCombie and colleagues (169), studied *in vitro* particularly by J. Moore and co-workers (169, 170) is now in clinical trials with very promising results. Still another approach, recently developed by T. Fouts and A. DeVico in our group, to block CCR5 is to utilize gp120-CD4 fusion proteins joined in a configuration that allows proper folding (*Fig. 13*) so as to tightly bind CCR5, thereby mimicking the binding process of HIV-1 gp120 to CCR5 (171). Joined at the COOH terminus by an IgG heavy chain fragment for prolonging half-life

and prepared as a single chain, such an immunoadhesion has been recently developed (unpublished data by A. DeVico and T. Fouts) (*Fig. 14*). Early results show specific inhibitory effects against R5 HIV-1 entry (*Fig. 15*). Others such as P. Maddon and his co-workers have considered targeting CCR5 with MoAbs to this molecule. Finally, Maddon and colleagues have also pioneered therapeutic approaches designed to block the first stage of entry, gp120 attachment. They employ a tetrameric CD4-IgG2 molecule which binds HIV-1 gp120 and consequently prevents HIV from binding CD4 on cells (172). This molecule is also in clinical trials, and it is under evaluation in combination with T20 as well (168). Finally, others are targeting CXCR4 with newly developed drugs.

There are several reasons to vigorously pursue entry inhibitors. They have the advantage of blocking virus before any damage can be done to the cell, and as already mentioned, results of clinical trials with some have shown impressive results. Targeting CCR5 is in some respects even more attractive in that like CD4, CCR5 is a cellular product, and as such it will not so easily mutate. Unlike CD4, under the conditions we are currently living, CCR5 is dispensable as evidenced by the normal life span of Δ32 CCR5 homozygous people. Thus, its' complete inhibition should be safe. The worry about driving HIV-1 toward a more pathogenic X4 virus may not be so important. Firstly, that X4 viruses are more pathogenic is unproven. As already discussed in the pathogenesis section, R5 HIV-1 alone can produce an aggressive clinical course. Thus, progression to AIDS may occur for other reasons and the change in clinical status and immune function may favor emergence of X4 viruses under some circumstances rather than X4 viruses causing the progression Moreover, individuals heterozygous for CCR5 do sometimes become infected, and their clinical course is better than other HIV-1 infected individuals. Finally, recent results of Trkola et al showed that the development of resistance of an R5 HIV-1 to a CCR5 inhibitor involved change in gp120, so that it more efficiently bound CCR5 even in the presence of the drug rather than development of a co-receptor switch to CXCR4 by emergence of a putatively more pathogenic X4 strain (170).

3. Targeting extracellular immune suppressive factors by therapeutic vaccines. The concept of using therapeutic vaccines for HIV-1 infected persons was introduced and championed by 3 individuals in the early years of AIDS research at about the same time: Jonas Salk and Robert Redfield in the U.S. and Daniel Zagury in France. The concept independently

promoted by Salk (173) and Redfield (174, 175) were that some HIV proteins might not expose important epitopes during infection, and it might be beneficial to select (by trial and error) different preparations of different HIV proteins and use them in an attempt to augment the immune response. This was not an unreasonable notion, particularly for non-toxic substances, especially at a time when little or no anti-HIV therapy was available. Proof of concept was obtained (175), but there was no evidence of substantive clinical efficacy. Zagury's approach differed, and he focused on targeting extracellular proteins, be they of viral or of cellular origin, that were thought to be important in HIV pathogenesis. As discussed under pathogenesis some viral proteins, e.g., Tat, perhaps Nef, and some over produced cytokines, e.g., IFN-α, TNF-α, and other inflammatory cytokines, are likely to be important to HIV pathogenesis. Cytokines are released into the extracellular fluid, and Tat can also be released from infected cells (151). It is also possible that Nef is released, but to my knowledge no studies have been directed to this possibility. These molecules, both extracellular viral proteins as well as some cytokines, can in theory be targeted by therapeutic vaccines that induce antibodies which neutralize their activities. This approach is attractive by virtue of its inexpensiveness and feasibility, even for the undeveloped nations. It must be acknowledged that targeting normal, albeit overproduced cytokines, by vaccinating against them seems potentially hazardous and difficult to achieve, since raising an immune response to self-protein may not be easy. However, we have recently described the reasoning for this approach in detail (176), and its feasibility in the case of vaccinating against IFN-α has already demonstrated in phase 1 and phase 2 clinical trials, showing safety and immunogenicity (177, 178) and suggesting clinical benefit (179, 180). Targeting Tat by vaccinating against it is another therapeutic vaccine that has reached clinical testing; safety and immunogenicity have been described (181, 182). The rationale for developing a Tat-mediated therapeutical vaccine is also suggested by studies showing that the intercellular traffic of Tat can be abolished by anti-Tat IgG (183). Finally, a recent study showed that only infected individuals without antibodies to Tat, or to the Tat epitope (N-Terminal 46-60) progressed to develop Kaposi's sarcoma (!84, 185).

4. Identifying and elucidating the mechanisms of action of naturally occurring HIV suppressive factors. The search for such factors led to the discoveries of RANTES, MIP-1α, and MIP-1β as HIV inhibitors (59), and after the discoveries of the chemokine co-receptor usage by HIV ultimately to agonists and antagonists of them for a new avenue of therapy (169, 170).

Their identification, brought to light first by E. Berger and co-workers and then by others, help to lead to the discoveries of the HIV co-receptors (CCR5 and CXCR4), a major development in HIV. Other HIV suppressor factors exist (186, 187), and we anticipate that their identification and elucidation of their molecular mechanisms for inhibiting HIV will help open other new avenues of therapy.

Development of an HIV Preventive Vaccine

The Approaches

The first thought for a preventive vaccine is to develop a live attenuated form, such as the Sabin oral polio vaccine. Such vaccines are usually more efficacious and simple. This was an approach taken by R. Desrosiers and co-workers in the field of AIDS research with some interesting results (188, 189). In theory, however, this was a vaccine approach with hazard, and one which would unlikely be approved by the FDA. The caution was vindicated when Ruprecht and co-workers demonstrated that monkeys vaccinated with one candidate developed disease (190, 191). A second approach is the use of killed whole virus, analogous to the Salk Polio vaccine. This kind of vaccine would also be relatively simple to develop and use, and it has been argued by J. Cohen that this kind of vaccine suffers from a lack of interest by the field (192). That may be a fair criticism, but again it is likely that killed whole HIV would not be FDA acceptable. Most results in animal experiments with this approach have not been encouraging. The third approach is to use proteins of HIV ("subunit" vaccines) or their DNA counterpart. A discussion of them can be complicated and confusing, because the number, types, and manner of presentations for HIV/SIV subunit vaccines have been numerous. For instance, the vaccine can consist of (1) the gp120 envelope alone (common), (2) the core proteins alone (uncommon), (3) the regulatory proteins, not carried by the virus but encoded by the HIV genome and expressed as the earliest made HIV proteins (usually including Tat, Rev, and Nef (uncommon)), or (4) any combination of these 3 (common). They may be delivered in their DNA form by bacteria (e.g., attenuated Salmonella), or viral (e.g. avian poxvirus, attenuated polio, rhinovirus, adenovirus, and alpha viruses) vectors, or by direct inoculation of the proteins or of the DNA encoding the proteins. Each of these delivery systems is selected for some advantage, e.g., mucosal immunity (adenovirues, Salmonella and alpha viruses); access to dendritic cells (Salmonella and alpha

viruses), etc. Often, a "prime and boost" procedure is used; this procedure involves priming with vector derived or DNA vaccines, which favor generation of cellular immunity and boosting with direct protein or peptide injections, which usually favor humoral immunity.

