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Virology 1

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determine that HIV could be transmitted through blood and blood products. Since 1985, Dr. Essex and colleagues have worked with collaborators in Africa and Asia, where they conduct biological, clinical, and epidemiological studies.

In the early 1980s, our laboratory was involved in the study of retroviruses and their association with cancer. Max Essex had been involved in years of investigation on the biology of feline leukemia virus (FeLV), a retrovirus of cats that was not only associated with cancer but also immunosuppressive disorders. FeLV was only one of many known and well-studied animal retroviruses at the time. Viruses in mice, chickens, cows, sheep, and horses had been studied for years in an effort to better understand and investigate possible viral causes of human cancer. Therefore in 1982, the discovery of human T-cell leukemia virus (HTLV) seemed to be the 'holy grail' that had been searched for. However, within a few years, descriptions of a new disease or syndrome in young male homosexual populations prompted the search for the causative agent. Infectious disease specialists involved with these populations, epidemiologists, and virologists were drawn into the investigation of this new disease. In the Boston area in the mid-1980s, investigators from the Harvard School of Public Health combined expertise with clinical investigators at Mass General Hospital, Beth Israel, and the New England Deaconess hospitals to conduct studies of suspect cases and controls in a search for the etiologic agent. Small scientific meetings were held at increasing frequency to stay ahead of the new data that were being generated. These meetings brought together investigators from many different disciplines, which in retrospect promoted the public health perspective of the field. New funding opportunities also encouraged multidisciplinary groups that would study this new disease entity.

Once human immunodeficiency virus (HIV) was recognized as the etiologic agent of the acquired immune deficiency syndrome (AIDS), the field recognized some of the technical challenges of further characterizing the viral infection and therefore implementing clinical care. Retrovirus infection had only been relatively recently described in humans with HTLV-I and HTLV-II, and methods for diagnosis through antibodies or virus isolation were relatively new and distinct from other viral systems. Therefore the need for new technologies to address this virus infection was recognized early in the epidemic. In later years, the use of polymerase chain reaction (PCR) technology to detect and quantitate virus provided the foundation for clinical management of this disease. As epidemiologists recognized the alarming increase of HIV/AIDS cases across the globe, the use of PCR-based sequencing techniques allowed the realization of the tremendous diversity of HIV viral strains that compose what we now recognize as the global HIV/AIDS pandemic.

History of the Discovery of HIV

AIDS was first recognized as a new and distinct clinical entity in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegal *et al.*, 1981). The first cases were recognized because of an unusual clustering of diseases such as Kaposi sarcoma and *Pneumocystis carinii* pneumonia (PCP) in young homosexual men. Although such unusual diseases had previously been observed in distinct subgroups of the population – such as older men of Mediterranean origin in the case of Kaposi sarcoma or severely immunosuppressed cancer patients in the case of PCP – the occurrence of these diseases in previously healthy young people was unprecedented. Since most of the first cases of this newly defined clinical syndrome involved homosexual men, lifestyle practices were first implicated and intensively investigated. These included the exposure to amyl or butyl nitrate 'poppers' or the frequent contact with sperm through rectal sex, which might have acted as immunostimulatory doses of foreign proteins or antigens.

However, AIDS cases were soon reported in other populations as well, including injection drug users (IDUs) (Anonymous, 1982) and hemophiliacs (Davis *et al.*, 1983; Poon *et al.*, 1983; Elliott *et al.*, 1983). Similar to investigations of male homosexual populations, these new risk groups were exposed to doses of foreign proteins and antigens but through the blood. In the case of IDUs, this would occur through intravenous injection of drugs and needle sharing, and in the case of hemophiliacs through the therapeutic infusion of Factor VIII. Asymptomatic hemophiliacs and IDUs were often found to have unusual immunological tests, such as the inverted T lymphocyte helper:suppressor ratios (i.e. less than the normal 1:1 ratio of helper to suppressor cells). These abnormal tests suggested a problem with the cellular immune system, with low numbers of helper T lymphocytes, also referred to as CD4+ lymphocytes, a finding similar to that observed in many AIDS patients.

Three new categories of AIDS patients were soon observed, including blood transfusion recipients (Curran *et al.*, 1984; Jaffe *et al.*, 1984), adults from Central Africa (Clumeck *et al.*, 1983; Piot *et al.*, 1984; Van de Perre *et al.*, 1984), and infants born to mothers who had AIDS or were IDUs (Rubinstein *et al.*, 1983; Oleske *et al.*, 1983; Scott *et al.*, 1984). The transfusion-associated cases had received blood donated from an AIDS patient at least three years before they began showing symptoms (Curran *et al.*, 1984; Jaffe *et al.*, 1984). Based on the disparate populations afflicted with this new malady and the emerging epidemiology of the disease, the possible infectious etiology for AIDS was considered (Francis *et al.*, 1983).

Multiple studies were initiated to determine the possible role of various microorganisms, especially viruses, in causing AIDS. These studies measured and compared seroprevalence rates for suspect viruses in AIDS patients and controls. The shortlist of candidate viruses included: cytomegalovirus, which was already associated with immunosuppression in kidney transplant patients; Epstein–Barr virus, which was a lymphotropic virus; and hepatitis B virus, which was known to occur

at elevated rates in both homosexual men and recipients of blood or blood products. However, based on the unique clinical syndrome and unusual epidemiology of AIDS, if one of these viruses was to be etiologically involved, it would presumably have been a newly mutated or recombinant genetic variant.

At the same time, our group (Essex *et al.*, 1983), Gallo and his colleagues (Gelmann *et al.*, 1983), and Montagnier and his colleagues (Barre-Sinoussi *et al.*, 1983) postulated that a variant T lymphotropic retrovirus (HTLV) might be the etiologic agent of AIDS. Indeed, HTLV, discovered by Gallo and his colleagues in 1980, was the only human virus known to infect T helper (CD4+) lymphocytes at that time (Poiesz *et al.*, 1980). This seemed reasonable since it was already clear that T helper lymphocytes were selectively depleted by the causative agent in clinical AIDS (Rubinstein *et al.*, 1983; Ammann *et al.*, 1983; Fahey *et al.*, 1984; Lane *et al.*, 1985). In addition, HTLV was known to transmit through the same routes as the etiologic agent of AIDS: sexual contact (with transmission apparently more efficient from males), by blood, and from mother to baby (Essex, 1982). Finally, HTLV-I was also known to induce immunosuppression (Essex *et al.*, 1984), as we had previously recognized in animal retrovirus systems (Essex *et al.*, 1985).

AIDS patient blood samples were repeatedly cultured in an attempt to find a virus related to HTLV-I or HTLV-II (Kalyanaraman *et al.*, 1982). Although antibodies cross-reactive with HTLV-I and HTLV-related genomic sequences were found in a minority of AIDS patients (Gelmann *et al.*, 1981; Barre-Sinoussi *et al.*, 1983; Essex *et al.*, 1983), the reactivity was weak, suggesting either AIDS patients were also infected with an HTLV, or that a distant, weakly reactive virus was the causative agent. Soon after, Gallo and his colleagues obtained proof that AIDS was linked to an HTLV (Popovic *et al.*, 1984; Gallo *et al.*, 1984; Schupbach *et al.*, 1984). Further characterization of the agent – now termed HIV-1 – revealed that it was the same as the isolate detected earlier by Montagnier and his colleagues (Barre-Sinoussi *et al.*, 1983). Despite controversy over the names and identity of certain isolates, this new and unique human pathogen was clearly not only a distant genetic relative of the known HTLV virus but may have been recently introduced to humans from a primate reservoir (Essex and Kanki, 1988).

