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HIV EVOLUTION AND ESCAPE

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ABSTRACT

Human immunodeficiency virus (HIV) exemplifies the principles of Darwinian evolution with a telescoped chronology. Because of its high mutation rate and remarkably high rates of replication, evolution can be appreciated over periods of days in contrast to the durations conceived of by Darwin. Certain selective pressures that drive the evolution of HIV include chemotherapy, anatomic compartmentalization and the immune response. Examples of these selective forces on HIV evolution are described.

INTRODUCTION

> "Natural selection is daily and hourly scrutinising, throughout the world, the slightest variations; rejecting those that are bad, preserving and adding up all that are good; silently and insensibly working, *whenever and wherever opportunity offers*. . . .. We see nothing of these slow changes in progress, until the hand of time has marked the lapse of ages, and the so imperfect is our view into long-past geological ages, that we see only that the forms of life are now different from what they formerly were."
> —*Darwin, "On the Origin of Species"* (1)

Charles Darwin with astute observation of the natural world and perspicacious insight proposed his theory of evolution by means of natural selection. The principles of Darwinian evolution were succinctly summarized in a brilliant review of a new edition of *On the Origin of Species* by Robert Pollack in 1997 (2) (Figure 1). Darwin generated these principles almost 150 years ago with neither a glim-

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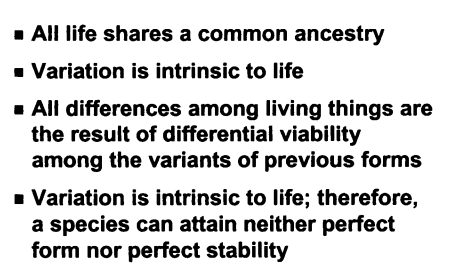

FIG. 1. Principles of Darwinian Evolution derived from reference 2.

mer of appreciation about the existence of nucleic acid nor its basis for genetic information. Nevertheless his insights have stood the test of time. The role (or opportunity) for those of us following his footsteps is to embellish his theory and to generate supportive documentation.

One application of his theory, which Darwin could not have foreseen, is the microbial world. Microbes outnumber us multicellular creatures both in numbers of species and population size. They also have more rapid replication rates. As a result they evolve more quickly. As Pollack observed, "The strategy our ancestors have followed since they first assembled into multicellular creatures bets on genetic stability and complexity to create a species made up of individuals, each with a reasonable chance of survival. The microbial strategy takes the opposite tack. Their genetic simplicity and malleability allow them to discard almost all progeny, always leaving a few genetic variants to survive any contingency." (2)

The introduction of human immunodeficiency virus (HIV) into the human species in the past century (3) has become a global scourge promising to be the primary infectious cause of mortality for years to come (4). Its success as a pathogen and in evading therapeutic and vaccination strategies is largely attributable to its ability to accelerate Darwinian evolution. HIV, like all microbes with single stranded RNA genomes, replicates with the high mutation rate of approximately one nucleotide change per genome per replication cycle (5,6). This is attributable to a reverse transcriptase enzyme with poor fidelity and the lack of proof reading mechanisms which are available to eukaryotic DNA. The rate of evolution of HIV is accelerated by a prodigious rate of replication with 10^{10}–10^{11} virus particles generated daily in each infected individual (7), thus every possible mutation and many combinations of multiple mutations are generated on a daily basis. Moreover, this high rate of mutation and high rate of replication is occurring today in close to 50 million people (4).

Much translational research on HIV has proven to represent the need to deal with the capacity of the virus to adapt to selection pressures. My colleagues and I have been investigating the evolution of HIV in response to three selective pressures: chemotherapy, anatomic compartments and neutralizing antibody. Selected aspects of these investigations are summarized.