The objective

There are 2 camps at the moment in the HIV vaccine field. The temporary majority are those focused solely on the generation of cell mediated immunity (CMl) with the thinking that sterilizing immunity is unattainable, therefore, accepting the notion of allowing infection but protecting against disease development by CTL attack against infected cells. This keeps the HIV/SIV titers low and disease at bay. Results in monkeys challenged with SIV or the SIV/HIV chimera called SHIV with some of these approaches, mainly employing DNA vaccines, have sometimes been impressive (193-195). The reason they have become the dominant approach in recent years is chiefly due to the years of vaccine failures in the early period of HIV research (1984-1990), which was focused upon induction of neutralizing antibodies by viral envelope. Those results almost always consisted of protection limited to the virus used in the vaccine and its closest relatives, and/or to viruses which were laboratory cell line adapted strains but not to most primary HIV isolates. Soon it was also shown that the neutralizing antibodies that gave positive results in many reports were directed to a cellular factor related to the cells used to grow the virus that was used for the vaccine, or the neutralizing antibodies were to the V3 loop of the envelope, a highly variant sequence, and thus highly dependent on the virus challenge and on the dose of the vaccine (see G. Hunsman & co-workers, 196, 197). Consequently, in more recent years most investigators have sought to develop vaccines that would induce CMI, deducing that it was improbable or impossible to develop neutralizing antibodies against a wide range of primary isolates. In this manner, the strategy for a CMI based vaccine was born, and as indicated above, some have produced impressive results in monkeys challenged with SIV or SHIV. They have held the virus to low levels for over 1 year and prevented disease development. The caveat is that we do not know how long CMI can hold the virus in check. A human vaccinated as a child could lose control of the virus years later. Based on these limitations, a second and temporarily much smaller group has stayed with the quest for development of vaccine which generates neutralizing antibodies that offer sterilizing immunity against a broad (multi-clade) variety of primary HIV isolates. Greater confidence in the possibility to do so has come from recent advances in the structural biology of the HIV-1 gp120 and its interaction with cell

receptors (198), better understanding of these receptors, the development of a number of monoclonal antibodies that show potent cross clade neutralization of HIV (199), and by more sophisticated approaches to mimic the natural trimeric version of the envelope (200, 201). Taking advantage of some of this information, a complex of gp120-linker sequence-CD4 has been made, which has produced cross clade neutralizing antibodies in monkeys. This is based on the same complex described above but without the Ig fragment. The concept is based on the idea of a gp120 - CD4 transition state, which takes a conformation that exposes sites on gp120 critical to its binding of CCR5, and that a specific immune response and one active against a broad range of HIV types can be generated against these sites. Early results suggest that the antibodies, which neutralize infection *in vitro* are directed against the complex of gp120 – CD4 and not to CD4 itself or gp120, though some non-neutralizing antibodies to both do develop (202). Two major challenges lie ahead: (1) evidence must be obtained to prove that any immune response to CD4 is without harm, or CD4 must be replaced with a sequence which mimics but does not lead to an immune response against CD4. (2) Prevention of infection of animals. Both objectives are currently being actively pursued.

There are some efficacy trials with simpler subunit vaccines already ongoing, but their possibilities for success are not viewed with enthusiasm. Their status and some of the other issues have been recently addressed by J.P. Moore (203), and a broader recent and critical review of various vaccine approaches by Berzofsky and colleagues (204).

Years ago the late Albert Sabin concluded that developing an HIV vaccine which prevented infection was impossible (205). It is the collective goal of the field to prove him wrong.

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Legends to the Figures

Fig. 1: The Faucet and Drain model for HIV pathogenesis. In this simplistic version HIV infects and kills CD4+ T-cells. Uninfected T cells respond, proliferate, but ultimately fail to keep adequate numbers of T cells because of "immune exhaustion". For several reasons discussed in the text, this model is not valid for major aspects of HIV pathogenesis.

Fig. 2: The beginning of HIV infection of a cell. Interactions with cell surface polysaccharides (not shown) is followed by gp120 binding to CD4, conformation change in gp120 involving at least the V3 loop, and engagement of chemokine receptors, generally either CCR5 or CXCR4. If it is the initial infection, CCR5 is almost always the receptor, and the variant of HIV is called a R5 virus. Following binding to this co-receptor, the gp41 portion of the envelope also undergoes conformational change leading to its eventual penetration of the cell membrane and fusion of the bilipid viral and cellular membranes.
Sometimes (usually late in disease development) variants of HIV emerge which particularly target CXCR4. These are called X4 viruses.

Fig. 3(A): A more detailed look at the events leading to fusion. Note the trimeric form of gp120.
Fig. 3(B): The same process but emphasizing the change in the form of gp41. This occurs when gp120 engages the HIV co-receptor and involves activation of gp41. Two coiled heptad segments of gp41 interact causing exposure and extension of its fusion domain, which then can insert into the cell surface membrane juxtaposing viral and cell surfaces to begin fusion.
These diagrams are from my colleague Dr. Anthony DeVico.

Fig. 4: After entry, the reverse transcriptase (RT) complex in the cytoplasm of the cell initiates DNA synthesis from the viral RNA (bottom left). With the double stranded DNA formed several viral proteins, RT, p17, integrase, and Vpr form the pre-integration complex, which penetrates the nuclear membrane. For most retroviruses this is limited to cells in cycle, but the DNA of HIV and other lenti-retroviruses can penetrate nuclear membranes of cells not in cycle. For HIV, these include macrophages and some "resting" CD4+ T cells. The mechanisms for entry into nuclei of resting cells is not yet clarified, but sometimes may involve nuclear herniations caused by Vpr. Indeed, Vpr is essential for macrophage infection, but not for resting T-cells. Integration is achieved rapidly after nuclear entry to form the provirus.

Fig. 5: The formation of virus from the integrated DNA provirus first involves transcription. Newly made RNA may be at very low levels, and most as incomplete transcripts until the cell receives signals to become activated. This basal level of expression is highly variable among different cells and depends at least in part on the site of chromosomal integration (50), which is highly variable. Otherwise, basal expression is completely under control of cellular factors and their influence on the promoter and enhancer elements within the 5' LTR. The enhancer region of the LTR markedly augments transcription when cells are activated. The enhancer sequence includes NF-kB sites, which are essential for the high activity of the enhancer. NF-kB is under control of its inhibitor, ikB, which is modified and then degraded by proteosomes freeing NF-kB for its role in influencing activity of the enhancer. These events occur upon cell activation or after direct exposure to cytokines like like TNF-α. When activated, the short mRNA for Tat (multiple spliced small transcripts) obtain levels sufficient for Tat synthesis. Tat with cellular cyclin T_1 (51) binds to a region of viral RNA called TAR (a short double stranded hairpin structure in the R region of the LTR located just after the transcription start site (U3/R junction)), and then binds cellular cyclin-dependent kinase 9 (Cdk-9). In this complex, Tat markedly increases RNA synthesis, especially causing elongation of short RNA transcripts. With

additional cellular proteins, different RNA transcripts are produced. When inhibitory sequences are not present, multiply spliced RNAs which encode Tat, Rev, and Nef enter the cytoplasm without difficulty (121). The unspliced full length RNA, which include genomic RNA, structural enzymatic, and a few other viral proteins, stay in the nucleus. Their ultimate transport to the cytoplasm is Rev dependent. This is mediated by Rev binding to the Rev response element (RRE), a complex stem loop of the *env* sequence of the viral RNA (121, 123). Rev then forms a multimer, and through its nuclear export sequence and host factors is ultimately able to transport these larger transcripts to the cytoplasm where the viral genomic RNA accumulates and precursors of the viral structural proteins and enzymes are made.

This area of research on HIV molecular biology is the premier example of basic HIV research now yielding fundamental insights to all molecular biology (see particularly references of Tang et al (121), Reddy et al (206), and Nappi et al (207)).

Fig. 6: The fate of an HIV infected cell varies. Sometimes the cell is killed, and this figure summarizes several mechanisms for this death to occur (upper panel). However, there are forces to contend with that inhibit death of the infected cell. Our information to date suggests that the HIV Nef protein is chief among them (lower panel).