After HIV-1 was recognized as the cause of AIDS, it was also recognized that this virus was new, at least to inhabitants of the Western Hemisphere. This raised the question of whether HIV-1 was also new to Old World human populations, such as Africa, or whether it recently entered humans from another species. Reports of diseases such as AIDS had not been reported in these populations. It is conceivable that if HIV-1 had been present in Africa for some time, the interplay between pathogen and host would have led to a less virulent virus and people with genetic resistance to the lethal properties of the virus. However, early clinical studies did not indicate differences in the pathogenicity of the virus–host interactions in Africa compared with the United States or Europe.

HIV-1 or a related virus was likely present in human populations in Central Africa at the same time or even before AIDS was diagnosed in the United States.

In the early 1980s, Africans residing in Europe were presenting with similar clinical signs and symptoms of AIDS (Clumeck *et al.*, 1983). Serum samples collected from Africans at earlier periods were also examined for the presence of antibodies reactive with HIV-1. In some cases, the examination of stored samples suggested elevated rates of infection in Africa during the mid-1960s to 1970s (Saxinger *et al.*, 1985). Subsequently, it was revealed that most of those surveys were conducted with first-stage tests that were imperfect. In addition, the reactors were mostly false positives, due either to contamination of the HIV antigen or to 'sticky sera' containing antibodies that reacted non-specifically because the sera had been repeatedly frozen and thawed and maintained under poor conditions.

While examining sera taken from Africa in the period 1955 to 1965, we found one antibody-positive sample that was clearly positive in a specific manner (Nahmias *et al.*, 1986). When tested with the highly specific test radioimmunoprecipitation, this sample contained high titers of antibodies that were reactive with virtually all the major antigens of HIV-1. This sample represented only a rare positive reactor in a high-risk group of individuals suffering from tuberculosis and AIDS-like illness in a region that subsequently had high rates of infection with HIV-1. However, only 1 per cent or fewer of the individuals who tested positive were from what is now classified as a region of moderate to high prevalence (Kinshasa, Zaire), which suggests that the virus was then only rarely present in places that would now be classified as within the 'AIDS Belt of Africa.'

AIDS Denial

The recognition that AIDS is caused by HIV was not easy for some to accept. A small group of scientists have persisted in denial of the overwhelming evidence for causation. Additionally, various conspiracy theories have emerged suggesting that HIV-1 was deliberately created by germ warfare scientists. While such proclamations seem silly or irrational to informed medical scientists, they interfere with constructive attempts to educate appropriate population groups.

One reason why some have been reluctant to accept that AIDS is an infectious disease caused by HIV-1 is the very prolonged induction period combined with a very high mortality rate. Most infectious diseases occur after a short induction period. Even for the small number of infectious diseases with a very long induction period caused by viruses, such as tropical spastic paraparesis, adult T cell leukemia or shingles, only a small fraction of infected people experience that clinical outcome. The definition of AIDS as an amalgamation of clinical outcomes ranging from tuberculosis to chronic diarrhea to cancer (e.g. lymphoma and Kaposi sarcoma) also may cause confusion for those trying to accept HIV-1 as the single etiologic cause. This only becomes logical when it is recognized that AIDS disease is fundamentally an irreversible destruction of the immune system. All of the other outcomes are secondary to the immune destruction.

Another problem in understanding AIDS etiology may be a lack of appreciation of the discipline of epidemiology and dissension about the proper definition of cause. For epidemiologists, a very high-risk association, such as for tobacco and lung cancer, is sufficient to ascribe cause. Using analogous logic, the causal association between HIV-1 infection and subsequent destruction of the immune system is overwhelming. In prospective cohort studies, almost all HIV-1-infected people eventually develop immune depletion. This association is much higher than for such viral infections as polio or flu. Concerning time and spatial geographic associations, clinical AIDS rates have exactly paralleled HIV infection rates, whether in Bombay, San Francisco or Nairobi, allowing for the 5- to 15-year induction period.

Until recently, a lack of understanding about how HIV-1 caused immune depletion helped those who reject epidemiology to deny causation. This situation has changed dramatically, however, with the recognition that HIVs-1 can be highly lytic for T4 lymphocytes (Yu *et al.*, 1994), and that very large numbers of T4 cells are killed by the virus *in vivo* (Wei *et al.*, 1995; Ho *et al.*, 1995). Further, while the very low rate of infected circulating lymphocytes was interpreted by some as incompatible with the destruction of large numbers of cells, recent studies reveal that most HIV-1 is in lymph nodes rather than blood, and up to 25–50 per cent of lymph node T cells may be infected (Pantaleo *et al.*, 1993). HIV-1 is also highly unusual as a virus that targets T4 lymphocytes and macrophages, both essential components of the immune system. Finally, HIV-1 is transmitted in exactly the same way as clinical AIDS: by blood, by sex, and from mother to infant. When taken together, these various correlations provide inescapable evidence that HIV-1 must be the cause of AIDS.

HIV-related Retroviruses of Monkeys

Soon after the recognition of clinical AIDS in people, several clinical reports described outbreaks of severe infections, wasting disease, and death in several colonies of Asian macaque monkeys housed at primate centers in the United States (Letvin *et al.*, 1983; Henrickson *et al.*, 1983). Due to their similarity to the human syndrome, these diseases were designated simian AIDS or SAIDS. As in the case of human AIDS, many possible causes were considered. Following the recognition that SAIDS appeared to be of infectious origin, cytomegalovirus of monkeys was also considered as a possible etiologic agent.

However, seroepidemiological screening revealed that a proportion of the SAIDS monkeys had antibodies that cross-reacted with HIV (Kanki *et al.*, 1985a,b) while healthy monkeys had no such antibodies. Although the antibodies cross-reacted with core antigens of HIV-1, they showed only very weak cross-reactivity with the envelope antigens. Further characterization of the cultures revealed the presence of HIV-like particles and antigens detectable with antibodies

from either SAIDS monkeys or people with AIDS. The sizes of the protein antigens detected by radioimmunoprecipitation analysis were similar to those of HIV-1. When these antigens were tested with sera from people with AIDS or healthy carriers, virtually all sera had antibodies cross-reactive to core antigens. This primate virus was named STLV-III due to its relationship to HIV-1 (which was then called HTLV-III and/or LAV), and later termed simian immunodeficiency virus or SIV (Biberfeld *et al.*, 1987).

An animal model for AIDS was an important advance at the time, particularly given the close similarities of both the disease and the animal host. However, the origin of the virus remained a puzzle. Although cases of AIDS had been reported in Africa, at the time AIDS cases in Asia were considered quite rare. We tested the hypothesis that the natural primate host for SIV would be able to sustain infection without significant disease, and that this primate would most likely reside in Africa. At least half of the healthy wild-caught African green monkeys showed evidence of exposure to SIV on the basis of antibodies (Kanki *et al.*, 1985a, 1986a). Although it was possible that SIV caused some type of disease that had not been recognized, it was clear that the disease did not resemble the lethal immunosuppressive syndrome found in Asian macaque monkeys. Similarly, captive African mangabey monkeys infected with SIV revealed no disease symptoms. The pathogenic effects of SIV appeared to be species-specific. Further studies of wild-caught Asian monkeys demonstrated that SIV was not a natural infection of these primates. It is generally believed that SIV was introduced to Asian macaques in captivity, explaining in part the unusual and high mortality induced by SIV.

More recent studies reveal that several African monkey species are infected with different SIVs. These include several species commonly described as African greens, such as vervet, grivet, sabaeus, and tantalus, as well as mona, diana, Sykes', mandrill, and sooty mangabey species (Tsujimoto *et al.*, 1988; Johnson *et al.*, 1989; Allan *et al.*, 1991). Thus far, SIVs have not been described in Asian species or in baboons, though these species can be infected in captivity with some primate lentiviruses. As a group, the SIVs are more closely related to HIV-2s than to HIV-1s, although some, such as the mandrill SIV, are evolutionarily distant (Tsujimoto *et al.*, 1988). The sooty mangabey monkey virus and the Senegalese human HIV-2, on the other hand, are essentially the same at the genetic level (Peeters *et al.*, 1989; Essex, 1994; Kirchhoff *et al.*, 1997). It is this virus that also apparently accidentally infected the Asian macaques in captivity (Essex and Kanki, 1988).

