AD_____

Award Number: DAMD17-95-C-5005

TITLE: Interactions of HIV-1 and HIV-2 in West Africa

PRINCIPAL INVESTIGATOR: Phyllis J. Kanki, D.V.M., Ph.D.

CONTRACTING ORGANIZATION: Harvard College
Cambridge, Massachusetts 02138

REPORT DATE: October 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Final (15 Nov 94 - 30 Sep 02)

4. TITLE AND SUBTITLE
Interactions of HIV-1 and HIV-2 in West Africa

5. FUNDING NUMBERS
DAMD17-95-C-5005

6. AUTHOR(S) :
Phyllis J. Kanki, D.V.M., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Harvard College
Cambridge, Massachusetts 02138

E*Mail: pkanki@hsph.harvard.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Since. the discovery of the second human immunodeficiency virus in 1985, considerable progress has been made in understanding the virology and epidemiology of HIV-2. The data suggests differences between HIV-2 and HIV-1 in geographic distribution, distinct epidemic trends, differences in perinatal transmission rates and incubation periods to the development of AIDS. The virologic determinants and mechanisms for these apparent biological differences are still unknown. However, an understanding of how HIV-2 differs from HIV-1 is essential for interpretations of comparative virologic studies. We have specifically studied the interactions between HIV-2 and HIV-1 and found that the attenuated phenotype of HIV-2 is apparently capable of providing protection from subsequent infection with HIV-1. In vitro studies suggest multiple mechanisms for such protection and we have characterized some of these virus and host determinants with an aim towards understanding correlates of immune protection. This further suggests that understanding HIV-2 immunity and cross-immunity may be useful for HIV vaccine design and development. A second aim of our studies has been the development of an HIV-2 based vaccine using the novel modified lethal factor toxin of anthrax. Several HIV-2 constructs have been made and demonstrated that this novel means of antigen delivery is capable of eliciting robust HIV-2 responses. We are hopeful that such information will be useful in future vaccine design for HIV/AIDS.

14. SUBJECT TERMS	15. NUMBER OF PAGES
human immunodeficiency virus, HIV-1, HIV-2, AIDS, Africa	40
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

Introduction

Human immunodeficiency virus type 2 (HIV-2) is the second human immunodeficiency virus in the class of human retroviruses, it constitutes the closest known virus related to the prototype AIDS virus, HIV-1. HIV-2 was first discovered based on its close serologic and antigenic relationship to Simian Immunodeficiency virus (SIV), in contrast to its weak cross reactivity with HIV-1 antigens [1] . Most HIV-2 isolates are essentially indistinguishable from the SIVs of such species as mangabeys and macaques, leading one to conclude that some SIVs and most HIV-2s are essentially the same virus [2, 3].

HIV-2 shares many virologic and biologic features with HIV-1. Both viruses are transmitted by the same routes of infection, they infect the same cells, and they both exhibit considerable genetic variation in the outer envelope gene. As with other retroviruses, both HIVs induce lifelong infection, with permanent integration of viral genetic material into the host cell's DNA. It was based on these initial similarities, that some believed that HIV-2 might cause a second worldwide AIDS epidemic. However, over 17 years since its discovery, research studies conducted both in the laboratory and in HIV-2 infected people have highlighted distinct biological differences between these related viruses [4-6] . Some of these unique properties include a distinct global distribution of the virus with limited spread, significantly reduced perinatal and sexual transmission, slower rates of progression to AIDS and the potential protective effect of HIV-2 from subsequent HIV-1 infection [6] . Over the past 8 years of funding by the DAMD, we have gained considerable information on the biology of HIV-2 infection in people and some of the potential consequences of its interaction with HIV-1 infection, the virus responsible for the global HIV-1/AIDS pandemic.

HIV-2 Virologic Characteristics

The antigenic relatedness of both SIV and HIV-2 to the prototype HIV-1 virus prompted both the discovery and further classification of these related viruses [1, 7, 8] . Similar to HIV-1, restriction site polymorphism and sequence data indicate significant genetic variability amongst HIV-2 strains [9, 10] . As more sequence data have become available from various HIV-2 and SIV strains, it has also become apparent that no branching order of divergence can be specified and that these virus types may in fact share a common ancestor [3, 11] .