Fig. 7: Expression of viral proteins follows 2 pathways. One, through endoplasmic reticulum and the golgi, involves the biosynthesis and maturation of the env protein, which includes its cleavage by a cellular protease and its glycosylation and later glycosyl trimming and transport to the plasma membrane. The second is on free polyribosomes. This is the synthesis of Gag and pol polyproteins, myristoylation of the Gag polyproteins leading to transport to the plasma membrane, where it is processed by the viral protease which is completed after budding and release.

Fig. 8: Viral components assemble at the plasma membrane. This is under

the influence of the maturing structural protein precursors. They promote attachment of 2 identical copies of the viral RNA genome and some VpR. Assembly is mainly at cholesterol rich regions of the plasma membrane where budding occurs. In this manner the viral membrane is cholesterol rich (ref. 160 or Greene and Peterlin).

Figs. 9-11: Cartoon schemes showing 3 mechanisms for killing of a bystander (uninfected) cell by contact with another (usually infected) cell.

Fig. 12: A summary of some of the mechanisms having the detrimental effects on bystander cells.

Fig. 13: Schematic representation of gp120 bound to CD4 emphasizing the distance between the COOH terminus of gp120 and the NH2 terminus of CD4. This figure was prepared by my colleagues Drs. George Lewis and Anthony DeVico.

Figs. 14-15: Schematic representations of an immunoadhesion, which targets CCR5 for use in inhibiting HIV. The text provides the rationale. *Fig. 14* shows the single chain for including R5-gp120, a linker sequence, 2 domains (D1 D2) of CD4, and a segment of IgG_1. *Fig. 15* is a cartoon representation of its bioactivity. The figures were prepared by my colleague Dr. Anthony DeVico.

Fig. 1

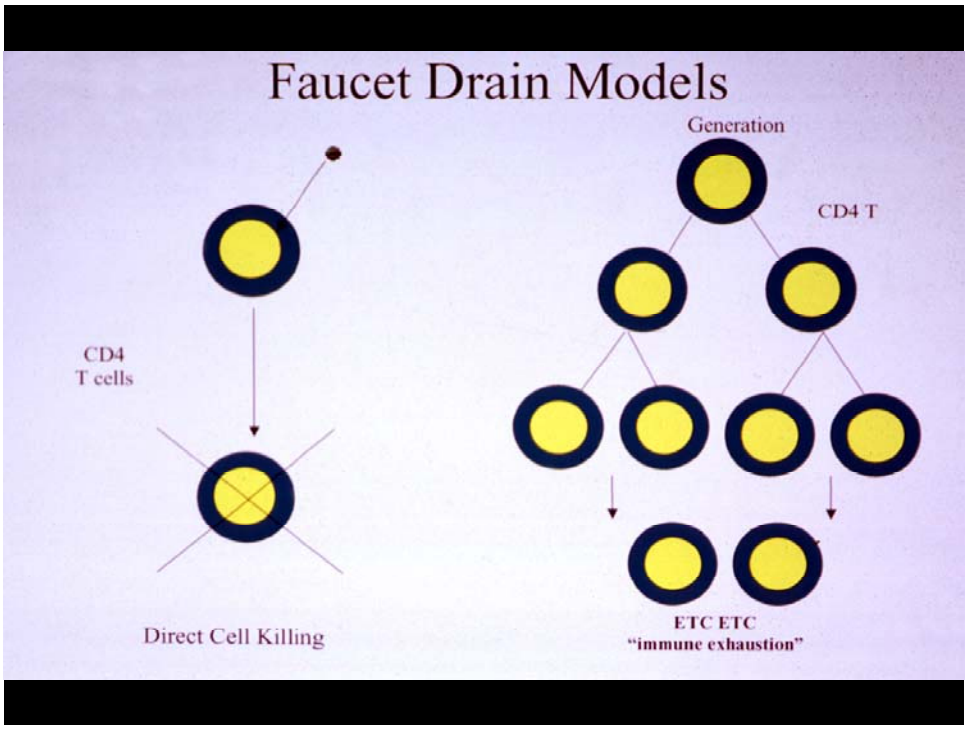
Faucet Drain Models
Generation
CD4 T
CD4
T cells
Direct Cell Killing
ETC ETC
"immune exhaustion"

Fig. 2

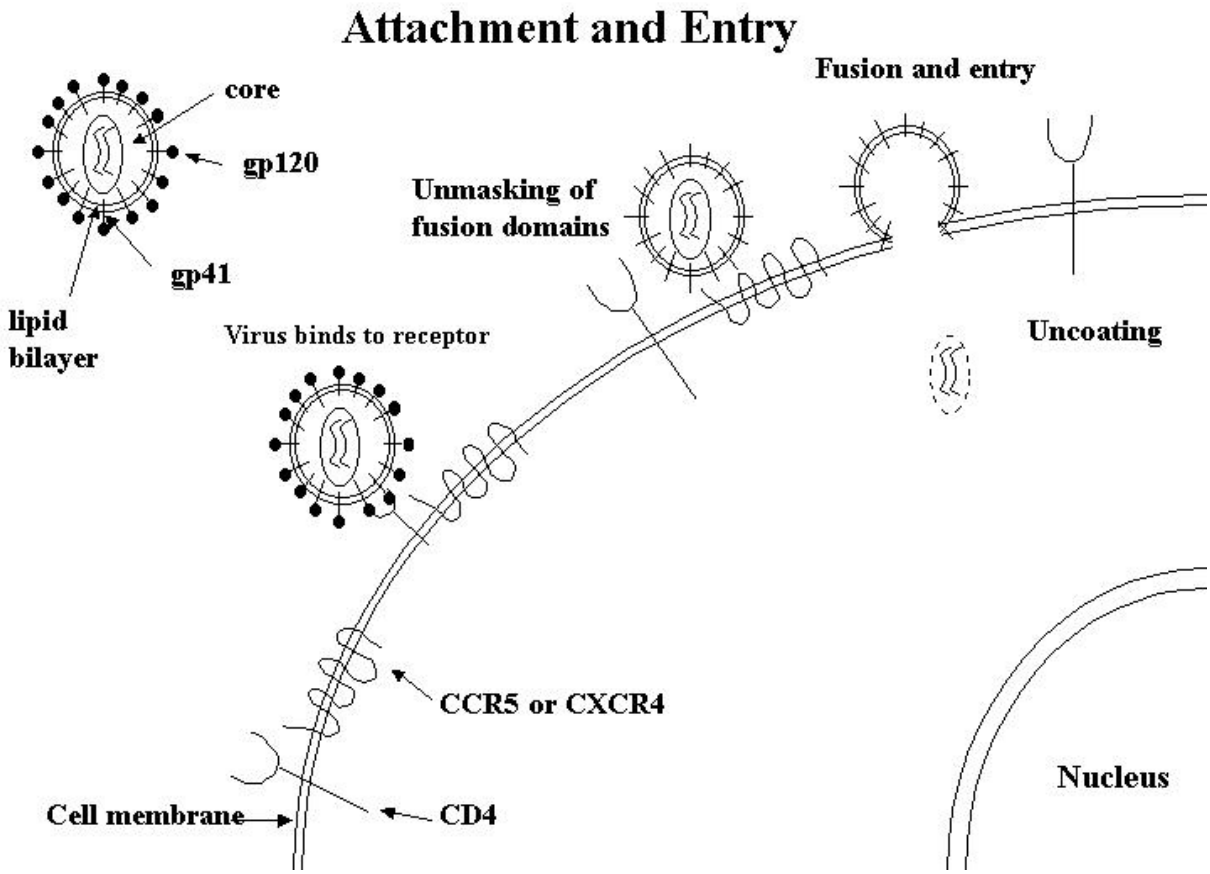
Attachment and Entry
core
gp120
gp41
lipid
bilayer
Virus binds to receptor
Unmasking of
fusion domains
Fusion and entry
Uncoating
CCR5 or CXCR4
CD4
Cell membrane
Nucleus