The possibility that SIV might cause disease rarely in African monkeys in advanced age could not be ruled out. The high prevalence of infection in wild-caught monkeys supported the notion that these African species of primates had evolved to a more benign coexistence with the virus in which infection did not affect survival. This was clearly different from the case of SIV in Asian primates or HIV-1 in people. Because wild macaques do not appear to be infected with

SIV, and because the virus is limited to African primates, it appears likely that the virus accidentally infected captive rhesus monkeys in recent times. New SIVs are still being described and it is possible that species specificity may play a role in the level of host–virus adaptation and therefore levels of population infection (Georges-Courbot *et al.*, 1998). Currently it appears that natural SIV infection of African primates does not result in significant disease, supported by the high seroprevalence rates in most species evaluated. It thus appears that virus–host interaction has resulted in a non-pathogenic infection and the genetic, immunological and virus determinants of this relationship are worthy of further study.

We could speculate that the virus had either moved from subhuman primates to people prior to the mid-1950s or had been introduced to the cities through the migration of a few resistant carriers from a previously isolated group of people. However if HIV-1 had been present in rural areas, we might expect to find that Africans demonstrate greater resistance to infection and disease development, owing to genetic selection and evolution of the human species. In prospective studies conducted to date, Africans infected with HIV-1 appear to develop clinical AIDS and other signs and symptoms of HIV disease as rapidly as individuals in the United States or Europe (Mann *et al.*, 1986; Marlink *et al.*, 1994). Furthermore, the degree of genomic variation seen in African isolates of HIV-1 was greater than that seen for viruses from Europe or the United States.

A virus that could be a progenitor of HIV-1 was isolated from a chimpanzee in Central Africa (Huet *et al.*, 1990). This finding, combined with the knowledge that all HIV-1 viruses tested appear to be avirulent when inoculated into chimpanzees, is also compatible with a subhuman primate origin for HIV-1. Some African isolates of HIV-1 appear to be as close to the chimpanzee isolate as to other prototype strains of HIV-1 (De Leys *et al.*, 1990; Zekeng *et al.*, 1994). These findings appear to have been confirmed by more detailed genetic analysis of a virus from a chimpanzee housed in the United States, although the exact history and pedigree of this animal is unclear (Gao *et al.*, 1999).

HIV-2 – HIV Closely Related to SIV

Because a relative of HIV-1 – SIV – had been found in wild African monkeys and was only about 50 per cent related to HIV-1 at the genomic level, it seemed logical that viruses more highly related to SIV might also be present in human populations. Serum samples from West African prostitutes were examined to determine if they had antibodies that were more highly cross-reactive with SIV than with HIV-1 (Barin *et al.*, 1985). Through Western blot and radioimmunoprecipitation methods, it became clear that a significant proportion of Senegalese prostitutes had antibodies that were highly reactive with all the major antigens of SIV detected by this technique. When the same SIV antigens were reacted with sera

from HIV-1-infected individuals of either European or Central African origin with classic disease manifestations of AIDS, little or no reaction was seen with the envelope antigens. The class of reactivity seen with serum samples from West African prostitutes was in fact virtually indistinguishable from that seen with serum samples from African monkeys or captive rhesus macaques (Kanki *et al.*, 1986b; Hirsch *et al.*, 1989; Essex, 1994).

HIV-1 Subtypes

The HIV virus lifecycle requires transcription of the viral RNA into a DNA copy that becomes randomly integrated into the host cellular DNA. The transcription process is performed by the virus enzyme reverse transcriptase, which is error-prone. As a result, each round of replication results in at least one mutation per genome copy. The genetic variation of HIV is hierarchical, as depicted in the simplified schematic of subtype variation, interpatient variation, and intrapatient variation (Figure 1.1). HIV-1 virus isolates from North Americans and Europeans showed distinct genetic variability compared with the variability in viruses from African patients (McCutchan *et al.*, 1996; Burke and McCutchan, 1997). This variability was more dramatic than the recognized genetic variability between viral isolates

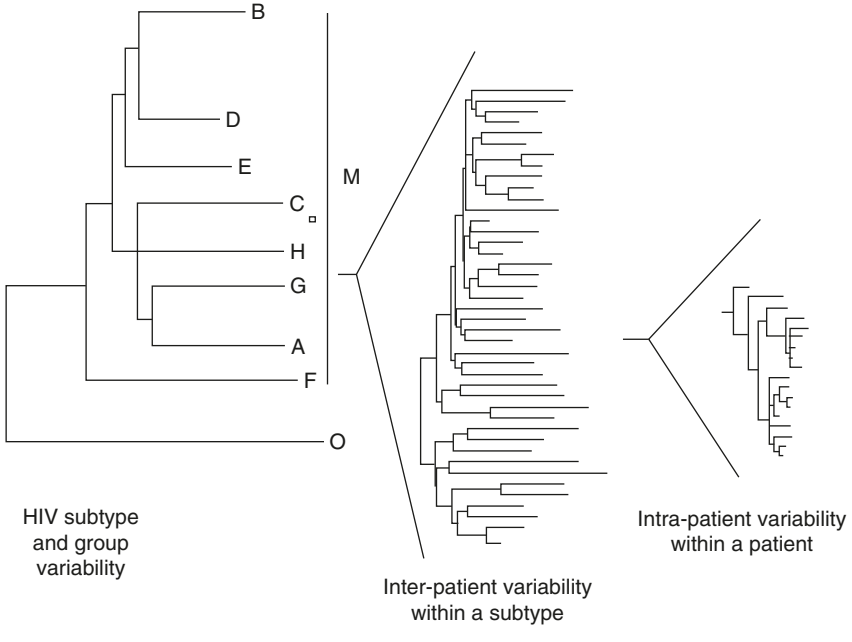

Fig. 1.1 Levels of HIV genetic diversity. Courtesy of Phyllis J Kanki.

from a single geographic region (i.e. inter-isolate variability) (Myers and Pavlakis, 1992; Korber *et al.*, 1995). In turn, this was also distinguishable from the genetic variation that was seen at the level of an individual patient (i.e. intrapatient variability). At the level of the individual patient, a swarm or quasi-species of highly related but distinguishable viral variants has been demonstrated throughout the course of HIV infection (Goodenow *et al.*, 1989; Balfe *et al.*, 1990; Delassus *et al.*, 1991). Genetic diversity is therefore a major characteristic of the HIV viruses and provides a major obstacle to drug and vaccine development.

Remarkably, all the HIVs-1 isolated from the United States and Western Europe through 1994 have been of a single subtype, B. Most of the diverse subtypes of HIV-1 have been found in sub-Saharan Africa. Subtypes A, C, and D in particular have been found more frequently than other subtypes in Africa. A high rate of spread of HIV-1 for Africa appeared during the 1980s, at about the same time the epidemic spread in the United States and Europe. The movement of populations and extensive international travel makes the likelihood of mixing subtypes inevitable, and non-B subtypes have already been identified, and are increasing in the United States and Europe.