HIV DRUG RESISTANCE

Following the clinical trials with zidovudine (AZT), the first drug shown to have antiretroviral activity (8,9), we examined serial isolates from patients receiving AZT monotherapy. Progressive incremental reductions in AZT susceptibility occurred in these isolates (Figure 2). These increases in drug resistance were later shown to be attributable to the cumulative acquisition of mutations in reverse transcriptase, the gene for the target enzyme (10,11). This observation is consistent with the adaptation of the virus during ongoing replication in the presence of the selective pressure of active drug. In 1996, we proposed that in the absence of selective drug pressure the probability of a resistant population emerging as predominant is negligible (Figure 3) (12). As the selective pressure of increasing drug activity is imposed, the probability of a resistant population emerging increases (13). At yet higher

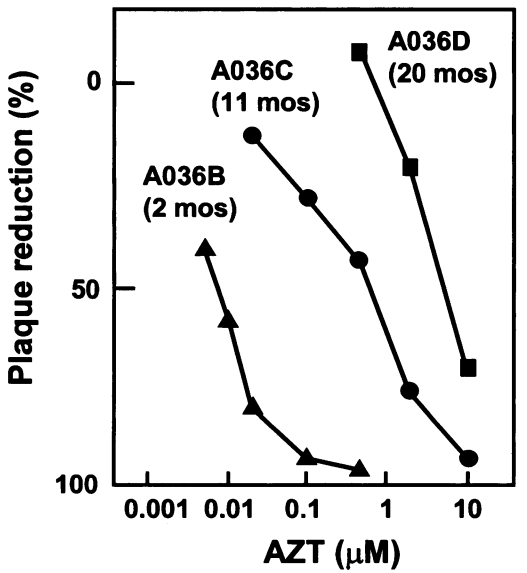

FIG. 2. Zidovudine (AZT) susceptibility of sequential isolates of HIV-1 from a patient administered AZT (from reference 10).

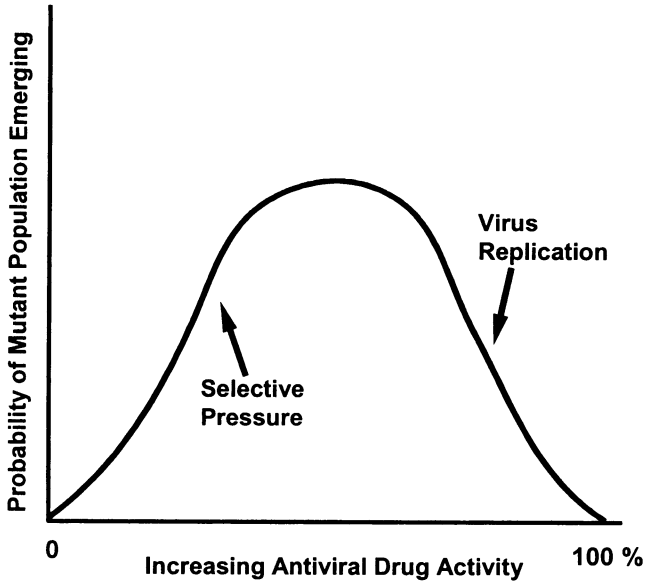

FIG. 3. Proposed theoretical relationship between antiviral drug activity and the probability of the emergence of a drug resistant population. The opposing factors of selective drug pressure and rates of virus replication are indicated (from reference 12).

levels of selective antiviral pressure replication is sufficiently restricted to diminish the likelihood of outgrowth of a resistant population. Finally, when replication is completely suppressed, although selective drug pressure is high, resistant mutants cannot emerge. This proposal was not confirmed until potent combination chemotherapy could be designed, which permitted the dramatic suppression of plasma HIV RNA below the levels of detection of standard assays (Figure 4) (14,15).

Over the past several years testing for HIV drug resistance has become the standard of practice in the management of the chemotherapy of HIV patients and their drug regimens (16–18). As with much antimicrobial chemotherapy, the selection for widespread resistance has become a consequence of the success of these drugs. Similarly transmission of drug resistance to newly infected patients is becoming all too common (19).

ANATOMIC COMPARTMENTS

The composition of cell types, the distribution of cellular and humoral immune responses, and drug disposition differ between the