By comparison to HIV-1, the genetic diversity of HIV-2 is less extensive and only two subtypes (A, B) have been well characterized, other studies have reported the existence of four additional subtypes (C, D, E and F), but different attempts to isolate viruses or obtain additional samples to sequence from

these identified subtypes have been unsuccessful [12] . Thus far, HIV-2 subtype A is the most characterized subtype and appears to be the major variant circulating in West Africa [13-15] Thus far, the Ghanaian isolates, subtype B [16, 17] are believed to be most distant from the prototype HIV-2/mangabey/macaque virus [2] . Only a recent study from Ivory Coast suggests a predominance of HIV-2 subtype B in this country [18] . Similar to the situation with HIV-1 subtypes, the potential impact of subtype differences on the epidemiology, pathogenicity and transmission of HIV-2 is not yet well appreciated.

AIM 1: HIV-1 and HIV-2 interactions at a population level

1.A. HIV-2 Epidemiology and Global Distribution

The isolation of a second retrovirus, HIV-2, led to fears that a second AIDS pandemic would occur, similar in scope and magnitude to that caused by HIV-1. However, the peculiar biologic properties of HIV-2, namely the lower transmissibility of this virus through both sexual and vertical routes, contributed to a more regionalized endemic distribution of the virus. Studies conducted shortly after the discovery of HIV-2 found significant rates of HIV-2 in a number of West African countries, frequently with low to absent rates of HIV-1 [19, 20] . In the late 1980's, countries such as Guinea Bissau, The Gambia, Cape Verde and Senegal, the prevalence of HIV-2 infection exceeded that of infection with HIV-1, but in most instances, HIV-1 infection has now increased and exceeded rates of HIV-2 infection [19, 21-23] . Rates of HIV-2 infection are highest in sexually active populations such as commercial sex workers, sexually transmitted disease patients, prisoners and people hospitalized with infectious diseases [19, 22, 24, 25]. Such risk groups usually have seroprevalence rates that are 5- to 10-fold higher than the general population, frequently represented by blood donors and pregnant women. Largely due to relatively low prevalence rates in most places outside of West Africa, it has been difficult to compare the prevalence rates in different countries due to differences in study design and diagnostic methodologies. Often times serosurveys were conducted with methods that failed to distinguish HIV-1 from HIV-2, thus erroneously reported type specific prevalence rates, or difficulties with diagnosis and distinction of HIV-dual infections resultant from the lack of HIV specificity in the serologic or nucleic-acid based assays.

In most other countries of West Africa such as Burkina Faso, Ghana, Ivory Coast, Nigeria, and Mali, infection with HIV-1 is more prevalent than with HIV-2; ranging from a 3-24 fold rate ratio (HIV-1 versus HIV-2) [6, 26-30] . For example, in a study from Bamako Mali, HIV-2 infection was found in 3.9% of 176 commercial sex workers, in contrast to the 20.45% HIV-1 infection. Comparison of HIV-1 and HIV-2 seroprevalence data from our study with previous data from Mali shows a significant rise in HIV-1

prevalence and a significant decrease in HIV-2 prevalence and confirms similar trends observed in neighboring countries [31] .

The existence of significant rates of HIV-1 and HIV-2 in many of these countries raised the question of what outcome would result from the interaction of these viruses at a population level. Anderson and May analyzed the available biological and epidemiological data on the pathogenicity, transmissibility and antigenic similarity of HIV-1 and HIV-2, and used simple mathematical models to study the competition between the two viral types [32] . The mathematical model of the concomitant transmission of the two viruses transmitted within the same sexually active population, suggested a positive association between pathogenicity and reproductive success, suggesting that HIV-1 would competitively displace HIV-2 in the longer term. This is supported by studies that measure and compare the rates of sexual and perinatal transmission of the two viruses, as well as the temporal falling trends of HIV-2 prevalence through the region [33-36] . Thus, the current data suggests that HIV-2 has been present in certain populations for a long time in order to establish endemic infection and its spread outside of these endemic areas is limited by a low transmission potential. It therefore seems unlikely that this virus will cause a global pandemic similar to that of HIV-1.