In Asia, the introduction and spread of HIV-1 appeared about a decade later than in the West (see Chapter 15 for details). In Thailand, HIV-1 subtype B was detected in intravenous drug users during the mid-1980s. During the late 1980s, subtype E was first detected. By the early to mid-1990s, HIV-1 subtype E had spread very rapidly throughout heterosexuals in Thailand, with the highest rates in the northern regions of the country (Weniger *et al.*, 1994). Although apparently present earlier in the region, HIV-1 subtype B never spread to cause a major heterosexual epidemic as did HIV-1 subtype E. In China, the epidemic in IDUs is a unique recombinant virus of C and B. In heterosexual populations, subtype E appears to be the predominant subtype, similar to that of much of South-East Asia. This complex mixture of HIV-1 subtypes in Asia has challenged vaccine development efforts, since distinct subtype epidemics are being observed in different high-risk populations.

A similar situation occurred in India with HIV-1 subtypes B and C. While subtype B appeared to be introduced earlier among IDUs, this subtype did not spread as rapidly among heterosexuals as did subtype C. Previously associated with the massive heterosexual epidemic in southeastern Africa, subtype C also caused a rapid heterosexual epidemic in western India, initially spreading from the Bombay region (Jain *et al.*, 1994; Weniger *et al.*, 1994). In the past 7–8 years, it is clear that subtype C is the sole viral subtype responsible for the newest and perhaps most frightening of HIV epidemics, worldwide. In most countries of southern Africa, rapid dissemination of HIV infection has been described with rates ranging from 10 to 40 per cent in pregnant women in most countries of the region. As a result of these significant increases in subtype C infection in Africa, and its predominance in places such as India and perhaps China, this viral subtype is responsible for over half of all HIV infections worldwide (UNAIDS, 2002).

An even more distant subtype, designated HIV-O, has been detected in Cameroon (Nkengasong *et al.*, 1994). The viruses isolated from this subtype are even less related to HIV-1 subtypes A through H than either of the other subtypes are related to each other, yet HIV-O is more related to HIV-1 than to HIV-2 (Gurtler *et al.*, 1994). To emphasize this distance, HIV-1 subtypes A through H are designated the major group (M), and HIV-O is designated the outgroup (O) (Charneau *et al.*, 1994). Despite extensive serosurveys, the distribution of HIV-O appears to be quite restricted (Peeters *et al.*, 1997). While HIV-1 subtypes A through H probably had a common human progenitor ancestor, HIV-O no doubt entered independently from a chimpanzee host. HIV-2 almost certainly entered independently from monkey species native to West Africa (Essex and Kanki, 1988; Hirsch *et al.*, 1989).

The movement and distribution of HIV-1 subtypes throughout the world is often perplexing, particularly when subtypes such as E to H appear to be isolated more frequently in such places as Asia, South America, or eastern Europe than in Africa, where they presumably originated (Louwagie *et al.*, 1993; Bobkov *et al.*, 1994). However, the viruses that have been isolated and characterized were acquired for analysis from convenience samples and therefore may suffer from extensive regional selection bias and inadvertent clustering. In the future, it will be important to develop more consistent surveillance methods and full-length sequence analysis to generate a true global map of HIV subtypes.

Emergence of HIV-1 Disease Phenotypes

Our understanding of the epidemiology and biology of different HIV-1 subtypes is critical to future intervention efforts, and further studies are clearly needed (Anderson *et al.*, 1996). Studies have demonstrated differences in the ability of non-B and B subtype viruses to infect Langerhans' cells, a critical cell in heterosexual transmission of HIV. This suggests that the viral properties of non-B subtype viruses would facilitate heterosexual transmission and may have contributed to the dramatic epidemic spread in Asia and Africa (Soto-Ramirez *et al.*, 1996). A cross-sectional study of heterosexual couples in Thailand suggests a higher risk of heterosexual transmission of subtype E compared with subtype B (Kunanusont *et al.*, 1995). Studies in many African countries have described multiple HIV-1 subtypes, but it is not known if subtypes enter populations at different time points, or if the distribution of subtypes reflects the dynamics of different subtype-specific transmission potentials. Studies in South Africa demonstrate the association of certain subtypes with different modes of HIV transmission: subtype B viruses are associated with homosexual transmission, and non-B subtypes are associated with heterosexual transmission (Vanharmelen *et al.*, 1997). Future detailed studies of prospectively followed cohorts will be necessary to determine differences in pathogenicity and transmissibility of different subtypes.

Host selection of HIVs-1 for efficiency of heterosexual transmission may partially explain the high rates of heterosexual transmission seen with subtypes C and E (Soto-Ramirez *et al.*, 1996). HIV-1 subtype B, the major subtype in the developed world, appears to have undergone counterselection (i.e. less likely to be selected for compared with more virulent or transmissible viruses) to lose the phenotypic property of efficient heterosexual transmission. If efficient heterosexual transmission requires a particular genotype for vaginal infection, such sequences may have been partially lost by these HIV-1 B 'strains' that have been repeatedly passaged by blood exposure or rectal intercourse. Many of the non-B subtypes, on the other hand, could theoretically maintain vaginal phenotypic properties through regular heterosexual transmission in Africa and Asia (Kunanusont *et al.*, 1995; Soto-Ramirez *et al.*, 1996).

Based on prospective studies of female sex workers in Dakar, Senegal, we have recently reported on disease progression in non-B subtype infections with known time of infection. In evaluating AIDS-free survival curves of women with incident subtype A, C, D, and G infection, we have shown distinct differences in AIDS-free survival (Kanki *et al.*, 1999). The comparison of non-A subtypes with subtype A demonstrated a significantly longer AIDS-free survival for women infected with subtype A. Due to the small sample size per subtype, our estimate of AIDS incidence should be considered imprecise, and further study is clearly warranted. Cross-sectional studies indicate a significant proportion of AIDS cases with subtype A infection in West and East Africa (PJ Kanki, unpublished data, 1998). Further study of HIV-1 subtype natural history and progression from different geographic regions is clearly needed to better evaluate the role of viral subtype differences and AIDS pathogenesis.

Viral Load and HIV Pathogenesis

Human immunodeficiency virus infection results in progressive loss of immune function marked by depletion of the CD4+ T lymphocytes, leading to opportunistic infections and malignancies characteristic of the syndrome termed AIDS. A number of host and viral factors influence the rate of disease progression. Studies in the West prior to the implementation of antiretroviral therapy suggested a median time to AIDS ranging from 8 to 10 years. Historically, CD4+ T lymphocyte counts provided the most reliable prognostic marker of HIV progression (Fahey *et al.*, 1984). In addition, other prognostic markers of progression to AIDS included immunologic markers of immune dysfunction which included cutaneous anergy (Redfield *et al.*, 1986; Blatt *et al.*, 1993), serum β2-microglobulin, and neopterin levels (Fahey *et al.*, 1990). In the past, quantitation of viral infection was performed with imperfect and/or laborious methods. The serologic quantitation of the viral core antigen, p24 was frequently considered as a surrogate marker of high viral burdens (Allain *et al.*, 1987; Dewolf *et al.*, 1997).

Quantitative viral culture was difficult to perform and not cost effective for clinical monitoring on a regular basis (Coombs *et al.*, 1989; Ho *et al.*, 1989). HIV viral expression as measured by mRNA was also considered an important marker that preceded immunologic compromise (Gupta *et al.*, 1993; Saksela *et al.*, 1994). More qualitative characteristics of the HIV virus infection included the syncytium-inducing properties of the virus, which were often coupled with viral burden; the syncytium-inducing viruses were associated with high viral loads and rapid progression and non-syncytium-inducing viruses associated with lower virus loads and slow progression (Tersmette *et al.*, 1988; Fenyo *et al.*, 1989).

The development of the sensitive PCR-based technology to reliably quantitate RNA levels of virus in the plasma revolutionized our abilities to track viral infection. PCR technology is based on the use of a temperature-sensitive DNA polymerase. A target sequence is identified in the DNA sequence and appropriate primers are designed to span the sequence of interest. The primers need to be as similar to the original target sequence as possible. Once the primers anneal to the target sequence the temperature-sensitive polymerase begins to transcribe sequences, matching the sequence that it has found in the sample. When the temperature increases, the strands of DNA separate and the primer binding and polymerase reaction occurs again. In this way, a large number of exact DNA copies can be made given the cycles of temperature and the availability of primer, nucleotides, and polymerase.

