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Prevention, Suppression or Eradication of Viral Infection:

Paradigms for dealing with the challenges of Human Immunodeficiency Virus

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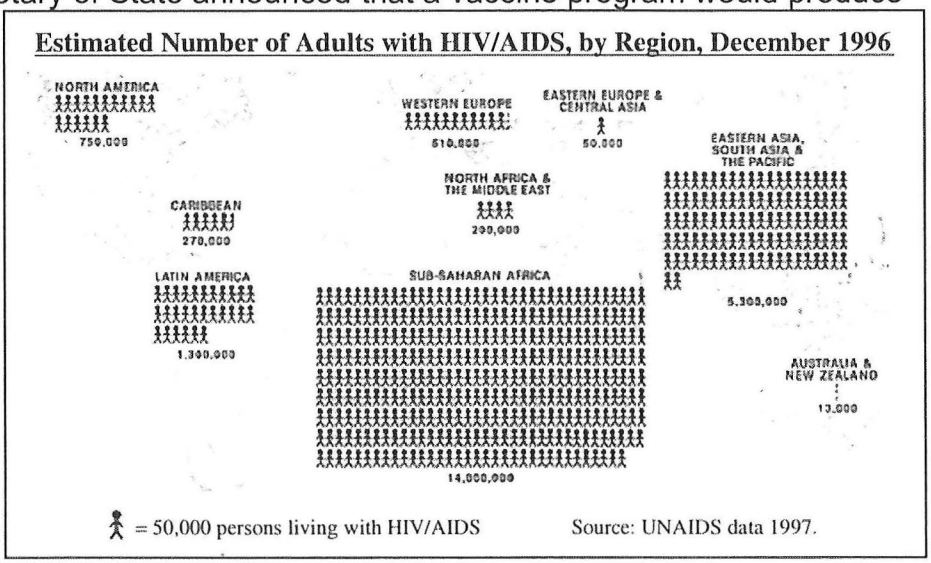
Interests:

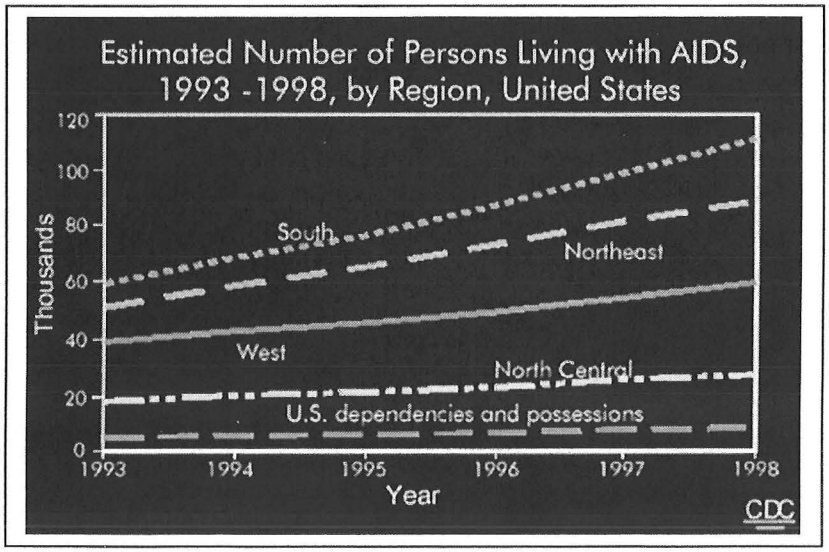
HIV Pathogenesis
Transcriptional Regulation
Host-Virus Interactions
HIV Therapeutics and Clinical Trials

I. A Persistent Pathogen of Enduring Importance

Since its explosive emergence in the early 1980's, the HIV pandemic has continued to defy expectations. First identified as a clinical entity by Gottlieb and colleagues in 1981 (Gottlieb et al. 1981), within two years the acquired immunodeficiency syndrome was associated with infection with a previously unknown retrovirus, human immunodeficiency virus type 1 (Barre-Sinoussi et al 1983, Gallo et al 1983). By 1985 the journal *Science* had published 108 papers describing in aspects of the genetics, molecular biology, virology and immunology of this newly recognized pathogen. At the time, when more than 1 million Americans were estimated to be HIV-infected, the U.S. Secretary of State announced that a vaccine program would produce an effective prophylactic vaccine in two years. A decade after this promise little progress had been made towards an effective vaccine, but potent antiviral chemotherapy capable of reversing immunodeficiency had become available.



As we note the passing of the 20th anniversary of the description of AIDS and the 15th anniversary of the licensing of AZT, more than 6 million people have died of AIDS, and more than 30 million are now living with HIV infection. WHO estimates that 100 million people will be infected with HIV in the next decade. According to UNAIDS, each day about 8,500 people, including 1,000 children, become newly infected. About 90 percent of these infections occur in developing countries, where the disease is likely to exacerbate poverty and inequality. However, it is clear that HIV will continue to impact those of us in the developed world throughout our careers in medicine.