A second epidemiologic pattern of HIV-2 infection has been suggested from reports of HIV-2 in Portugal, Mozambique, Angola, southwestern India and Brazil, all areas with former ties to Portugal [37] [38, 39] which appear to have low but stable rates of HIV-2 in the population [40] . These countries once shared common historical-political ties, with economic relationships existing even today. HIV-2 has also been detected in the large cities of southwestern India [39] , perhaps because of exchange with the former Portuguese colonies of Africa. Goa, a former Portuguese colony, situated south of Bombay on the western coast, has reported 4.9% HIV-2 and 9.8% HIV-1 infection in STD patients [41] . Whereas, significant HIV-2 infection has not been reported in other parts of Asia to date.

Geographically, the distribution of HIV-2 seems to be totally independent from the distribution of HIV-1 [6, 19, 27, 37] . Countries in central or eastern Africa appear to be relatively free of HIV-2, as are most regions of Europe and North America. In these very low risk regions, almost all of the rare infections observed are either in West African immigrants or in individuals who had contact with West Africans.

1B. TRANSMISSION OF HIV-2

Although HIV-2 is transmitted by sex and blood, as is HIV-1, the rate of infection in West Africa appears more stable than for HIV-1. In Senegal, during an 8-year period of follow-up, there was a

26-fold increase in HIV-1, whereas HIV-2 remained relatively constant [33, 42] . This implies that HIV-2 may have been in the human population in Africa at least as long as HIV-1, and in West Africa HIV-2 has apparently been present considerably longer. The relative lack of significant HIV-2 prevalence in Europe, North America and Asia in the face of HIV-1 expansion also supports the general observation that HIV-2 is spread less efficiently than HIV-1 [19, 32, 33, 37, 43-46, 47, 48, 49] .

1C. HIV-1 Genetic Subtype Distribution in Dakar, Senegal.

The frequency of HIV-1 genetic subtypes in incident and prevalent HIV-1 infected individuals has been regularly evaluated in the cohort of commercial sex workers in Dakar over the past 17 years. In many instances, more detailed studies of the complete viral envelope or gag region were performed to characterize subtype A infections into the appropriate sub-subtype or Circulating Recombinant Form (CRF). This distribution of HIV-1 subtypes is quite similar to other West African countries, and so experiences with ARV therapy in these subtype infections may be informative to a large number of infected individuals throughout much of this geographic region of the continent.

1C1. HIV-1 Disease Progression Differs by Viral Subtype

The recognized differences in transmission and virulence of HIV-2 compared to HIV-1 indicate that HIVs can have different pathogenic potentials. This lends support to the hypothesis that genetically distinct subtypes of HIV-1 might also differ in their biological properties. Subtype differences in transmission and disease potential would drastically alter our predictions of the future of the HIV epidemic and its impact. Given the known differences in the geographic distribution and associated epidemics of different HIV subtypes, we asked whether HIV-1 subtypes might differ in their rate of disease development [50] .