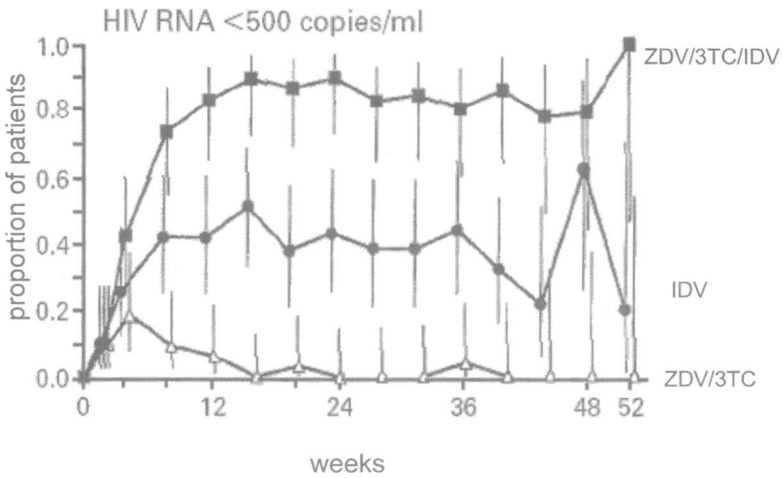

FIG. 4. The suppression of HIV replication in patients receiving potent combination antiretroviral therapy. Patients were randomized to zidovudine plus lamivudine ZDV/3TC which was the standard of care in 1995, to indinavir (IDV) an investigational protease inhibitor, or all 3 drugs. The proportion of patients achieving the virological endpoint of plasma HIV RNA being below the limits of detection is depicted (from reference 14).

circulation and anatomic compartments. The central nervous system (CNS) is important both as a target for HIV pathology and as a drug, and possibly an immunologic, sanctuary. The genital tract is important as the primary source of new HIV transmissions.

Since the early 1990s, investigators have described distinctive envelope HIV sequences in the CNS compared to the circulation among patients with established disease (20–24). This contrasts with subjects studied during primary infection when partial sequence of the viral envelope from CSF and blood are indistinguishable (unpublished data) consistent with the transmission of a single genetic variant during primary infection and the subsequent independent evolution of virus populations during the course of infection. The selection for these variants could be distinctive host cell tropism or possibly differential immune selection. Doms and colleagues observed a trade-off between resistance of HIV variants to antibody neutralization and their ability to infect cells with a low density of the primary or secondary viral receptors (25,26). This observation may explain the intra-host evolution of HIV in the CNS where putative target cells, the microglia and to lesser extent astrocytes and microvascular endothelial cells, show low primary and secondary receptor expression while effective neutral-

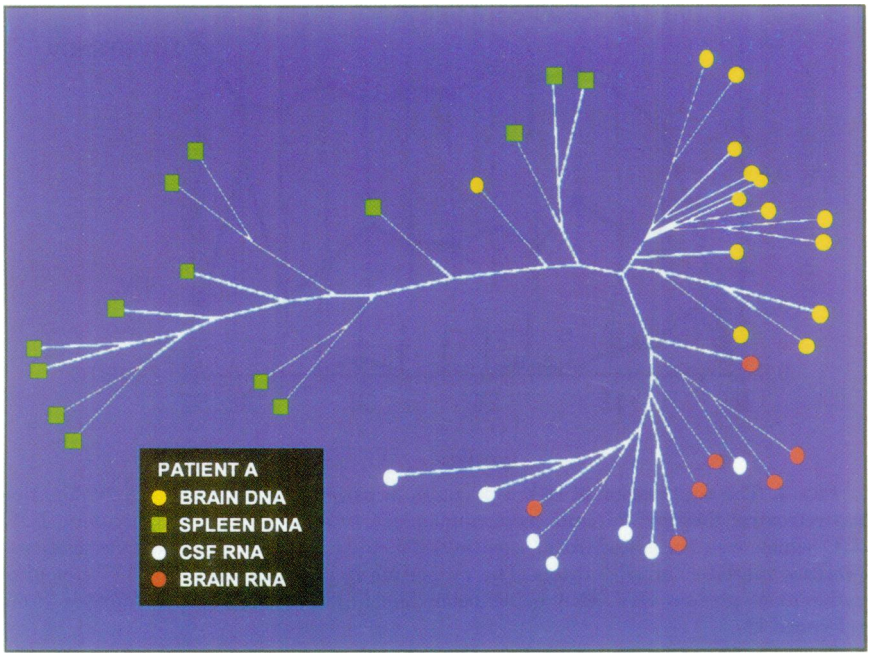

FIG. 5. The anatomic compartmentalization of genetic variants examining the sequence of the reverse transcriptase of HIV is depicted. In this unrooted phylogenetic tree variants found in the lymphoid and central nervous systems have evolved independently (from reference 30).

izing antibody concentrations may differ from that in the systematic circulation (27,28).