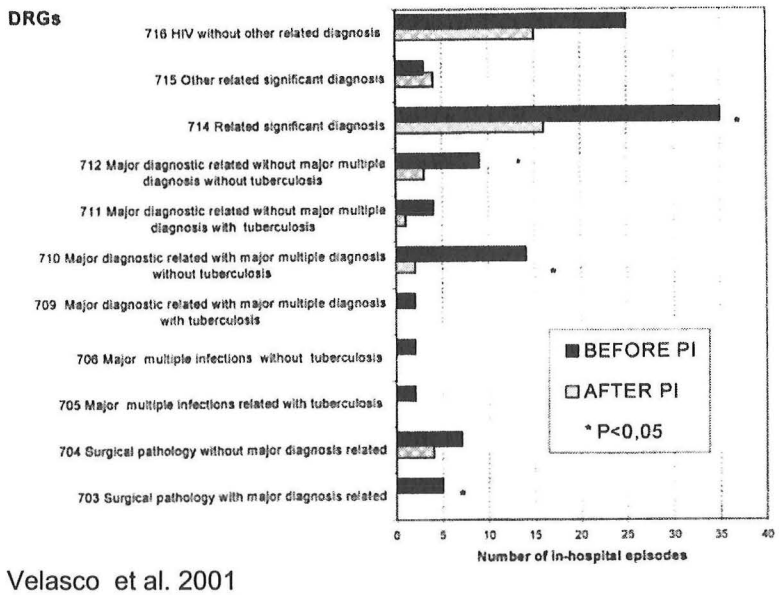
In Dallas County itself 14,207 cases of AIDS or HIV infection have been reported between 1999 and September of 2001, with 7480 people currently reported to be living following an HIV infection or AIDS diagnosis. The epidemic continues to grow at a modest pace here, with 1441 cases of AIDS or HIV infection reported in Dallas in 2000, and approximately 1700 projected in 2001 (TDH HIV/STD surveillance report, Q3 2001).

The 10 leading states or territories reporting the highest number of cumulative AIDS among residents as of June 2001 are as follows:

State/Territory	# of Cumulative AIDS Cases
New York	144,106
California	121,831
Florida	83,005
Texas	**55,292**
New Jersey	43,017
Illinois	25,665
Puerto Rico	25,459
Pennsylvania	25,264
Georgia	23,575
Maryland	22,432

CDC report 2001

A cost analysis performed in Chicago in 2001 found that the mean total cost for a year of HIV care was $21,367. The mean costs when stratified by CD4 count ranged from $31,519 to $14,824/year for patients with CD4 counts below 50/µl to those with counts above 500/µl. The annual cost of care for this 2800 patient cohort was $55.4 million (Roberts et al., 2001). As with other areas of medicine and infectious diseases, the cost of care is likely to increase over time due to new therapies and technologies used to manage drug-resistant HIV.



Velasco et al. 2001

HIV continues to persist and spread within human populations, and is expected to have continued impact on U.S. healthcare. It is therefore appropriate to re-examine current approaches to HIV infection in the light of the history of the medical approach to viral infections, and to consider the future of HIV therapies.

II. Medical Management of Viral Infection: Historical Paradigms

Vaccination

Historically, prophylactic vaccine approaches have been the most effective medical interventions for diseases due to viral infections. The vaccine era began when Edward Jenner observed that milkmaids exposed to cowpox were resistant to smallpox infection. This led to the medical use of vaccinia to induce cross-reactive immunity to

the closely related but more virulent pathogen. Both T-cell mediated responses and antibody production are thought to result from immunization, and prevent smallpox infection. The use of vaccinia preparations has been so successful that variola or smallpox was declared eradicated in the wild in 1980.

Poliovirus is another example of a viral pathogen that can be well controlled by vaccination. In the pre-vaccine era, more than 20,000 cases of paralytic polio were reported in the U.S. Live-attenuated or inactivated/killed polio vaccine can induce effective humoral immunity. No cases of wild-type polio have been reported in the U.S. since 1979. Given sufficient investment, the worldwide eradication of polio is thought be achievable within this decade (Children's Vaccine Initiative, 1997).

However, effective vaccines for other viral pathogens have been more difficult to develop. Despite the wide prevalence of asymptomatic herpes virus infection in humans, illustrating the ability of the human immune response to control herpes virus replication, vaccines to prevent herpes virus infection have not yet emerged. A varicella vaccine was approved in 1995 for use in healthy varicella-susceptible children and adults. While vaccination induces persistent neutralizing antibodies, it does not prevent infection but rather attenuates disease. In one study, 9% of vaccinees developed breakthrough chickenpox 8 weeks to 11.8 years after vaccination. However, breakthrough chickenpox was mild, even when vaccinees did not seroconvert or lost detectable antibody (Ampofo et al., 2002). On the other hand, vaccines for herpes simplex virus have thus far not been shown to be capable of preventing infection or ameliorating disease.

Similarly, there is little precedent for effective prophylactic vaccines against retroviral infection. Inactivated or subunit vaccines for ungulate lentiviruses such as the sheep pathogen visna, the equine infectious anemia virus, or the caprine arthritis/encephalitis virus have successfully ameliorated disease, but have not proved capable of preventing infection. The best effect so far has been seen with feline immunodeficiency virus, as more than 90% of cats immunized with killed FIV, an oncogenic retrovirus distantly related to HIV, can be protected from homologous but heterologous challenge (Elyar et al., 1997).

Antiviral Therapy

While vaccinia was used prophylactically in the 19th century, effective, safe, and practical pharmacologic antiviral therapy only emerged in the last few decades. Acyclovir, and the related antiherpesvirus drugs valacyclovir and famciclovir, may be considered the current paradigm of effective antiviral therapy. These agents are well tolerated, orally bioavailable drugs that are converted by herpesvirus thymidine kinase into an acyclic guanosine inhibitors of viral DNA polymerase. Initial or disseminated herpes simplex infection is now routinely treated, and recurrent herpetic outbreaks prevented with these agents. Although acyclovir therapy does not eradicate infection in this setting, minimal toxicity has been experienced by thousands of patients with recurrent symptomatic disease maintained on long-term suppressive acyclovir therapy

(Goldberg et al. 1993). Consideration has been given to allowing non-prescription sale of acyclovir.

III. Prevention of HIV Infection

Current Methods:

While the number of AIDS cases in the US is declining, the number of people living with HIV infection is growing. Increased prevalence of HIV in the population means that even more prevention efforts are needed. Past prevention efforts have resulted in behavior change for many individuals and have helped slow the epidemic. Many studies find that high-risk behaviors, especially unprotected sex, are continuing at far too high a rate.