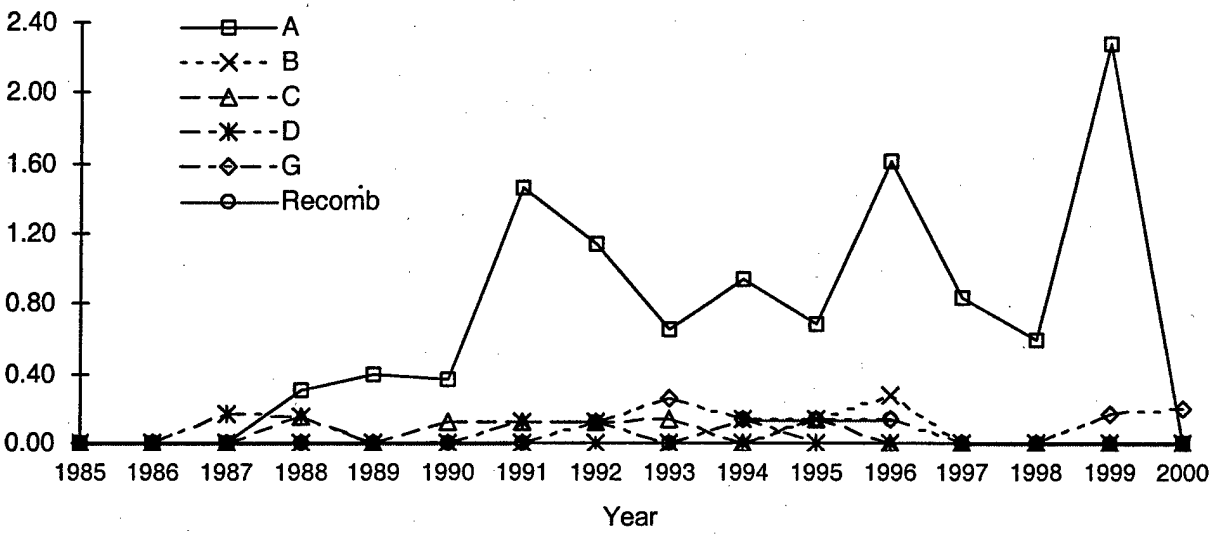
Table 1. HIV-1 subtypes in Dakar sex workers by serohistory (updated 2002)

HIV-1 subtype	Seroincident	Seroprevalent	Total
A	88	86	174
B	3	7	10
C	6	1	7
D	5	1	6
G	8	21	29
Complex recombinants	6	2	8
TOTAL	115	118	233

We have tracked the rates of HIV-2 and HIV-1 subtypes in the Dakar cohort for 17 years of study - in addition to the rate and dynamics of these viruses – we have also generated incidence curves for all virus types and subtypes (Figure 1).

By prospective clinical study of individuals with known type of infection, we have been able to evaluate the subtype specific disease causing potential of viruses in our cohort. The AIDS-free survival curves were distinct with subtype A demonstrating a longer AIDS-free survival than non-A subtypes; the 5 year AIDS-free survival probability was 87% in subtype A infected women compared to 52% for non-subtype A infected women (Figure 2: log rank test, p = 0.02; Wilcoxon-Gehan p =0.03). The Cox proportional hazards model estimated that women infected with a non-A subtype were 8.23 times more likely to develop AIDS compared with those infected with subtype A (95%CI= 1.70 to 39.8, p value = 0.009)

Figure 1: Annual Incidence (IR per 100 PYO) by HIV-1 Subtype



This was the first study that has evaluated disease progression in non-B subtype infections with known time of infection. In evaluation of the AIDS-free survival curves of women with incident A, C, D, and G infection, we have shown distinct differences in AIDS-free survival. Due to the small sample size per subtype, with few AIDS cases despite a lengthy study period, our estimate of AIDS incidence for each subtype should be considered imprecise, and further study is clearly warranted. The further investigation of differences between HIV-1 subtypes may be central to our understanding the diversity of HIV-1 pathogenesis and epidemiology, and its impact on HIV intervention strategies.