Minute copies of DNA can be detected in large volumes of DNA with this very sensitive technology.

Mellors and colleagues provided important new evidence that such quantitation of plasma levels of RNA virus, or viral load, were important predictors of disease progression (Mellors *et al.*, 1997). Based on the large US cohort studies of homosexual men, the risk of developing AIDS and death was significantly associated with baseline plasma viral loads, independent of CD4+ lymphocyte counts (Mellors *et al.*, 1995, 1996). It is worth noting that these studies and many of the ensuing viral load studies were conducted in the US and Europe, where subtype B HIV-1 infection is predominant. In viral load studies, as we will discuss further, it is critical to consider the populations studied, HIV viral subtypes, and methodologies employed.

Many studies that have followed the natural history of HIV infection over time have now reported on the utility of viral load determinations to track and predict disease progression. Since a variety of other markers, most notably lymphocyte subset data, were frequently available the relationship of viral load with CD4+ lymphocytes counts has been regularly evaluated. It has been important to evaluate the viral load's predictive value over the full course of HIV's natural history, and this has required analysis from long-standing cohort studies, where time of infection has been known and where observation times predated the use of antiretroviral therapy.

The large US Multi-center AIDS Cohort Study (MACS) has demonstrated a strong correlation in initial HIV RNA loads with declines in CD4 lymphocyte

counts. Both initial HIV RNA levels and slopes were associated with AIDS-free times. HIV RNA load at the first seropositive visit, similar to three months after seroconversion, was highly predictive of AIDS, and subsequent HIV RNA measurements showed even better prognostic discrimination. However, HIV RNA slopes in the three years preceding AIDS and HIV RNA levels at AIDS diagnosis showed little variation according to total AIDS-free time (Lyles *et al.*, 2000). The MACS study population is largely a white homosexual male cohort, and it may not be possible to generalize all of these findings to other population groups with different modes of transmission and different viral subtypes.

Sabin and colleagues have reported a similar predictive value of plasma viral loads in the large European hemophilia cohorts where their results confirm the importance of the HIV RNA level in assessing the long-term prognosis in individuals infected with HIV (Sabin *et al.*, 1998). The risk of developing AIDS and death remained low when the HIV-1 RNA level was below 4 log 10 copies/ml, but increased rapidly thereafter, supporting current guidelines for the initiation of antiretroviral therapy after the viral load has exceeded this level (Sabin *et al.*, 2000). In the French SEROCO study of HIV seroconverters ($n = 330$), patients who remained AIDS-free had lower early viral loads and, on average, a longer period of viral load decline after infection (36 versus 18 months), followed by a slower viral load increase compared with those who progressed to AIDS (Hubert *et al.*, 2000). A true plateau-phase after the seroconversion period was observed, lasting approximately four years, identified only in patients who remained AIDS-free for at least 90 months. In multivariate analysis, both early viral load and later changes were significant predictors of progression to AIDS (Hubert *et al.*, 2000).

Primary HIV Infection and Viral Setpoint

In recent years, through the study of primary HIV infection, we have learned that some degree of virus containment occurs in the very early phases of HIV infection *in vivo*. During this critical period, a complex dynamic of infecting virus and responding host and immune factors leads to the establishment of a level of viremia, or viral setpoint, that appears predictive of subsequent HIV progression rates and survival (Mellors *et al.*, 1995, 1996). The incubation period from initial infection to onset of symptoms is an average of 21.4 days (SD = 9.6 days, range: 10–55 days) and the self-limited illness resolves within 1–3 weeks. Current data suggest that HIV viral load in the blood reaches a peak in the first 15–30 days, concurrent with a precipitous drop in CD4+ T cell count and increase in absolute number of CD8+ lymphocytes (Clark *et al.*, 1991; Clark and Shaw, 1993; Clark and Wolthers, 2000). Subsequent to these early events and tied to the resolution of the acute clinical syndrome is the lowering of HIV load and rebound of CD4+ T lymphocyte levels.

Corey and colleagues have reported considerable variability in the viral burden during these early phases of HIV infection, after 120 days after acquisition, followed by a rapid decrease in plasma HIV RNA levels to an inflection point, after which they gradually increase (Schacker *et al.*, 1998). The early infection phase continues over the next 6–12 months, with seeding and establishment of HIV viral load in blood and lymphoreticular tissues (Fauci, 1993, 1996; Haynes *et al.*, 1996). The establishment of 'steady-state viremia' or viral setpoint is attained during this phase of infection and remains relatively invariant throughout much of the long incubation period.

It is now well established that the level of HIV-1 plasma viremia early in infection is highly predictive of future clinical course (Mellors *et al.*, 1995, 1996; Stein *et al.*, 1997). Cross-sectional studies of long-term non-progressors (LTNPs) as a group have demonstrated a significantly lower cell and plasma viral burden when compared with rapid progressors (Cao *et al.*, 1995; Rinaldo *et al.*, 1995; Pantaleo *et al.*, 1995). Increases in plasma viremia are correlated with increase in proviral burden, quantitative virus isolation, and quantity of virus in lymphoreticular tissue (Haynes *et al.*, 1996). The stability of virion-associated HIV-1 RNA levels suggests that an equilibrium between HIV-1 replication rate and efficacy of immunologic response is established shortly after infection and persists throughout the asymptomatic period of the disease. Thus, defective immunologic control of HIV-1 infection may be as important as the viral replication rate for determining AIDS-free survival.

HIV Therapy and Viral Load

In HIV-1 infection, treatment with highly active antiretroviral therapy (HAART) has been shown to drastically lower plasma viremia, and this is currently used as an indicator of the effectiveness of treatment (Obrien *et al.*, 1998; Panther *et al.*, 2000). After discontinuing HAART, individuals had rebounds in their viral burdens approximating pre-HAART levels, even after a significant lapse of time approaching five years (Hatano *et al.*, 2000). In addition, the viral load at baseline is predictive of the rate of HIV-1 decline following antiretroviral therapy (Notermans *et al.*, 1998). Multiple studies have shown that the current repertoire of antiretroviral drugs is insufficient to completely eradicate HIV-1 from infected individuals (Wong *et al.*, 1997; Finzi *et al.*, 1997; Dornadula *et al.*, 1999).

In the era of HIV treatment, the use of sequential HIV RNA measurements may be more meaningful than any single measurement. The pre-treatment slopes of HIV-1 RNA decline in the acutely infected individuals increased significantly ($p = 0.0001$) after initiation of antiretroviral therapy. However, these post-treatment slopes were lower than those found in the chronically infected individuals ($p = 0.012$). Slopes were inversely correlated ($p = 0.012$) with baseline HIV-1 RNA (Putter *et al.*, 2000).

The combination of multiple adverse side-effects associated with HAART and the stringent demands for regimen adherence have prompted the design of new treatment strategies. Several studies have examined the longitudinal effects of multiple scheduled treatment interruptions where the long-term safety of this experimental protocol is largely unknown, particularly with respect to the generation of resistant viruses. However, such a treatment strategy would improve the likelihood of long-term adherence; the use of individual viral load plateaus will serve as an important guide in the aggressiveness and timing of treatment interruptions (Hatano *et al.*, 2000).