Between 1998 and 200 the AIDS Prevention Project of the University of Texas Southwestern Medical Center at Dallas, as part of a nationwide project, surveyed gay and bisexual men between the ages of 23 and 29 in the Dallas area (personal comm. A. Freeman, UTSW). Despite the fact that HIV prevention efforts were in place in the community, the rate of HIV infection in the survey group was 18%, and 32% among African American men. More than half of the participants reported unprotected sex in the past six months, and nearly 25% reported a previous STD infection. While prevention efforts may have had some effect, it is clear that current intervention methods must be improved.

Secondary prevention of HIV infection is an important method not to be overlooked. Models have suggested that infection can be particularly widely spread by those with acute infection or advanced disease (DeGruttola et al., 1989, Downs et al., 1996). Therefore outreach to at-risk populations, coupled with effective testing and counseling programs offer the opportunity to identify infected patients, provide risk-reduction education, and decrease transmission of HIV infection.

Antiretroviral prophylaxis following an occupational exposure to HIV is a familiar concept to the medical community. As the rate of seroconversion following exposure is roughly 1:250, and the effect of prophylaxis incomplete, it is difficult to demonstrate an effect of prophylaxis. A very large observational database constructed in the 1990's suggested a reduction in risk of seroconversion when zidovudine (AZT) prophylaxis was prescribed after occupational exposure (Kahn, 1998). Combination antiretroviral therapy is now recommended following occupational exposure, based on expert panel recommendations. Two or more drugs are prescribed depending on the adjudged level of seroconversion risk (MMWR Recomm. Rep., 2001). Prophylaxis is surely effective in some settings, but due to tolerability issues the prescribed a four-week course of prophylaxis is completed by less than 50% of health care workers (Quirino et al., 2000). Neither is prophylaxis completely effective, as cases of the acute seroconversion syndrome have been reported following the completion of a course of prophylactic therapy (Hawkins et al., 2001).

Antiretroviral prophylaxis following sexual exposure may also be considered in some settings. Recommendations are based on rationale similar to that used for occupational prophylaxis. However, there is no data so far that addresses the efficacy of this approach, and is less widely practiced than occupational prophylaxis. Concerns have been voiced that the widespread use of prophylaxis following sexual exposure might encourage risky sexual behavior.

Finally, antiretroviral prophylaxis to prevent mother-to-child transmission has been the most succesful preventive intervention thus far, significantly decreasing the incidence of pediatric HIV infection in the U.S. Mother-to-child prophylaxis has been successfully implemented in pilot programs in resource-poor settings (Newell 2001, Peiperl 2001). Initially as chronic therapy was thought to be unavailable in such settings, the issue of the effect of short-term exposure to sub-optimal therapy was not problematic. As efforts to provide antiretroviral therapy in the undeveloped world expand, mother-to-child prophylaxis has become more challenging.

Prophylactic vaccines

Effective prophylactic vaccines for retroviruses such as HIV present a great challenge. In general, the high random mutational error rate of retroviral reverse transcriptase rapidly generates escape mutants to both neutralizing antibodies and cytotoxic T cell responses. Further, the ability of these viruses to remain integrated within the host genome with little or no expression of viral antigens impedes the clearance of virally infected cells. Finally, absence of effective mucosal immunity in the unexposed host may allow establishment of infection before a mature immune response can develop.

It is thought that an effective HIV vaccine should be capable of inducing both broad and persistent cytotoxic T cell immunity, and broadly neutralizing antibodies, and must be capable of protecting against mucosal challenge. In animal models of HIV infection, high doses of passively transferred neutralizing antibodies have prevented HIV infection via both parenteral and mucosal routes (Mascola et al 1999, Mascola et al 2000, Baba et al, 2000). However, it is unclear if such high titers of antibody could be persistently induced by immunization. Although CTL responses are known to be critical for the control of viral

HIV Vaccine Development: Key Advances in Fundamental Knowledge

- **Structure of HIV envelope**
- **Entry of HIV envelope into target cells**
- **Structure of broadly neutralizing antibodies**
- **Improved understanding of specificity and role of CTL in HIV/SIV infection**
- **Role of antibodies and CTL in animal model challenge studies**

from: AS Fauci 2001

replication, vaccines must induce CTL responses after challenge that are prompt and potent enough to completely protect from infection.

Protection against experimental HIV challenge has been achieved, although in experiments using homologous strains. Superinfection with divergent strains of HIV-1 is uncommon, suggesting that protective immunity can be induced, but this immunity does not prevent progressive immunodeficiency. Transient infection has been reported, as evidenced by HIV-specific CTL in the absence of infection, but these reports are controversial. Acquired resistance has been demonstrated in animal models with use of attenuated *Nef*-deleted live-virus vaccines, but progressive immunodeficiency has been reported in juvenille animals infected with these viral vaccines.

Overall, it is important to note that the immunological correlates of protection from HIV infection are still unknown. It is expected that the development of a vaccine with efficacy against the broad diversity of worldwide HIV clade and strain diversity will be very difficult. Driven by federal funding, international health organizations, and large private philanothropic foundations (notably the Gates Foundation) infrastructure for worldwide testing of HIV vaccines is developing. However, expansion of human clinical trials to meet the challenges of clinical-grade test vaccine production, and efficient prioritization and analysis of vaccine candidates will be difficult. In view of this array of scientific and practical challenges, there is currently little expectation in the field that a vaccine candidate with any likelihood of inducing protection from infection will be available for testing in the next 3-5 years. In fact, the vast majority of current clinical trials for HIV vaccines seek to test *therapeutic* vaccines, which would improve immune control of HIV replication once infection has occurred (discussed below).