Variable penetration of antiretroviral drugs into the CNS constitutes an additional and potentially clinically important selective pressure shaping viral evolution (29). Wong et al examined autopsy tissues, including brain, from 4 patients previously on failing antiretroviral therapy and found that genetic resistance patterns differed between virus populations in brain and those in lymph node and spleen (Figure 5) (30). Examples were found of the discordant absence of some resistance conferring mutations in brain when non-CNS-penetrating antiretrovirals were used and of the discordant persistence of some mutants in brain from past drug exposure, documenting the role of both the imposition and withdrawal of antiretroviral drug selection on the evolution of reverse transcriptase in the CNS (30). Recently, several groups have observed that the prevalence of discordant resistance between virus in cerebrospinal fluid and plasma approach 50% in selected patient populations (31,32). These studies provide some in-

sight into the complexity of viral evolution in vivo but there is clearly a need for more systematic and longitudinal studies in the future to better understand the rates and determinants of viral genetic divergence in the CNS and their impact on disease progression and treatment response.

Similar to the central nervous system, the male genital tract is an anatomic compartment with a distinct hormonal, cytokine and immunologic milieu, which is specific for the development and maturation of sperm (33–35). This specialized environment also shapes the viral population that is harbored there. Sequence analysis has revealed that in some individuals, virus extracted from seminal plasma is similar to that found in blood plasma, while in others it differs, with few correlates to indicate why (35–39). It has been suggested that free virus may be exchanged between the blood and genital tract compartments while a subpopulation replicates in cells that are specific to the tissues of the genital tract (34,39–41). Reasons for a viral population that is specific for the genital tract could include adaptations that enhance interactions with cells important in the infection process such as dendritic cells. These cells have recently been shown to express a lectin, DC-SIGN (42) that interacts with HIV envelope and potentiates infection. Currently, we are using machine learning and phylogenetic techniques (43) in an attempt to identify conserved genetic regions of HIV derived from semen. Identification of such "sequence signatures" may have significant bearing on future vaccine development, since the vast majority of new HIV infections occur through exposure to HIV contained in genital secretions (40).

The male genital tract is not a homogenous compartment. It is comprised of many different tissues contributing fluids and cells to male genital secretions. Each of these tissues may represent subcompartments with their own environments for the development of HIV, including differential pharmacologic penetration (38,40,44). Since many antimicrobial medications do not penetrate well into the fibromuscular prostate, allowing bacterial and fungal pathogens to be harbored there, we investigated it as a reservoir for HIV when individuals are treated with antiretrovirals (39,45). Individuals who were treated with antiretroviral medications and had HIV RNA viral loads that were below the level of detection in the blood (<50 copies/ml) were also undetectable in their seminal plasma (<25 copies/ml) except when their prostate was stimulated by a digital massage before ejaculation (46). The recovery of cell free virus only after prostate stimulation implicated the prostate as a sanctuary for HIV during antiretroviral treatment, which could have significant transmission implications in both treated and untreated individuals (39).

NEUTRALIZING ANTIBODY

The protective efficacy of most viral vaccines correlates with the elicitation of neutralizing antibody (47). The induction of neutralizing antibody to HIV by a candidate vaccine has been hampered by the inability to design immunogens that induce neutralizing antibody to most primary isolates of HIV (48). Even the role of neutralizing antibody in the natural history of HIV infection has been difficult to characterize because assays for neutralizing antibody have been labor intensive, slow and imprecise and the isolation of primary isolates from each patient to assay autologous antibody responses is slow, expensive and often difficult to achieve.

The development of an assay for neutralizing antibody, which is precise, applicable to virus from the plasma of patients, and amenable to high throughput, has permitted us to characterize the neutralizing antibody response of patients with HIV infection (49). A recombinant virus assay initially developed to measure antiretroviral drug resistance during a single round of virus replication was adapted to measure virus entry and its inhibition by neutralizing antibody (50). Briefly, full length envelope is amplified from plasma HIV RNA and co-transfected in an expression vector with an HIV-genomic vector deleted in envelope and expressing an indicator firefly luciferase gene. The pseudovirions expressing patient HIV envelope that are produced are used in an assay for neutralizing antibody using serial dilutions of patient plasma. The inhibition of infectivity is measured by reduction of luciferase activity.