Fig. 3A

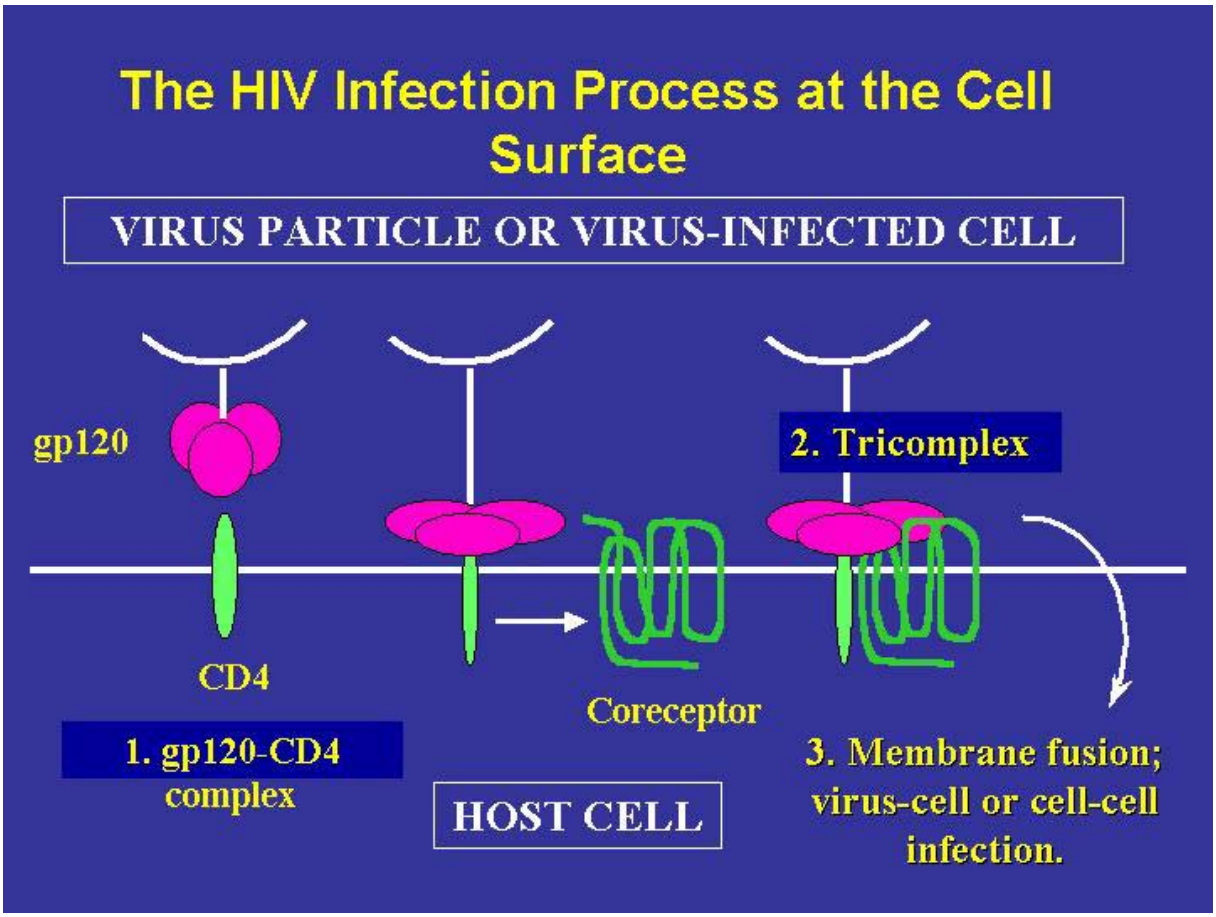
The HIV Infection Process at the Cell Surface
VIRUS PARTICLE OR VIRUS-INFECTED CELL
gp120
2. Tricomplex
CD4
Coreceptor
1. gp120-CD4 complex
HOST CELL
3. Membrane fusion; virus-cell or cell-cell infection.

Fig. 3B

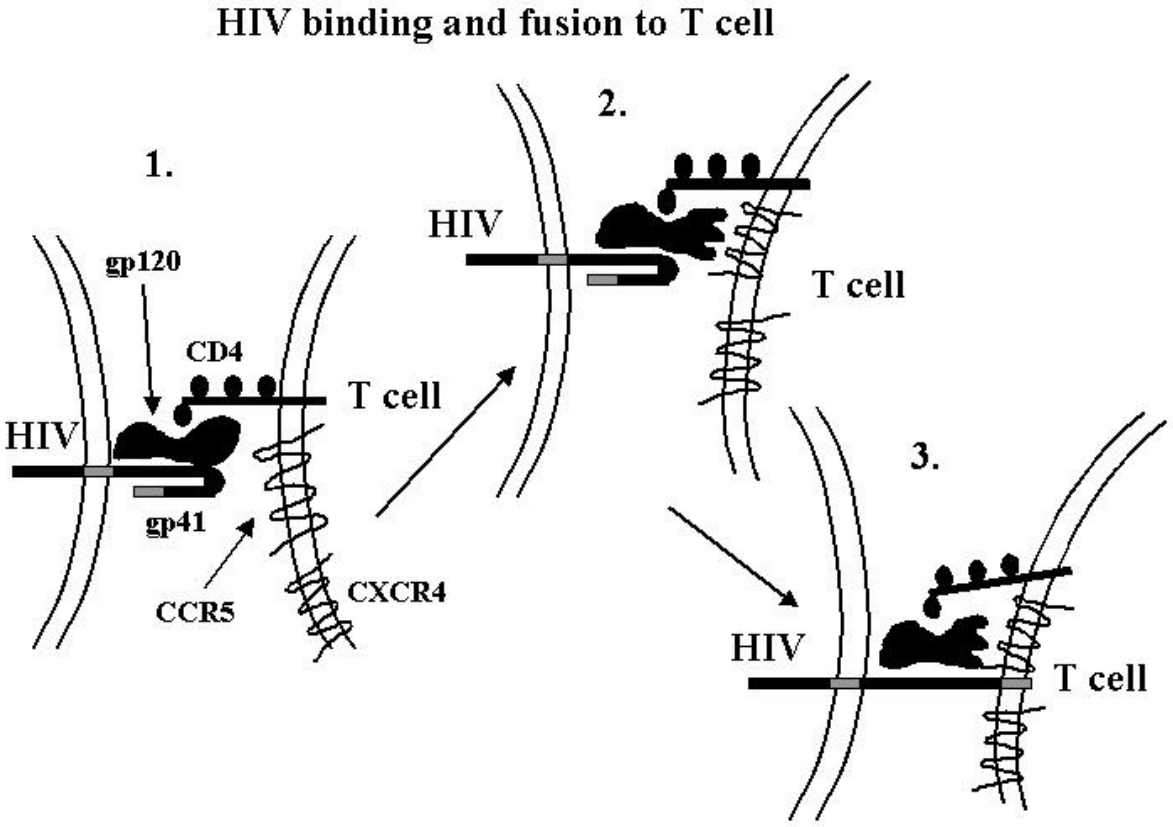
HIV binding and fusion to T cell
1.
gp120
CD4
T cell
HIV
gp41
CCR5
CXCR4
2.
HIV
T cell
3.
HIV
T cell