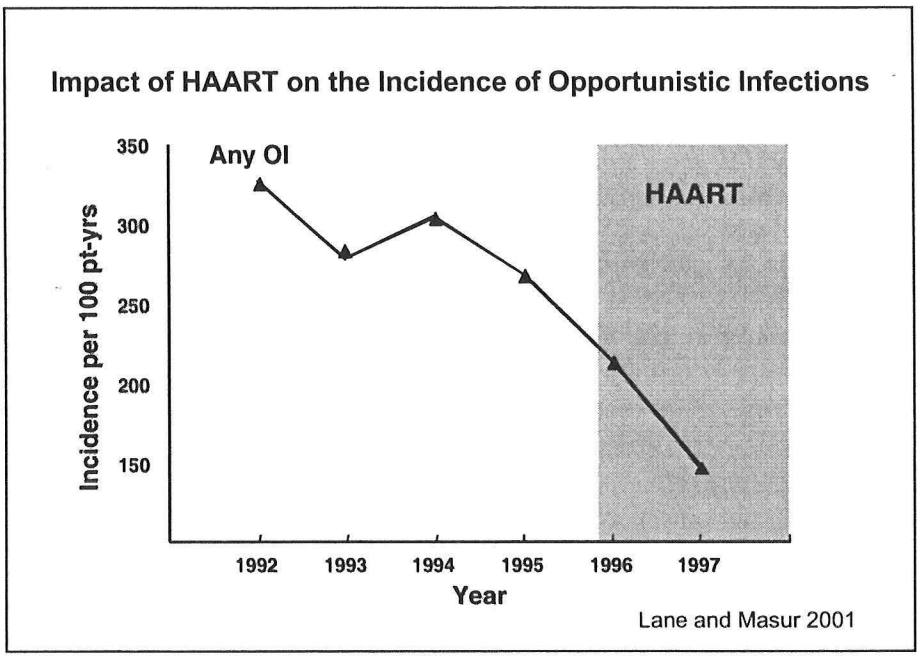
IV. Suppression of HIV Infection

Antiretroviral Therapy: the good

The development of potent combination antiretroviral therapy is one of the few encouraging highlights of the HIV pandemic. The growth of therapeutics from a single antiretroviral in 1987 to the current armamentarium of 16 drugs is unprecedented in medical history. HAART has resulted in striking decreases in AIDS-associated hospitalization and mortality. Growing evidence suggests clinically significant immune recovery occurs in the majority of HIV-infected patients treated with HAART in whom viremia is durably suppressed. Four observations suggest that increased CD4 counts are a marker of meaningful functional immune reconstitution (Gea-Banacloche and Lane, 1999):

1. The resolution of opportunistic infections previously refractory to treatment (e.g., oral candidiasis, molluscum, contagiosum, cryptosporidiosi/microsporidiosis, mycobacterium avium complex bacterium) and regression of Kaposi's sarcoma lesions and lymphoma.
2. Syndromes of unusual inflammatory immune responses against opportunistic pathogens such as mycobacterial lymphadenitis (Race et al, 1998).
3. Rarity of opportunistic infections

4. Successful discontinuation of prophylaxis or suppressive therapy for opportunistic infections



Recent healthcare analyses have estimated the value of antiretroviral therapy for HIV. Freedberg et al. (2001) calculated the ratio of lifetime cost of therapy to the increases in life expectancy. The population analyzed had advanced disease with a mean CD4 cell count of 87/μl. Life expectancy of patients receiving HAART increased from 1.97 to 3.51 years. When this estimate was adjusted for quality of life, the adjusted gain was from 1.53 to 2.91 years. For each quality-adjusted life years gained, the incremental cost of care was $23,000. Perhaps surprisingly, gains became <u>less</u> expensive when only patients with higher CD4 counts were analyzed. Patients with CD4 cell counts of 500/μl, the incremental costs of care per year of life gained were $13,000.

The finding that initial CD4 cell count and drug costs are the most important determinants of costs, clinical benefits, and cost-effectiveness confirmed the previous work from this institution (Keiser et al 2001). Using data from the Veteran's Administration, total costs of care decreased despite a significant increase in antiretroviral cost. Decrease in inpatient utilization outweighed pharmacy costs. It has been estimated that HAART is more cost-effective than other common medical treatments, such as radiation therapy for early-stage breast cancer and treatments for hypercholesterolemia.

<u>Antiretroviral Therapy: the bad and the ugly</u>

Despite the great benefits wrought by HAART, the 2001 revision of the National committee's recommendation for antiretroviral therapy reflected a growing recognition in the field of the shortcomings of long-term therapy. Current guidelines now suggest that therapy be deferred in most patients until significant T cell depletion occurs. Two commonly encountered problems have led to this change in approach: the induction of antiretroviral resistance, and long-term antiretroviral toxicity.

Several categories of long-term antiretroviral toxicity have been recognized. Neuropathy, myopathy, pancreatitis, hepatic steatosis, lactic acidosis, and fat wasting or

lipoatrophy have been primarily associated with nucleoside analog reverse transcriptase inhibitors. Metabolic complications such as the fat redistribution or lipodystrophy syndrome, insulin resistance, hyperlipidemia, and bone disease (osteopenia and/or osteoporosis) are commonly thought to relate primarily to the use of protease inhibitors, with a variable contribution by nucleoside analog reverse transcriptase inhibitors. HIV infection itself is also likely to contribute to some of these abnormalities, as some had been seen prior to the advent of HAART (Powderley, 2002).