What the Future Holds

In just over two decades, the world has come to recognize a uniquely pathogenic new virus. The early epidemiologic pattern of the HIV/AIDS epidemic presented unique challenges to virologists, epidemiologists, clinicians, and public health officials. Throughout its 20-year history the epidemic continues to challenge us within our respective disciplines and in many ways has promoted an integration of these fields in an effort to curb its spread. Although the discovery of this new pathogen occurred relatively quickly after the first cases, there are still many questions as to how such a chronic virus infection can be so uniformly virulent 8–10 years post infection.

Many mysteries still surround the origin of the HIV virus and its relatives. The prevalence of the related simian viruses in African primates is so high that it suggests an ancient and stable virus–host relationship, and one that has developed into a virtually innocuous virus infection. As an intermediate, the HIV-2 virus is significantly less pathogenic than HIV-1, taking 20–30 years to develop AIDS, although the end-stage disease appears similar. We are still trying to discover the viral and host mechanisms by which this virus is able to persist for such a long period of time in an asymptomatic phase and yet somehow be triggered to cause fatal immunosuppression decades later.

Through molecular techniques such as PCR diagnostics and high-throughput sequencing we can now further appreciate the genetic diversity of HIV and SIVs. A decade ago it would take months to generate and analyze the sequence of a single HIV virus, a feat that this now readily achieved in a few days. We have also seen these technologies brought to bear on other epidemics of infectious diseases such as the recent severe acute respiratory syndrome (SARS) epidemic in Asia. There is no doubt that such new techniques will expedite our characterization of the ever-changing HIV epidemic and assist researchers in predicting the viral genotype that is relevant for targeting.

The genetic diversity of the virus at the level of the infected individual is now appreciated as a quasi-species rather than a single genotype that continues to challenge the immune system and clearly plays a role in the pathogenicity of the

virus. It also presents a significant barrier to the current armamentarium of antiretroviral drugs that we can provide to our patients with the emergence of viral resistant variants that may continue to require better and different therapies to control virus infection over the long term. At present, we do not have such therapies to affect a cure and the various viral reservoirs and significant viral replication rate suggest that this will be nearly impossible in the near future. Genetic diversity in HIV evidenced by different strains or subtypes has yielded a complex global map of variant viruses, affecting our ability to diagnose, treat, and ultimately vaccinate against an ever-changing set of viruses. We already recognize that these subtypes infecting a single population can generate recombinant viruses at an incredible rate which might predict that vaccine development may never keep pace to provide sterilizing immunity.

New technologies have made significant advances in our research to better understand and control this virus. The ability to measure HIV-1 viral load has revolutionized our ability to track virus infection and replication in the patient. The advent of viral load assays has been both a technological as well as pragmatic feat, supplying a sturdy clinical measure for the management of AIDS patients. Formerly, clinicians and scientists relied on difficult and cumbersome plasma and peripheral blood mononuclear cell cultures for virus isolation or p24 antigen quantitation to monitor infection in the individual patient. The development of PCR-based methods to measure viral RNA has been particularly useful in following patients and their therapeutic management. Importantly the measurement of viral burden has provided new insights into the mechanisms of HIV transmission and pathogenesis.

The twenty-first century will no doubt bring new technologies and research advances to HIV/AIDS research. In the past few years we have seen the global community at least acknowledge the inequalities of the HIV/AIDS global epidemic, and renewed interest and support for prevention, treatment and vaccines are being directed to the developing world. This is where the HIV/AIDS epidemic is most complex from a virological point of view, with multiple subtypes and types of HIV viruses and the ever-growing proportion of complex recombinants. The need to test HIV interventions and vaccines in these parts of the world is therefore critical, since their effects on the HIV/AIDS epidemic in these populations will have the largest impact. As scientists we need to continue our efforts to insure that good science, public health, and effective health policy will end the HIV/AIDS epidemic in the next century.

References

Allain J, Laurian Y, Paul D *et al.* (1987) Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. Potential clinical importance. *N Engl J Med* 317: 1114–21.

Allan JS, Short M, Taylor ME *et al.* (1991) Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J Virol* 65: 2816–28.

Ammann AJ, Abrams D, Conant M *et al.* (1983) Acquired immune dysfunction in homosexual men: immunologic profiles. *Clin Immunol Immunopathol* 27: 315–25.

Anderson RM, Schwartlander B, McCutchan F, Hu D (1996) Implications of genetic variability in HIV for epidemiology and public health. *Lancet* 347: 1778–9.

Anonymous (1982) Epidemiologic aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. *N Engl J Med* 306: 248–52.

Balfe P, Simmonds P, Ludlam CA, Bishop JO, Brown AJ (1990) Concurrent evolution of human immunodeficiency virus type 1 in patients infected from the same source: rate of sequence change and low frequency of inactivating mutations. *J Virol* 64: 6221–33.

Barin F, M'Boup S, Denis F *et al.* (1985) Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of West Africa. *Lancet* ii: 1387–90.

Barre-Sinoussi F, Chermann J-C, Rey F *et al.* (1983) Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220: 868–70.

Biberfeld G, Brown F, Esparza J *et al.* (1987) Meeting report: WHO working group on characterization of HIV-related retroviruses: Criteria for characterization and proposal for a nomenclature system. *AIDS* 1: 189–90.

Blatt S, Hendrix C, Butzin C *et al.* (1993) Delayed-type hypersensitivity skin testing predicts progression to AIDS in HIV-infected patients. *Ann Intern Med* 119: 177–84.

Bobkov A, Cheingsong-Popov R, Garaev M *et al.* (1994) Identification of an env G subtype and heterogeneity of HIV-1 strains in the Russian Federation and Belarus. *AIDS* 8: 1649–55.

Burke DS, McCutchan FE (1997) Global Distribution of Human Immunodeficiency Virus-1 Clades in: Rosenberg SA, ed. *AIDS: Biology, Diagnosis, Treatment and Prevention*. Philadelphia, PA: Lippincott-Raven Publishers, pp. 119–26.

Cao Y, Qin L, Zhang L, Safrit J, Ho DD (1995) Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med* 332: 201–8.

Charneau P, Borman AM, Quillent C *et al.* (1994) Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group. *Virology* 205: 247–53.

Clark DR, Wolthers KC (2000) T-cell dynamics and renewal in HIV-1 infection. *Aids Pathog* 28: 55–64.

Clark SJ, Shaw GM (1993) The acute retroviral syndrome and the pathogenesis of HIV-1 infection. *Immunology* 5: 149–55.

Clark S, Saag M, Decker W *et al.* (1991) High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med* 324: 954–60.

Clumeck N, Mascart-Lemone F, de Maubeuge J, Brenez D, Marcelis L (1983) Acquired immune deficiency syndrome in Black Africans [letter]. *Lancet* 1: 642.

Coombs RW, Collier AC, Allain J *et al.* (1989) Plasma viremia in human immunodeficiency virus infection. *N Engl J Med* 321: 1621–31.

Curran JW, Lawrence DN, Jaffe H *et al.* (1984) Acquired immunodeficiency syndrome (AIDS) associated with transfusions. *N Engl J Med* 310: 69–75.

Davis KC, Horsburgh CR, Jr, Hasiba U, Schocket AL, Kirkpatrick CH (1983) Acquired immunodeficiency syndrome in a patient with hemophilia. *Ann Intern Med* 98: 284–6.

De Leys R, Vanderborght B, Vanden Haesevelde M *et al.* (1990) Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin. *J Virol* 64: 1207–16.

Delassus S, Cheynier R, Wain-Hobson S (1991) Evolution of human immunodeficiency virus type 1 nef and long terminal repeat sequences over 4 years in vivo and in vitro. *J Virol* 65: 225–31.

Dewolf F, Spijkerman I, Schellekens PT *et al.* (1997) Aids prognosis based on HIV-1 RNA, CD4+ T-cell count and function – markers with reciprocal predictive value over time after seroconversion. *AIDS* 11: 1799–806.

Dornadula G, Zhang H, VanUitert B *et al.* (1999) Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* 282: 1627–32.