Fig. 4

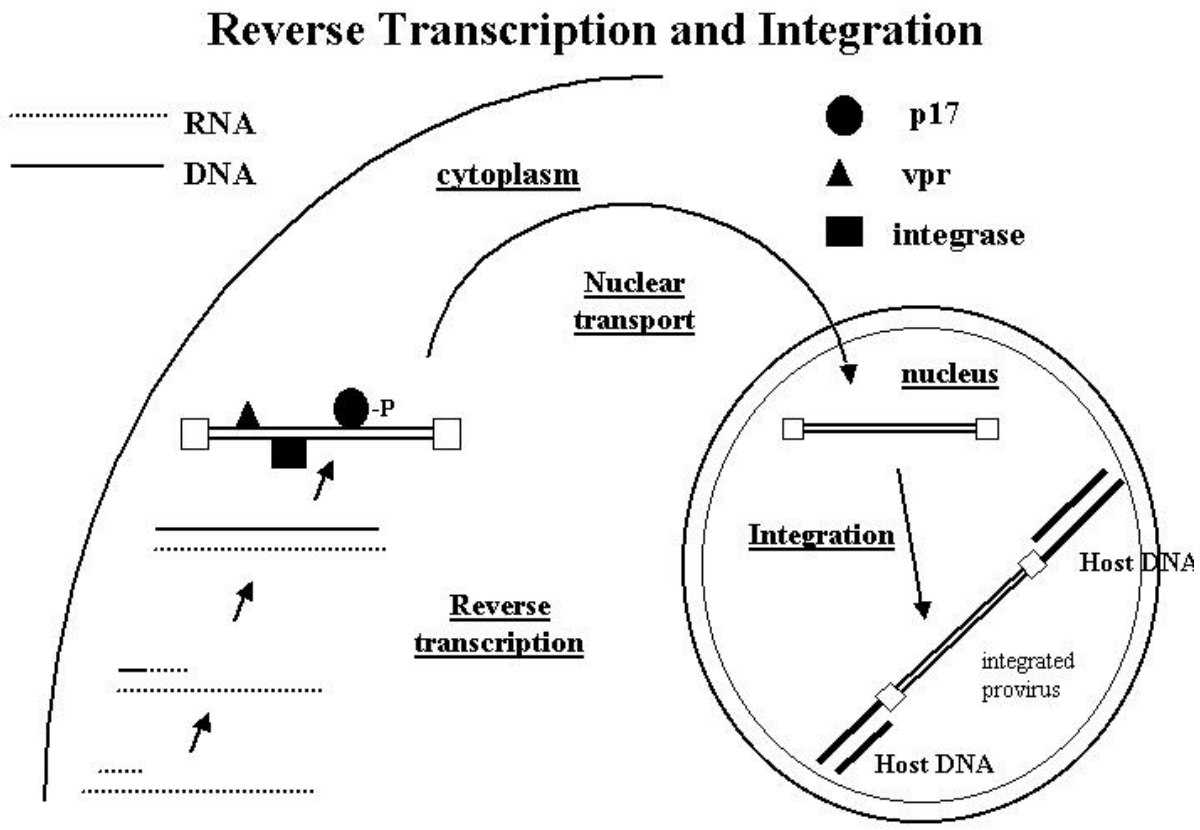
Reverse Transcription and Integration
RNA
DNA
cytoplasm
p17
vpr
integrase
Nuclear transport
nucleus
-P
Integration
Host DNA
Reverse transcription
integrated provirus
Host DNA

Fig. 5

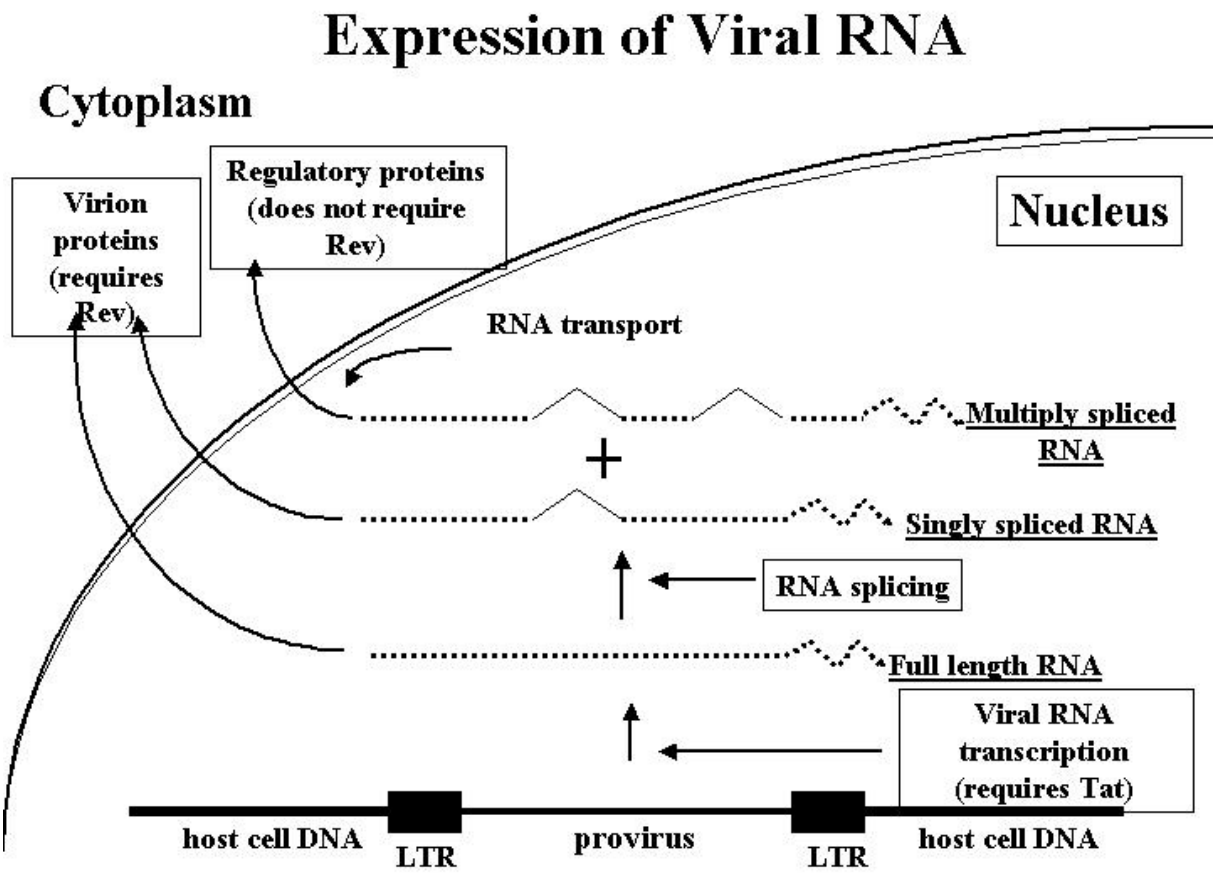
Expression of Viral RNA
Cytoplasm
Nucleus
Virion proteins (requires Rev)
Regulatory proteins (does not require Rev)
RNA transport
Multiply spliced RNA
Singly spliced RNA
RNA splicing
Full length RNA
Viral RNA transcription (requires Tat)
host cell DNA
LTR
provirus
LTR
host cell DNA