Updated DHHS Guidelines, Feb 2001: Initiation of ART in the Chronically HIV-Infected Patient

Clinical Category	CD4+ (cells/μl)	Plasma HIV RNA	Recommend
Symptomatic (AIDS, severe symptoms)	**Any**	**Any**	**Treat**
Asymptomatic AIDS	**<200**	**Any**	**Treat**
Asymptomatic AIDS	**200-350**	**Any**	**Offer treatment?**
Asymptomatic AIDS	**>350**	**>55,000**	**Offer treatment?**
Asymptomatic AIDS	**>350**	**<55,000**	**Defer treatment?**

The conservative use of HAART has also come about as the challenges of long-term adherence to HAART are recognized. In most patients, given the pharmacodynamics of current therapies, studies suggest that adherence to 95% of prescribed doses is needed to avoid resistance. In the context of a simple BID regimen, this translates into adherence to 13 of 14 doses per week. Population surveys suggest that the incidence of antiretroviral resistance is increasing in primary infection (Little 2001) and that more than 50% of patients on therapy had evidence of resistance to at least one class of antiretrovirals. The increasing need for multiagent therapy to treat drug-resistant HIV, and the cost of clinical drug resistance assays to guide therapy threatens to erase both the clinical and economic benefits gained by HAART.

Immunotherapy: IL2

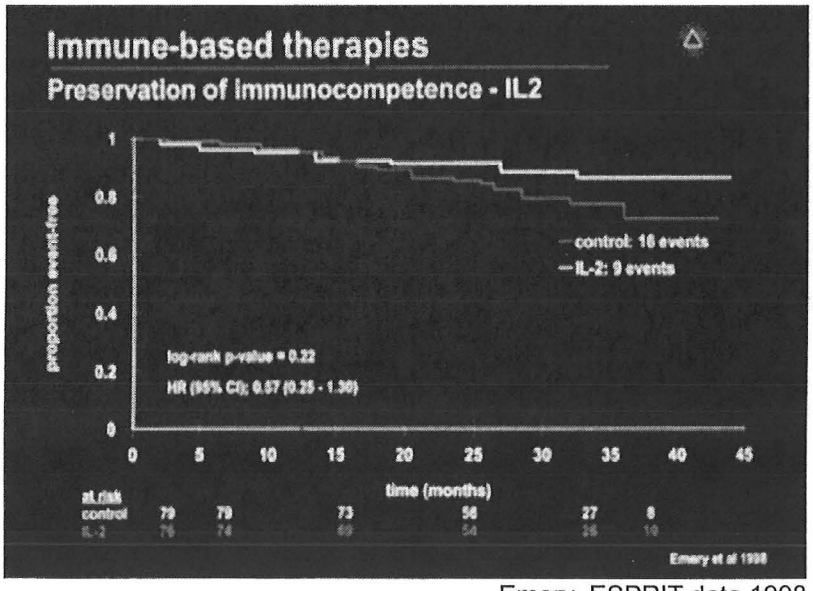
A variety of attempts to suppress HIV replication and ameliorate HIV disease via immunomodulatory therapy have been explored. Studies of interleukin 2 have the longest record and greatest success thus far (Mitsuyasu 2001). A variety of studies performed in different patient populations and clinical settings, have demonstrated that, when coupled with antiviral therapy, adjunctive IL-2 therapy results in substantial rises in CD4 cell count (Emery et al. 2000). The side effects of IL2, such as nausea, vomiting, fever, and fatigue, are well known.

The largest randomized study of IL2 in advanced HIV disease is the AIDS Clinical Trials Group 328 study, a study of the effects of HAART with or without continuous IV (CIV) or subcutaneous (SC) IL-2. Protease inhibitor-naive patients with CD4 cell counts 50 to 350/µl were treated with the protease inhibitor indinavir and two nucleoside reverse transcriptase inhibitors for 12 weeks (HAART). Patients with HIV RNA <5000 c/ml after receiving 12 weeks of HAART were randomized to continued HAART (n = 52), HAART + CIV IL-2 at 9 million units (MIU) qd for 5 days q8wks (n = 54), or HAART + SC IL-2 at 7.5 MIU bid for 5 days q8wks (n = 55) for up to 84 wks. As allowed by protocol, most patients on CIV (76%) switched to SC IL-2 after 3 or 6 cycles of IL-2.

While CD4 counts rose progressively on HAART alone, increases were substantially larger in patients receiving IL-2. There was no overall change in HIV RNA across the arms of the study. Median CD4 at week 84 and changes from baseline to wk 84 were as follows:

	HAART	HAART & CIV IL2	HAART & SC IL2
Median CD4/µl week 84	396	800	614
Change CD4/µl week 0-84	121	480	302
% subjects with >50% rise CD4	41	86	77

Significant increases in CD4 counts were seen with both CIV and SC IL-2 compared to HAART alone after 60 wks of therapy, which continued to increase and grow larger to week 84. Only 1 AIDS-related infection was seen in both of the IL-2 arms, and 5 in the HAART arm, giving a hint that IL2 may have clinical benefits, but direct proof of this still awaits the completion of ongoing clinical endpoint studies.



Emery, ESPRIT data 1998

Overall, evidence continues to accumulate that intermittent IL2 can be tolerated and that virologic suppression can be maintained on IL2, if

not aided by it. The overarching question is whether or not one is better off living with a T cell count of 1000 or 500, given the cost and side effect profile of IL2. Whether this therapy is worth its cost and toxicity awaits the outcome of ongoing clinical endpoint studies in both early (ESPRIT) and later-stage patients (SILCAAT). If these studies are favorable when they reach their endpoints in 2004 or 2005, the broad experience outlined will validate the use of IL2 in a variety of settings.

Immunotherapy: vaccines as another drug

Therapeutic vaccination is currently an area of intense investment and investigation. More than 70 phase I (dose-escalation safety and toxicity), five phase II (expanded safety and dose optimization) and two phase III (efficacy) clinical trials involving more than 3500 subjects worldwide have evaluated the safety and immunogenicity of HIV vaccines (Nabel 2001). A variety of recombinant gp120/140 envelope proteins, V3 peptide-based vaccines, vaccinia and canarypox vectors, and a combination of poxvirus vectors and envelope sub-units in prime-boost immunization schedules have been and are being tested.