We first investigated 14 study subjects who presented to the San Diego Acute and Early Infection Disease Research Program 30–65 days after their estimated date of HIV infection and who elected to defer or delay antiretroviral therapy. Plasma samples (3–13 per patient) were obtained at presentation to the clinic and at regular intervals for 6–39 months of follow-up. Autologous envelope-antibody pairs were assessed to characterize the development and evolution of autologous neutralizing antibody. Figure 6 demonstrates the ability of this assay to detect the emergence of autologous neutralization activity directed against the virus present at presentation of primary HIV infection (month 0) and in serial plasma samples (0, 6, and 12 months).

The neutralization activities of sequential plasma samples against sequential virus envelope proteins from the same patient (autologous responses) or against two reference viruses (heterologous responses), are displayed in Figure 7. This patient exemplified 12 of 14 patients who developed appreciable neutralizing antibody responses shortly after HIV infection. Of note each sequential virus tested escaped the

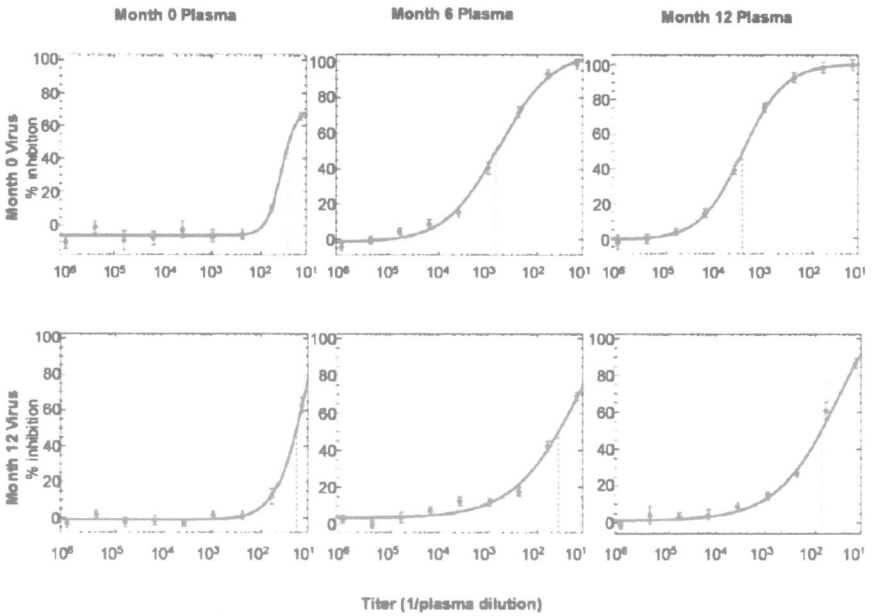

FIG. 6. Neutralization of autologous HIV. The neutralizing activity of plasmas obtained from an untreated patient 0, 6 and 12 months after presentation with primary infection is assayed against virus from months 0 and 12. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus replication (dashed lines). The error at each dilution reflects the standard error of duplicate wells (from reference 49).

concurrent neutralizing antibody response in that patient. The neutralizing antibody responses to early viruses were continually boosted as an example of Original Antigenic Sin (51,52).

There was no relationship between the neutralizing antibody response and the disease course. The magnitude of the neutralizing antibody response correlated neither with steady state plasma HIV RNA level nor with CD4 cell count in the year after infection. Thus the neutralizing antibody response does not significantly restrict HIV replication. Rather it is an immunologic response to a constantly escaping antigen. In fact these rates exceed those observed with the emergence of HIV drug resistance mutations. Immunoglobin has a half life of three to four weeks while HIV virions in the plasma have a half life of hours (7). Studies in progress suggest that the rate of evolution in the envelope gene of HIV is determined by the magnitude of each patient's neutralizing antibody response. These observations further support the argument that the magnitude of the neutralizing antibody re-

Virus (months)	Plasma (months)								
	0	3	6	9	12	15	18	21	25
0	26	219	675	1403	2670	2089	2190	2363	2411
3	29	179	1024	2151	3733	3152	2808	2953	3086
6	27	35	78	358	1769	1939	2247	3112	4345
9	36	67	82	200	795	1078	1371	2208	3375
12	19	48	36	64	76	166	556	937	1407
15	29	43	64	76	90	119	374	721	1234
18	42	65	61	152	117	134	122	289	526
21	41	66	82	84	85	113	78	107	296
25	42	62	56	62	85	77	55	61	95
Controls									
NL43	17	138	294	956	1172	953	1584	1868	2143
JRCSF	24	37	35	60	87	97	105	152	209
AMPHO	<10	32	14	13	14	13	<10	<10	31

FIG. 7. Neutralizing HIV antibody titers of sequential plasma specimens against autologous virus. Serial plasmas were obtained from an untreated patient presenting with primary HIV infection. The titer of each plasma against its concurrent virus specimen is shaded. Control viruses include an amphotropic murine leukemia virus (AMPHO), a neutralization-sensitive X4—tropic virus (NL4-3), and a relatively neutralization-resistant R5 - tropic virus (JR-CSF) (from reference 49).

sponse does not effectively impact disease course, but rather drives the rate of escape and evolution of the virus.