Fig. 6

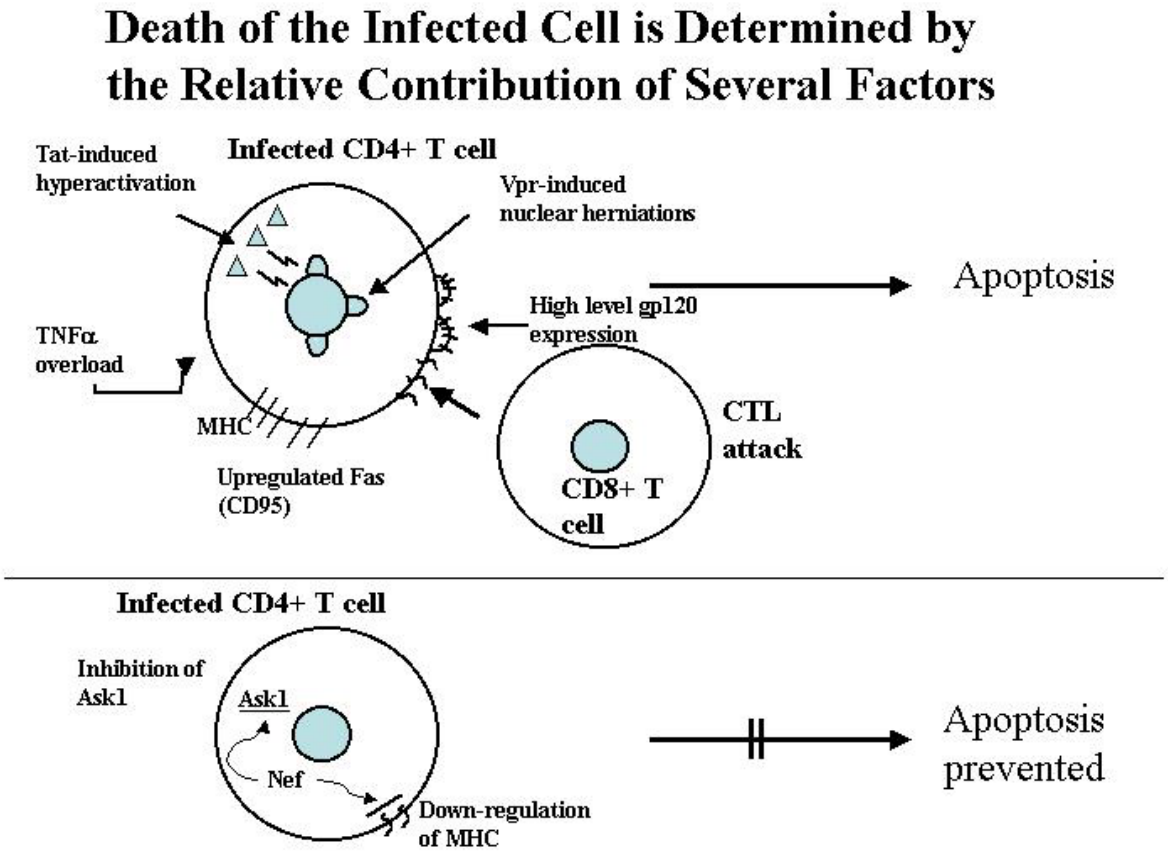
Death of the Infected Cell is Determined by the Relative Contribution of Several Factors
Infected CD4+ T cell
Tat-induced hyperactivation
Vpr-induced nuclear herniations
Apoptosis
High level gp120 expression
TNFα overload
MHC
CTL attack
Upregulated Fas (CD95)
CD8+ T cell
Infected CD4+ T cell
Inhibition of Ask1
Ask1
Nef
Down-regulation of MHC
Apoptosis prevented

Fig. 7

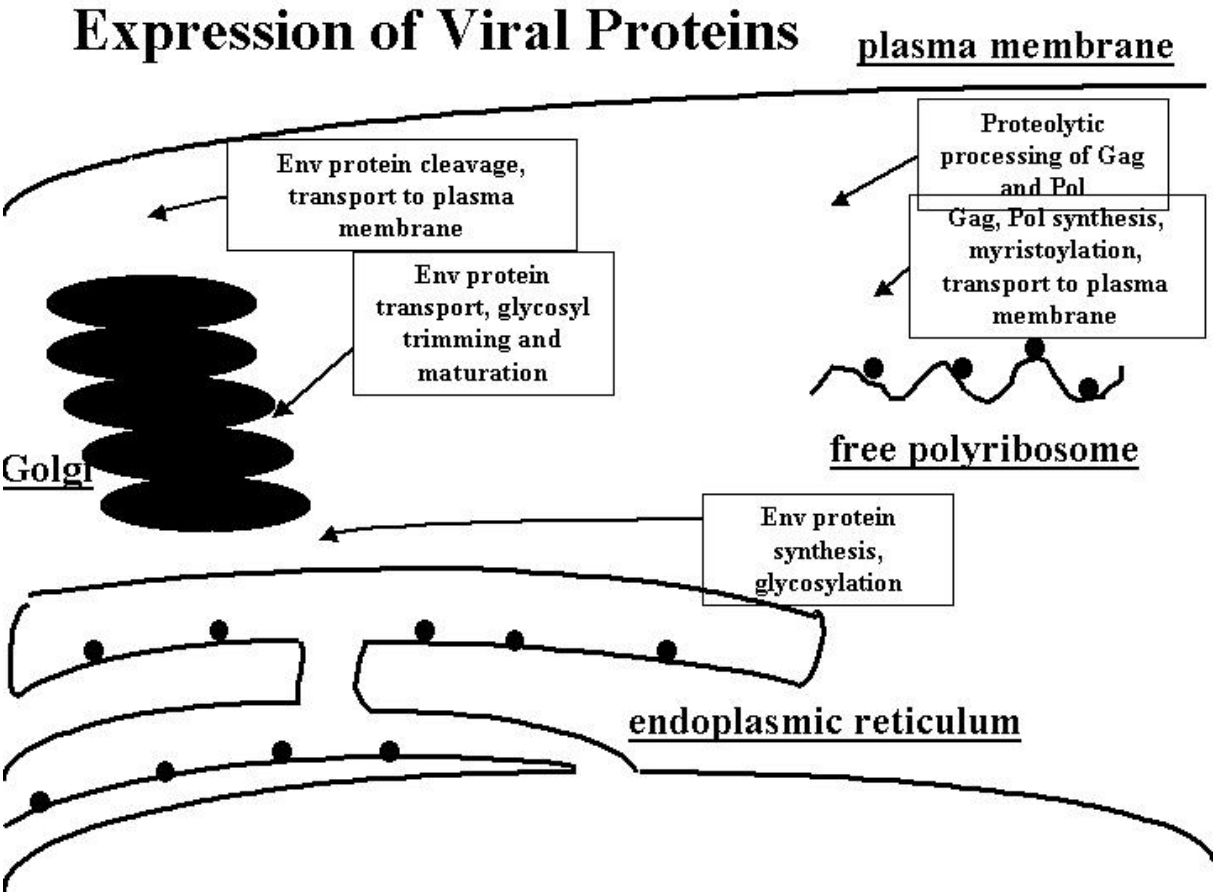
Expression of Viral Proteins
plasma membrane
Proteolytic processing of Gag and Pol
Env protein cleavage, transport to plasma membrane
Gag, Pol synthesis, myristoylation, transport to plasma membrane
Env protein transport, glycosyl trimming and maturation
free polyribosome
Golgi
Env protein synthesis, glycosylation
endoplasmic reticulum

Fig. 8

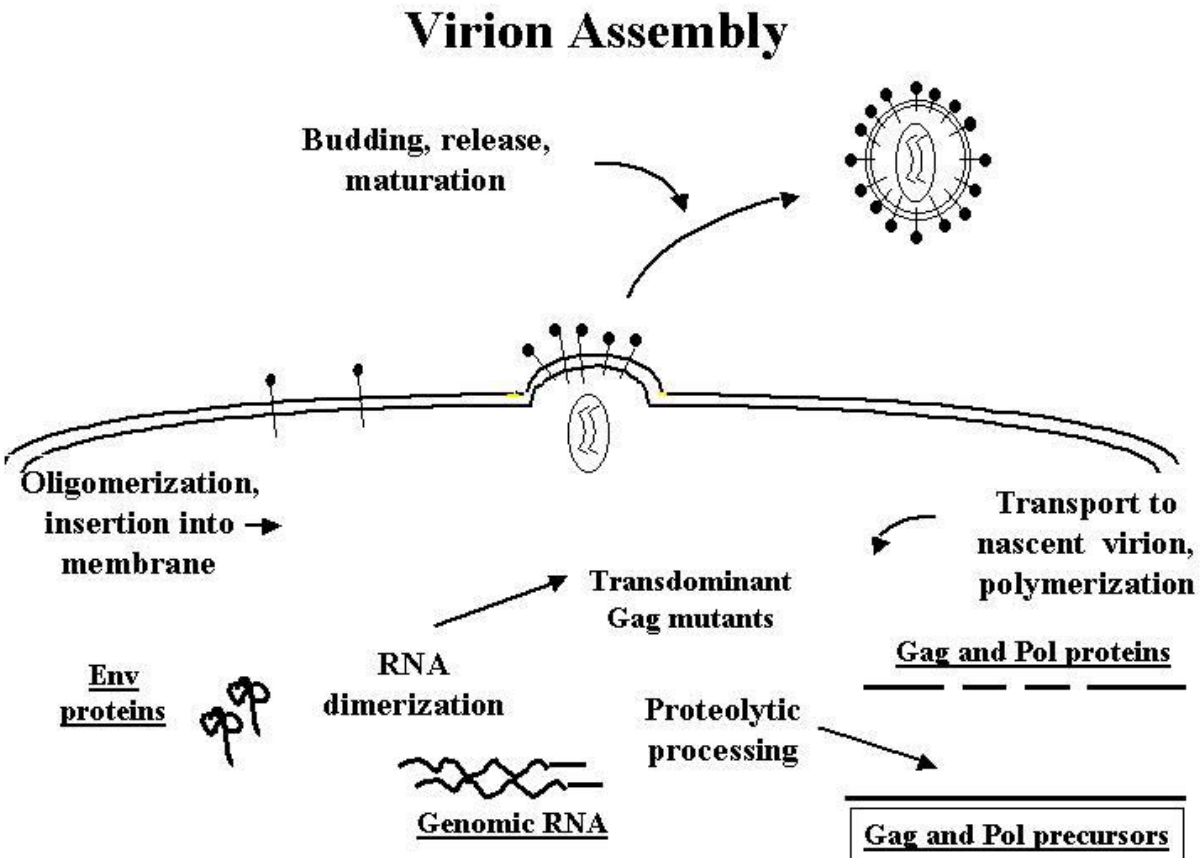
Virion Assembly
Budding, release, maturation
Oligomerization, insertion into membrane
Transport to nascent virion, polymerization
Transdominant Gag mutants
Gag and Pol proteins
Env proteins
RNA dimerization
Proteolytic processing
Genomic RNA
Gag and Pol precursors