As discussed above, data thus far suggests that a vaccine capable of inducing sterilizing immunity or complete protection from retroviral infection may be difficult or impossible to develop. Indeed, a vaccine that induced incompletely protective immunity might do more harm than good if it encouraged risky behaviors in vacinees. However, due to the cost and potential long-term toxicities of antiretrovirals, and the global deficiencies in health infrastructure, a vaccine that improved the immune response, decreased viremia, and slowed disease progression could confer many of the same benefits as antiviral therapy.

It is hypothesized that vaccination of an aviremic individual receiving HAART may allow the restoration and/or broadening of HIV-specific cell-mediated immunity. Once induced, such immunity may contain viral replication and delay viral rebound if HAART is discontinued. A recent pilot study (Rosenberg et al, 2000) found a transient increase in viral load observed after cessation of therapy in patients treated early after acute infection, and that CD8+ T-cell responses were augmented. Long-term follow-up of 14 such subjects with acute infection treated with highly active antiretroviral therapy (HAART) who then underwent treatment interruption demonstrated that 6 maintained persistent control of HIV viremia out to day 600, suggesting more durable immune control of the virus and cell-mediated immune responses against HIV-1 antigens. Two other clinical studies demonstrate the favorable effect of early ART on antiviral cellular immunity, particularly the preservation of CD4+ T helper cell responses (Oxenius et al. 2000, Malhotra et al., 2000). As early HAART is not feasible for most acutely infected humans, a similar beneficial clinical effect might be induced by an HIV-1 vaccine priming for immune responses that preserve host CD4 + T cells.

Somewhat encouraging data supporting this hypothesis has been reported from SHIV challenge studies in vaccinated primates (Barouch et al, 2000). Macaques immunized with plasmid DNA vaccines or DNA with pox virus vector vaccines, generated CTL and neutralizing antibody responses to the immunogens. CD4+ cell

counts were maintained along with partial containment of virus replication in all cases, with durability of effect lasting out to 1.5 years. Animals receiving plasmid DNA vaccines (gag, pol, env) with cytokine augmentation (IL-2) appeared to have greater viral suppression and longer survival than those given vaccine alone. Durability of response and suppression of viremia exceeded 1.5 years. These studies suggest that a vaccine that generates HIV-specific CTL responses in humans could similarly protect against viral replication and HIV disease progression. However, it should be noted that in a follow-up report, the same group has recently reported the emergence of a single nucleotide mutation within an immunodominant Gag CTL epitope resulted in a burst of viral replication, clinical disease progression, immunodeficiency, and death (Barouch et al 2002).

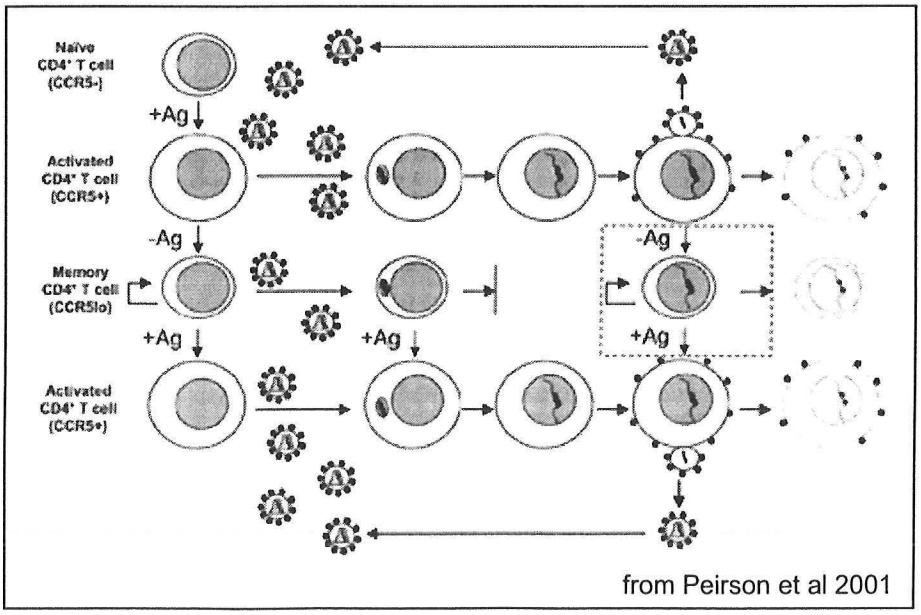
V. Eradication

With the development of HAART, it was theorized that sufficiently potent antiretroviral therapy might effect eradication of HIV infection (Perelson et al. 1997). However, reservoirs of HIV-1 have been identified that represent major impediments to achieving eradication. Clinical experiments to clear HIV infection have so far been unsuccessful. On the other hand, many hurdles are yet to be overcome in the prevention of HIV infection. The containment of HIV replication over decades of life is likely to be fraught with difficulty and cost. Therefore a careful re-evaluation of the prospects for clearing HIV infection is indicated.

Within a HIV-seropositive patient, millions of CD4+ cells are infected each day (Embertson et al 1993, Ho et al., 1995) resulting in:

1) integrated provirus producing viral particles and proteins that result in viral- or host-mediated cell death
2) unintegrated proviral DNA, or rarely
3) stably integrated provirus with little or no viral gene expression.