Elliott JL, Hoppes WL, Platt MS, Thomas JG, Patel IP, Gansar A (1983) The acquired immunodeficiency syndrome and Mycobacterium avium-intracellulare bacteremia in a patient with hemophilia. *Ann Intern Med* 98: 290–3.

Essex M (1982) Adult T-cell leukemia/lymphoma: Role of a human retrovirus. *J Natl Cancer Inst* 69: 981–5.

Essex M (1994) Simian immunodeficiency virus in people [editorial; comment]. *N Engl J Med* 330: 209–10.

Essex M, Kanki P (1988) The origins of the AIDS virus. *Sci Am* 259: 64–71.

Essex M, McLane MF, Lee TH *et al.* (1983) Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. *Science* 220: 859–62.

Essex M, McLane MF, Tachibana N, Francis DP, Lee TH (1984) Distribution of antibodies to HTLV-MA in patients with AIDS and related control groups in: Gross L, ed. *Human T-cell Leukemia Viruses*. New York: Cold Spring Harbor Press, pp. 355–62.

Essex M, McLane MF, Kanki PJ, Allan JS, Kitchen LW, Lee TH (1985) Retroviruses associated with leukemia and ablative syndromes in animals and in human beings. *Cancer Res* 45: 4534s–4538s.

Fahey JL, Prince H, Weaver M *et al.* (1984) Quantitative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immune deficiency syndrome from other immune subset disorders. *Am J Med* 76: 95–100.

Fahey JL, Taylor JM, Detels R *et al.* (1990) The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med* 322: 166–72.

Fauci AS (1993) Immunopathogenesis of HIV infection. *J Acquir Immune Defic Syndr* 6: 655–62.

Fauci AS (1996) Host factors and the pathogenesis of HIV-induced disease. *Nature* 384: 529–34.

Fenyo EM, Albert J, Asjo B (1989) Replicative capacity, cytopathic effect and cell tropism of HIV. *AIDS* 3: 5S–12S.

Finzi D, Hermankova M, Pierson T *et al.* (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278: 1295–300.

Francis DP, Curran JW, Essex M (1983) Epidemic acquired immune deficiency syndrome (AIDS): Epidemiologic evidence for a transmitted agent. *J Natl Cancer Inst* 71: 1–6.

Gallo RC, Salahuddin SZ, Popovic M *et al.* (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224: 500–3.

Gao F, Bailes E, Robertson DL *et al.* (1999) Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397: 436–41.

Gelmann EP, Wong-Staal F, Kramer RA, Gallo RC (1981) Molecular cloning and comparative analyses of the genomes of simian sarcoma virus and its associated helper virus. *Proc Natl Acad Sci USA* 78: 3373–7.

Gelmann EP, Popovic M, Blayney D *et al.* (1983) Proviral DNA of a retrovirus, human T-cell leukemia virus, in two patients with AIDS. *Science* 220: 862–5.

Georges-Courbot MC, Lu CY, Makuwa M *et al.* (1998) Natural infection of a household pet red-capped mangabey (*Cercocebus torquatus torquatus*) with a new simian immunodeficiency virus. *J Virol* 72: 600–8.

Goodenow M, Huet T, Saurin W, Kwok S, Sninsky J, Wain-Hobson S (1989) HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. *J Acquir Immune Defic Syndr* 2: 344–52.

Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA (1981) *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 305: 1425–31.

Gupta P, Kingsley L, Armstrong J, Ding M, Cottrill M, Rinaldo CR (1993) Enhanced expression of human immunodeficiency type 1 correlates with development of AIDS. *Virology* 196: 586–95.

Gurtler LG, Hauser PH, Eberle J *et al.* (1994) A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. *J Virol* 68: 1581–5.

Hatano H, Vogel S, Yoder C *et al.* (2000) Pre-HAART HIV burden approximates post-HAART viral levels following interruption of therapy in patients with sustained viral suppression. *AIDS* 14: 1357–63.

Haynes BF, Pantaleo G, Fauci AS (1996) Toward an understanding of the correlates of protective immunity to HIV infection. *Science* 271: 324–8.

Henrickson RV, Maul DH, Osborn KG *et al.* (1983) Epidemic of acquired immunodeficiency in rhesus monkeys. *Lancet* 1: 388–90.

Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR (1989) An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339: 389–92.

Ho DD, Moudgil T, Alam M (1989) Quantitation of human immunodeficiency virus in the blood of infected persons. *N Engl J Med* 321: 1621–5.

Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373: 123–6.

Hubert JB, Burgard M, Dussaix E *et al.* (2000) Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. *AIDS* 14: 123–31.

Huet T, Cheynier R, Meyerhans A, Roelants G, Wain Hobson S (1990) Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* 345: 356–9.

Jaffe HW, Francis DP, McLane MF *et al.* (1984) Transfusion-associated AIDS: serologic evidence of human T-cell leukemia virus infection of donors. *Science* 223: 1309–12.

Jain MK, John TJ, Keusch GT (1994) Epidemiology of HIV and AIDS in India. *AIDS* 8: S61–75.

Johnson PR, Gravell M, Allan J *et al.* (1989) Genetic diversity among simian immunodeficiency virus isolates from African green monkeys. *J Med Primatol* 18: 271–7.

Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC (1982) A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 218: 571–3.

Kanki PJ, Kurth R, Becker W, Dreesman G, McLane MF, Essex M (1985a) Antibodies to simian T-lymphotropic retrovirus type III in African Green monkeys and recognition of STLV-III viral proteins by AIDS and related sera. *Lancet* i: 1330–2.

Kanki PJ, McLane MF, King NWJ *et al.* (1985b) Serologic identification and characterization of a macaque T-lymphotropic retrovirus (HTLV) type III. *Science* 228: 1199–201.

Kanki P, Hunt RD, Essex M (1986a) The Pathobiology of Macaque Retroviruses closely related to Human T-cell Lymphotropic Viruses in: Salzman, LA, ed. *Animal Models of Retrovirus Infection and Their Relationship to AIDS*. Orlando, FL: Academic Press, pp. 223–32.

Kanki PJ, Barin F, M'Boup, S *et al.* (1986b) New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type IIIAGM (STLV-IIIAGM). *Science* 232: 238–43.

Kanki PJ, Hamel DJ, Sankalé J-L *et al.* (1999) Human immunodeficiency virus type 1 subtypes differ in disease progression. *J Infect Dis* 179: 68–73.

Kirchhoff F, Pohlmann S, Hamacher M *et al.* (1997) Simian immunodeficiency virus variants with differential T-cell and macrophage tropism use CCR5 and an unidentified cofactor expressed in CEMX174 cells for efficient entry. *J Virol* 71: 6509–16.

Korber BT, Allen EE, Farmer AD, Myers GL (1995) Heterogeneity of HIV-1 and HIV-2. *AIDS* 9: S5–18.

Kunanusont C, Foy HM, Kreiss JK *et al.* (1995) HIV-1 subtypes and male-to-female transmission in Thailand. *Lancet* 345: 1078–83.

Lane HC, Masur H, Gelmann EP *et al.* (1985) Correlation between immunologic function and clinical subpopulations of patients with the acquired immune deficiency syndrome. *Am J Med* 78: 417–22.

Letvin NL, Eaton KA, Aldrich WR *et al.* (1983) Acquired immunodeficiency syndrome in a colony of macaque monkeys. *Proc Natl Acad Sci USA* 80: 2718–22.

Louwagie J, McCutchan F, Mascola J (1993) Genetic subtypes of HIV-1. *AIDS Res Hum Retroviruses* 9 (Suppl 1): 147s–150s.

Lyles RH, Munoz A, Yamashita TE *et al.* (2000) Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. *J Infect Dis* 181: 872–80.