Fig. 9

Some mechanisms of Anergy and/or Apoptosis of Uninfected Bystander Cells

A. By cell-cell contact through mechanisms mediated by Nef

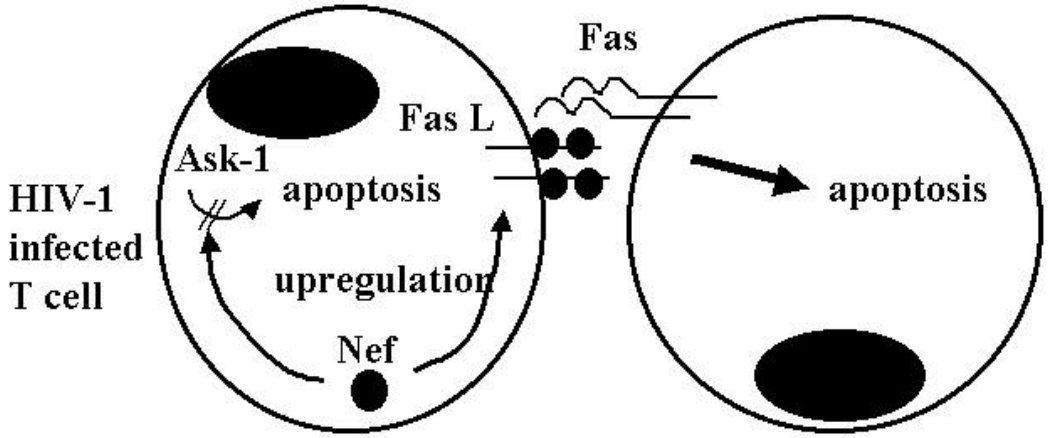

Fig. 10

cont…Some mechanisms of Anergy and/or Apoptosis of Uninfected Bystander Cells

B. By cell-cell contact through mechanisms mediated by gp120 (free gp120 or gp120 on infectious or inactive virions

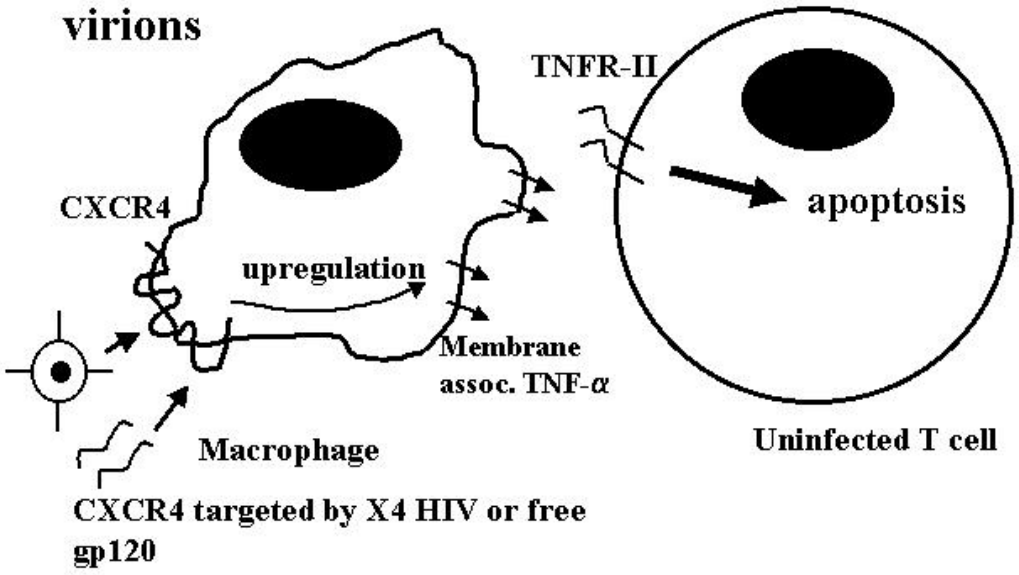

Fig. 11

cont…Some mechanisms of Anergy and/or Apoptosis of Uninfected Bystander Cells

C. By cell-cell contact again mediated by gp120 interaction with an HIV-1 co-receptor

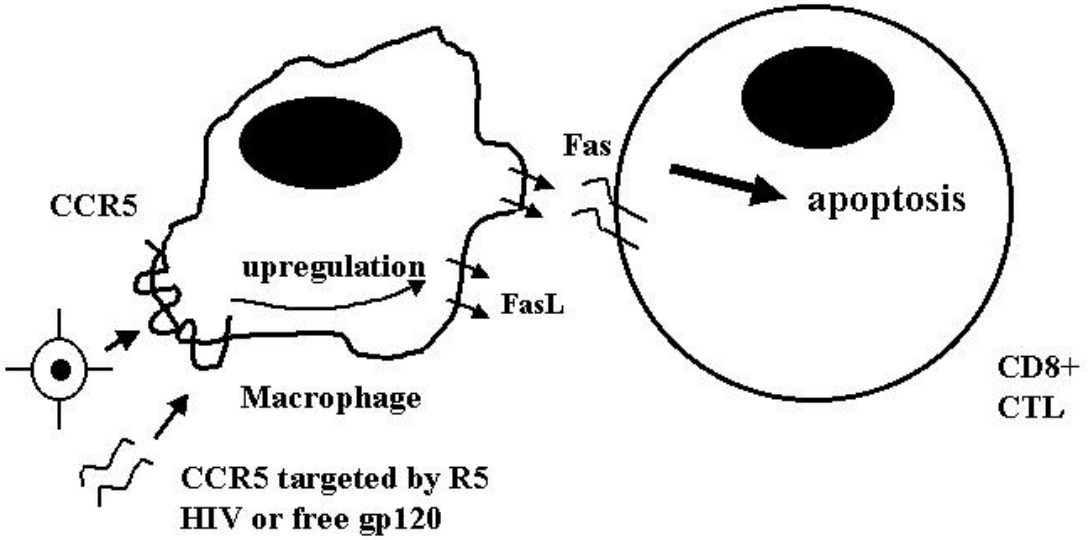

Fig. 12

- Gp120 of X4 virus binding to CXCR4 of macrophages and upregulation of membrane bound TNF-α on macrophage surface and of TNF-RII in CTLs.

- Gp120 of R5 virus binding to CCR5 of macrophages and upregulating FasL on the macrophages in a setting of high level Fas expression of bystander CD4 T cells.