The third state, sometimes called latency, has been quantitated in peripheral blood and lymphoid tissues of HIV-infected subjects. Some integrated proviral species within resting cells may be defective or integrated into quiescent regions of host chromatin, and may be incompetent for viral production.



from Peirson et al 2001

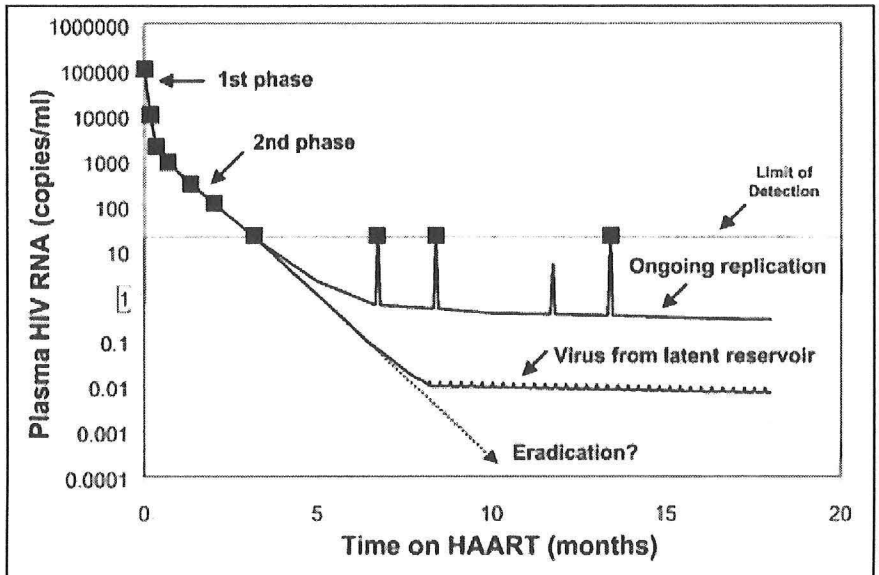
However, upon appropriate stimulation provirus within resting cells is capable of reverting to the productive state in vivo, as it can in vitro (Chun et al, 1995, Chun et al, 1997, Finzi et al, 1997, Wong et al, 1997). These rare cellular reservoirs are unaffected by current antiretroviral therapy and unrecognized by the host immune response.

While antiretroviral drugs allow significant inhibition of active HIV-1 replication, it is clear that the persistence of low-level HIV replication in the face of what is now called HAART is related in part to inadequate drug potency, suboptimal antiviral pharmacodynamics, and evasion of the immune response. Expected advances in drug therapy are likely to improve the tolerability and efficacy of HIV therapy. In the setting of future therapy, rational therapeutic approaches to the persistent provirus might allow the clearance of HIV infection. Conceptually, time-limited but intensive therapy designed to achieve clearance might be more tolerable and cost-effective than life-long suppressive therapy.

When viremia is suppressed by HAART, 3 phases of plasma virus decay are observed (Pierson et al 2001):

1. the first phase: half-life less than 1 day, representative of infection in activated cells
2. the second phase, half-life ca. 14 days, representative of production of HIV in macrophages and sub-maximally activated T cells, and
3. a third phase, half-life 16-44 months, representative of virus production from resting CD4 T cells.

In addition to infected cells that are long-lived, anatomic or pharmacologic reservoirs may allow persistent active viral replication at sites protected from the immune response or antiviral therapy. The key anatomic and pharmacologic reservoirs for HIV-1 appear to be the central nervous system, the gastrointestinal mucosa, and the genital tract.



from Peirson et al 2001

Clinical Attempts at Eradication

In 1998 a cohort of patients on HAART plus subcutaneous IL-2 were tested for the presence of latently infected cells. In a minority of the patients (3 of 14), virus could not be isolated from 10 million purified resting CD4+ T cells, suggesting a very low

frequency of latently infected cells (Chun et al 1999). It was hypothesized that the use of IL2 had induced activation of potentially productive resting T cells, and "drained" the reservoir of virus. However, upon cessation of HAART viral rebound was observed within 2-6 weeks.

Subsequently, as small cohort of subjects were treated with antiretrovirals, IL-2 and 5 days of OKT3 antibodies, in an attempt to activate and purge infected cells while simultaneously blocking new infection. The high dose of OKT3 used induced severe toxicities, including the pulmonary leak syndrome, and control of viral replication was not achieved (Prins et al, 1999).

Another attempt at eradication was recently reported at the annual Conference on Retroviruses and Opportunistic Infections (Pomerantz et al., 2002). 3 patients with HIV-1 infection on HAART, with less than 50 copies/mL of plasma viral RNA for more than 1 year, added DDI and hydroxyurea to intensify their therapy. After at least a month of therapy, the patients were with low dose OKT3 antibody (400 mg), followed by two weeks of subcutaneous IL-2 to stimulate latent provirus. The dose of OKT3 used (400 mg) stimulates T-cells rather than depleting these cells in vivo. All treated patients had only modest side effects. The HU and DDI were continued with HAART throughout this protocol, and for 5-6 months after OKT3 and IL-2 stimulation. Replication competent virus was undetectable after treatment and plasma viral RNA also became undetectable in each of these patients, using an ultrasensitive RT-PCR assay that detects 5 copies/ml of HIV RNA. Tonsillar biopsies were performed and no in situ hybridization for HIV-1-specific RNA was detected in lymphocytes or on follicular dendritic cells. After stopping all antiretroviral therapy, plasma viremia recurred after four weeks. Although this trial was unsuccessful, it should be noted that no serious toxicities were incurred and patients are currently stable on HAART.

New strategies for HIV Eradication

Some investigators have hypothesized that the major hurdle to clearance of HIV infection is the HAART is not highly active enough. Ramratnam and colleagues (2000) found that replication competent virus in resting CD4 T cells had a half life of only 6.3 months in highly adherent patients who maintained plasma HIV RNA of less than 50 copies/ml. Studies are in development to measure the decay of virus within the resting cell pool in patients on maximally potent therapy who add an investigational drug that inhibits viral fusion to their regimen.