Mann JM, Bila K, Colebunders RL *et al.* (1986) Natural history of human immunodeficiency virus infection in Zaire. *Lancet* 2: 707–9.

Marlink R, Kanki P, Thior I *et al.* (1994) Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* 265: 1587–90.

Masur H, Michelis MA, Greene JB *et al.* (1981) An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 305: 1431–8.

McCutchan F, Salimen MO, Carr JK, Burke DS (1996) HIV-1 genetic diversity. *AIDS* 10: S13–S20.

Mellors J, Kingsley L, Rinaldo C, Jr *et al.* (1995) Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* 122: 573–9.

Mellors JW, Rinaldo CR Jr, Gupta, P, White RM, Todd JA, Kingsley LA (1996) Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272: 1167–70.

Mellors JW, Munoz A, Giorgi JV *et al.* (1997) Plasma viral load and CD4(+) lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 126: 946–54.

Myers G, Pavlakis GN (1992) In: Levy JA, ed. *The Retroviridae*. New York: Plenum Press, pp. 1–37.

Nahmias AJ, Weiss J, Yao X *et al.* (1986) Evidence for human infection with an HTLV-III/LAV-like virus in Central Africa. *Lancet* 1: 1279–80.

Nkengasong JN, Janssens W, Heyndrickx L *et al.* (1994) Genotypic subtypes of HIV-1 in Cameroon. *AIDS* 8: 1405–12.

Notermans DW, Goudsmit J, Danner SA, Dewolf F, Perelson AS, Mittler J (1998) Rate of HIV-1 decline following antiretroviral therapy is related to viral load at baseline and drug regimen. *AIDS* 12: 1483–90.

Obrien TR, Rosenberg PS, Yellin F, Goedert JJ (1998) Longitudinal HIV-1 RNA levels in a cohort of homosexual men. *J Acquir Immune Defic Syndr* 18: 155–61.

Oleske J, Minnefor A, Cooper R *et al.* (1983) Immune deficiency syndrome in children. *JAMA* 249: 2345–9.

Pantaleo G, Graziosi C, Demarest JF *et al.* (1993) HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 362: 355–8.

Pantaleo G, Menzo S, Vaccarezza M *et al.* (1995) Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med* 332: 209–16.

Panther LA, Tucker L, Xu C, Tuomala RE, Mullins JI, Anderson DJ (2000) Genital tract human immunodeficiency virus type 1 (HIV-1) shedding and inflammation and HIV-1 env diversity in perinatal HIV-1 transmission. *J Infect Dis* 181: 555–63.

Peeters M, Honore C, Huet T *et al.* (1989) Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* 3: 625–30.

Piot P, Quinn TC, Taelman H *et al.* (1984) Acquired immunodeficiency syndrome in a heterosexual population in Zaire. *Lancet* 2: 65–9.

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 77: 7415–19.

Poon MC, Landay A, Prasthofer EF, Stagno S (1983) Acquired immunodeficiency syndrome with *Pneumocystis carinii* pneumonia and *Mycobacterium avium-intracellulare* infection in a previously healthy patient with classic hemophilia. Clinical, immunologic, and virologic findings. *Ann Intern Med* 98: 287–90.

Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224: 497–500.

Putter H, Prins JM, Jurriaans S *et al.* (2000) Slower decline of plasma HIV-1 RNA following highly suppressive antiretroviral therapy in primary compared with chronic infection. *AIDS* 14: 2831–9.

Redfield R, Wright D, Tramont E (1986) The Walter Reed staging classification for HTLV-IIILAV infection. *N Engl J Med* 314: 131–2.

Rinaldo C, Huang X-L, Fan Z *et al.* (1995) High levels of anti-human immunodeficiency virus type 1 (HIV-1) memory cytotoxic T-lymphocyte activity and low viral load are associated with lack of disease in HIV-1-infected long-term nonprogressors. *J Virol* 69: 5838–42.

Rubinstein A, Sicklick M, Gupta A *et al.* (1983) Acquired immunodeficiency with reversed T4/T8 ratios in infants born to promiscuous and drug-addicted mothers. *JAMA* 249: 2350–6.

Sabin CA, Devereux H, Phillips AN, Janossy G, Loveday C, Lee CA (1998) Immune markers and viral load after HIV-1 seroconversion as predictors of disease progression in a cohort of haemophilic men. *AIDS* 12: 1347–52.

Sabin CA, Devereux H, Phillips AN *et al.* (2000) Course of viral load throughout HIV-1 infection. *J Acquir Immune Defic Syndr* 23: 172–7.

Saksela K, Steven C, Ribinstein P, Baltimore D (1994) Human immunodeficiency virus type 1 mRNA expression in peripheral blood cells predicts disease progression independently of the numbers of CD4+ lymphocytes. Proc Natl Acad Sci USA 91: 1104–8.

Saxinger WC, Levine PH, Dean AG *et al.* (1985) Evidence for exposure to HTLV-III in Uganda before 1973. *Science* 227: 1036–8.

Schacker TW, Hughes JP, Shea T, Coombs RW, Corey L (1998) Biological and virologic characteristics of primary HIV infection. *Ann Intern Med* 128.

Schupbach J, Popovic M, Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC (1984) Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* 224: 503–5.

Scott GB, Buck BE, Leterman JG *et al.* (1984) Acquired immunodeficiency syndrome in infants. *N Engl J Med* 310: 76–81.

Siegal FP, Lopez C, Hammer GS *et al.* (1981) Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med* 305: 1439–44.

Soto-Ramirez L, Renjifo B, McLane MF *et al.* (1996) HIV-1 Langerhan's cell tropism associated with heterosexual transmission of HIV. *Science* 271: 1291–3.

Stein D, Lyles R, Graham N *et al.* (1997) Predicting clinical progression or death in subjects with early-stage human immunodeficiency virus (HIV) infection – a comparative analysis of quantification of HIV RNA, soluble tumor necrosis factor type i receptors, neopterin, and beta(2)-microglobulin. *J Infect Dis* 176: 1161–7.

Tersmette M, De Goede R, Al B *et al.* (1988) Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J Virol* 62: 2026–32.

Tsujimoto H, Cooper RW, Kodama T *et al.* (1988) Isolation and characterization of simian immunodeficiency virus from mandrills in Africa and its relationship to other human and simian immunodeficiency viruses. *J Virol* 62: 4044–50.

UNAIDS (2002) *UNAIDS Report on the Global HIV/AIDS Epidemic 2002*. Geneva: Joint United Nations Programme on HIV/AIDS (UNAIDS).

Van de Perre P, Rouvroy D, Lepage P *et al.* (1984) Acquired immunodeficiency syndrome in Rwanda. *Lancet* 2: 62–5.

Vanharmelen J, Wood R, Lambrick M, Rybicki EP, Williamson AL, Williamson C (1997) An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa. *AIDS* 11: 81–7.

Wei X, Ghosh SK, Taylor ME *et al.* (1995) Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373: 117–22.

Weniger BG, Takebe Y, Ou C-Y, Yamazaki S (1994) The molecular epidemiology of HIV in Asia. *AIDS* 8: 13s–28s.

Wong JK, Xignacio CC, Torriani F, Havlir D, Fitch NFS, Richman DS (1997) In vivo compartmentalization of human immunodeficiency virus: evidence from the examination of pol sequences from autopsy tissues. *J Virol* 71: 2059–71.

Yu X, McLane MF, Ratner L, O'Brien W, Collman R, Essex M, Lee TH (1994) Killing of primary CD4+ T cells by non-syncytium-inducing macrophage-tropic human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 91: 10237–41.

Zekeng L, Gurtler L, Alaneze A *et al.* (1994) Prevalence of HIV-1 subtype O infection in Cameroon: preliminary results. *AIDS* 8: 1628–9.