- Nef induced down regulation of MHC-CII on the surface of CD4+ T cells resulting in impaired CTL recognition of infected cells.

- Nef upregulation of FasL in infected CD4+ T cells

- The hyperactive state and its promotion of apoptosis.

- Extracellular Tat inducing hyperactivation, angery and apoptosis of bystander cells.

- Increased levels of some cytokines such as soluble TNF-α which can be proapoptotic and of IFN-α and TGF-β which can be immune suppressive.

- Regenerative abnormalities of the thymus and bone marrow.

Fig. 13

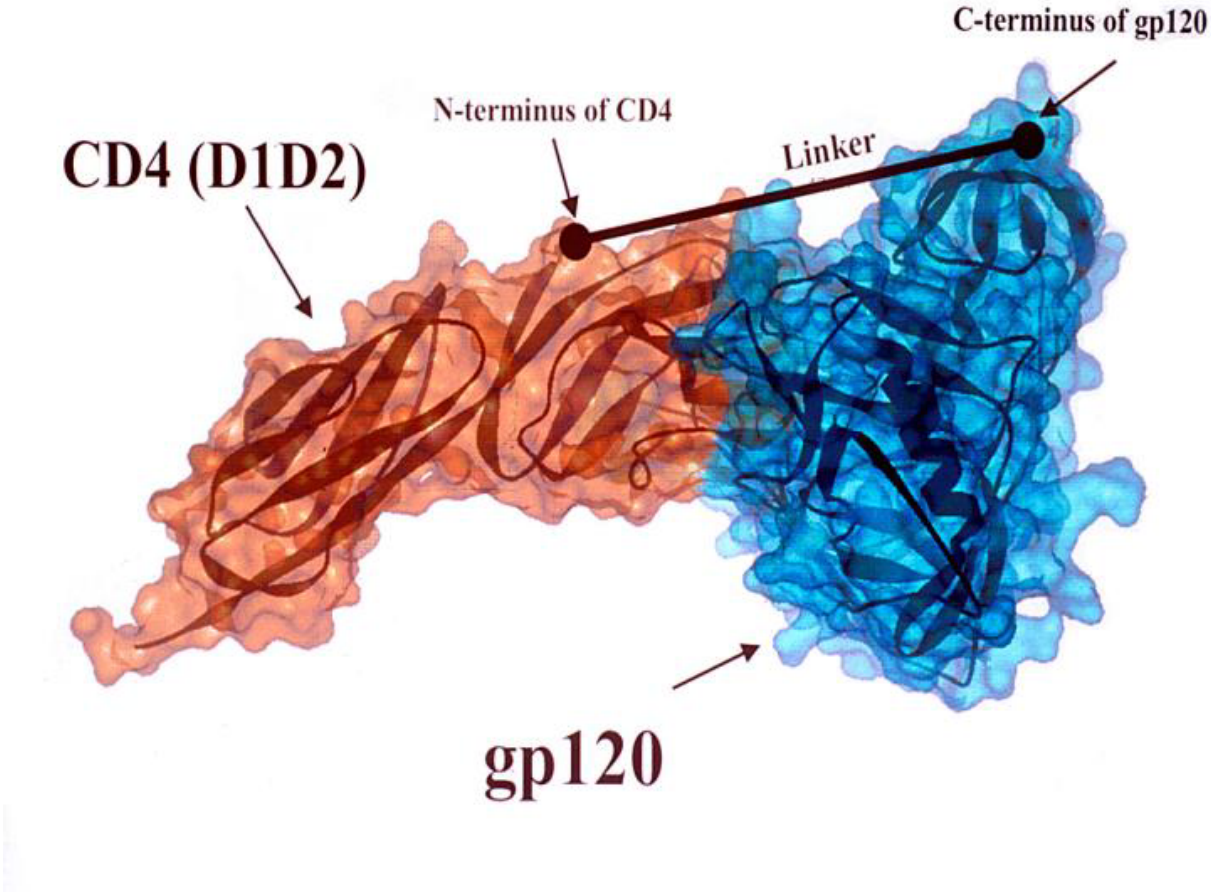

Fig. 14

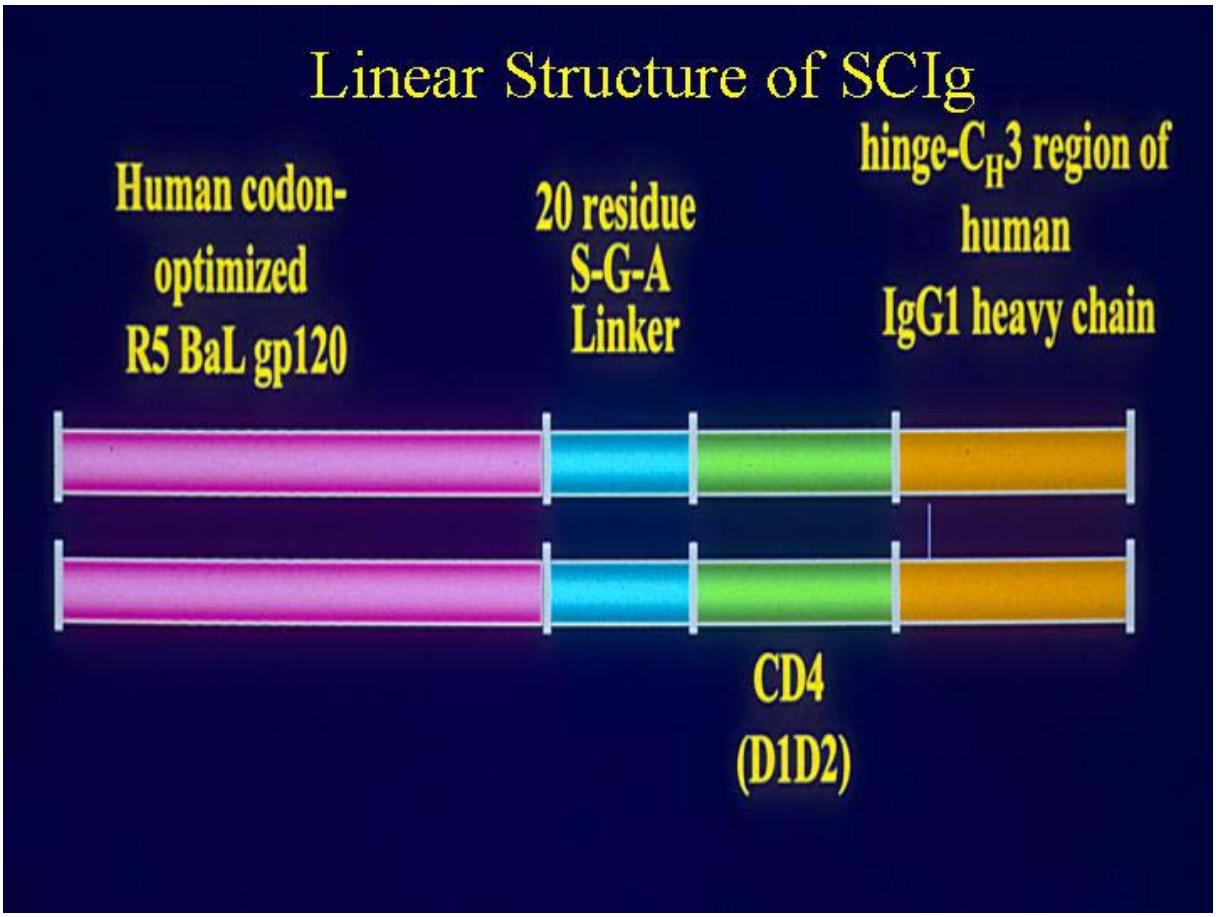
Linear Structure of SCIg
Human codon-optimized R5 BaL gp120
20 residue S-G-A Linker
hinge-C_H3 region of human IgG1 heavy chain
CD4 (D1D2)

Fig. 15

Developing the Single Chain complex for Therapy