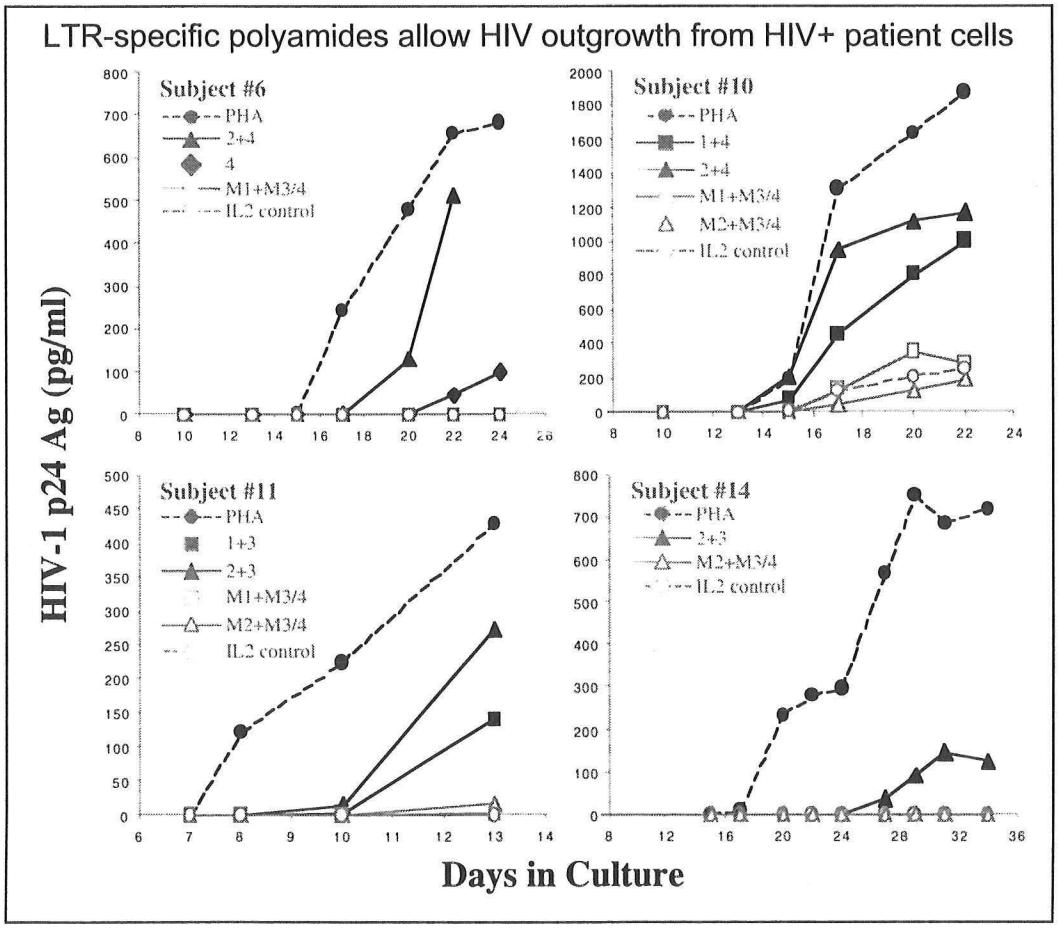
Vitetta and colleagues at UTSW have proposed to directly target resting CD4 cells within an infected individual on HAART with an immunotoxin molecule directed against CD45RO. When modeled in vitro, this approach was successful in eliminating HIV from primary cells in culture (Saavedra-Lozano et al., 2002).

Prostratin is a non-tumor promoting phorbol ester which inhibits de novo human immunodeficiency virus type-1 (HIV-1) infection at some concentrations through the down-regulation of CCR5 HIV co-receptor expression (Kulkosky et al 2002). Prostratin can also upregulate expression of latent proviral HIV genomes DNA by induction HIV

promoter expression. Prostratin can induce expression of HIV-1 from purified populations of cells derived from patient PBMCs on virally suppressive HAART. Studies are underway to develop prostratin for use in humans to induce the expression of latent provirus.

Our laboratory has studied the molecular mechanisms through which HIV may remain silent within resting CD4 cells. We have shown that the human transcription factors YY1 and LSF cooperate in repression of transcription from the HIV-1 LTR. LSF recruits YY1 to the LTR, and YY1 in turn recruits histone deacetylase 1(Margolis and Green, 1994; Romerio et al, 1997; Coull et al. 2000; He and Margolis 2002). The modulation of histone architecture within the host genome is required for activation of HIV gene expression, and is likely to regulate HIV quiescence. The integrated LTR is unresponsive to activation by NF-κB prior to histone acetylation, implying a role for histone deacetylase in establishing or maintaining quiescence (El-Kharroubi et al. 1998).

We have sought definitive evidence for the role of host factors in maintaining the quiescence of HIV within resting T cells through experiments which selectively block repressor function in primary cells from patients. Pyrrole-imidazole polyamides are synthetic small molecules containing N-methylpyrrole and N-methylimidazole amino acids, designed to bind to specific DNA sequences with affinities comparable to those of natural DNA-binding transcriptional regulatory proteins (Bremer et al. 1998; Dickinson et al. 1999). Polyamides have been shown to be capable of modulating gene expression by preventing the binding of transcriptional activators or



repressors *in vitro*. We found that HIV outgrowth was induced by polyamides that inhibit LSF binding (Coull et al 2001). These findings suggest that host factors which inhibit HIV gene expression may be a valid therapeutic target to "unmask" the latent reservoir of replication-competent virus, and allow clearance of infection in concert with an intact immune response and potent antiviral therapy.

V. Conclusion

In almost every aspect, the HIV pandemic present both enormous challenges and opportunities. The field has moved so far and so rapidly, but not as rapidly as the virus. Improvements in prevention efforts can and must be made both domestically and internationally, especially as a prophylactic vaccine will not be available in the near future. Antiretroviral therapy will continue to be a central part of care for those with infection, and must address the complex and interrelated issues of access, adherence, resistance, and long-term toxicity. Immunotherapies, in particular vaccines which ameliorate disease progression, may be available within the next decade. Finally, the possibility of eradication of HIV infection should not be discarded, as the obstacles to this goal appear no less daunting than the obstacles long-term therapy.

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