Using a second group of subjects with recent HIV infection, we investigated the impact of the administration of potent antiretroviral therapy on the neutralizing antibody response. To conduct these studies, a genomic HIV vector was constructed using a *pol* gene derived from a patient virus that was highly resistant to protease and reverse transcriptase inhibitors. This vector, in conjunction with patient virus envelope expression vectors, was used to measure neutralizing antibody accurately despite the presence of inhibitory drugs in plasma of treated patients that confound standard neutralization assays. Autologous antibody neutralization activities were measured in longitudinal plasma samples collected from five patients who were administered antiretroviral drugs shortly after presentation and who had sustained suppression of plasma HIV RNA below 50 copies/ml. In all five subjects, antibody titers plateaued at relatively low titers (<1:500) and their spectrum of activity evolved very little.

Of particular concern was the failure of patients to generate appreciable heterologous neutralizing antibody responses in the first year of infection despite generating high titers to their autologous virus. Cross-neutralization assays were performed with isolates shortly after infection from 13 patients. Compared with autologous viruses, neutralization of heterologous viruses was absent or at best negligible during

the first year of HIV infection. This observation is a particular concern regarding the ability of a candidate vaccine to generate broadly reactive neutralizing antibody.

DISCUSSION

Because of its high rates of replication and mutation, HIV generates a remarkably wide array of genetic variants every day in each patient. Recombination readily occurs as well in vitro and in vivo which further drives the generation of variants (53). The imposition of selective pressures accelerates the evolution of HIV at rates never imagined by Darwin. The extensive use of antiretroviral therapy provides one of the most dramatic examples of the impact of human intervention on evolution in an ecological system (54). Evolution in different anatomic compartments like the central nervous system and genital tract impacts pathogenesis, drug resistance and transmission. Neutralizing antibody drives remarkable variability in the surface glycoprotein of HIV and imposes a major challenge to the development of an effective vaccine.

These observations provide a retrospective appreciation of Darwin's brilliant insights and a prospective need to address the challenges of designing effective treatments and vaccines. The lessons taught by HIV may help to address other microbial challenges like hepatitis C virus and emerging infections.

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DISCUSSION

DuPont, Houston: Doug, it was a thorough and helpful presentation, with depressing results. I wonder if you would speculate down 10, 20, 30 years from now where you think we'll be with AIDS control. Will we be successful with either drug therapy or immunotherapy in this disease? AIDS looks more like cancer than an infectious disease to me the way it will be conquered.

Richman, LaJolla: I didn't have a chance, obviously, to describe all the aspects of treatment and pathogenesis of HIV disease. I think the accomplishments of chemotherapy have been one of the most dramatic accomplishments in American medicine. In just twenty years since the discovery of the virus the introduction of antiretroviral therapy in the developed world has resulted in a dramatic impact on morbidity and mortality. Medicine wards fifteen years ago were like a war zone, and now the only patients that are being admitted are either those with emotional or substance abuse problems who can't take their medicines, or frighteningly, a third of patients in this country who are infected and didn't know it until they first presented with their infection with AIDS related complications. We do need better ways to recognize our hundreds of thousands of infected, asymptomatic citizens who are unaware of or denying their condition. Access to therapy and the proper use of therapy can prevent disease and turn it into a manageable, chronic disease like hypertension or diabetes. The hope is that these drugs can be distributed and made available in the developing world, where 97% of the infected patients now exist. I think we can significantly impact infected patients; moreover, if we can suppress virus replication, patients will no longer be likely to be transmitters. To have a major impact on the epidemic globally; however, the development of a vaccine is ultimately the only hope. This is going to be a long, long, hard struggle. Despite promising research, vaccine is not going to impact the epidemic I would think for at least a decade.