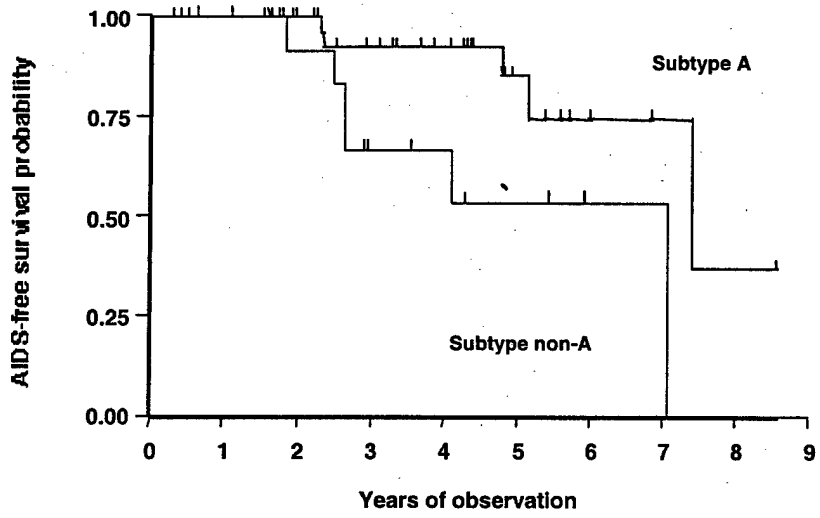


Figure 2: Kaplan-Meier AIDS-free survival in seroincident women comparing subtype A to all non-A subtypes. Log rank statistic p value = 0.02; Wilcoxon-Gehan statistic p value = 0.03

1C2. New HIV-1A Sub-subtype A3

Over the 17 year prospective study (1985-02) of high risk and HIV infected women in Senegal, West Africa we have demonstrated the dynamics of HIV-1 CRF 02 A/G (Ibng) as the predominant HIV-1 infection. In studies of HIV-2 protection, we first observed a novel HIV-1 subtype A variant that was seen in dually infected individuals, significantly more frequently than the more common HIV-1 CRF 02 A/G virus. This seemed to indicate that HIV-2 protection was sensitive to viral characteristics of the infecting HIV-1 subtype. Further genetic characterization of the gp120 and gag sequences of these women have allowed the identification of a novel subcluster of HIV-1 subtype A; termed sub subtype A3 [51] .

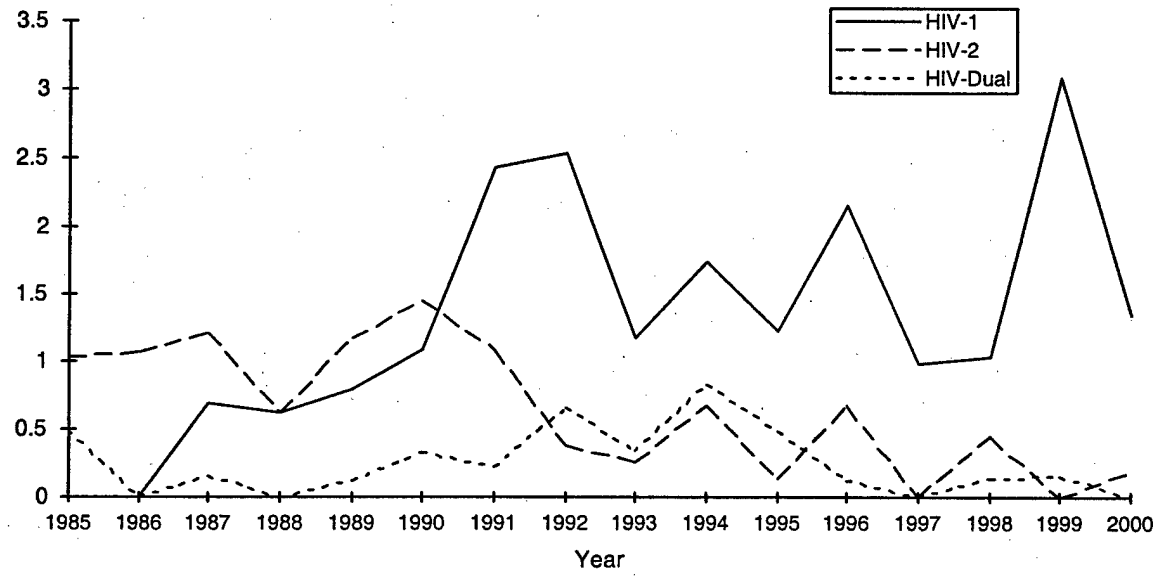
This novel virus variant arose in the early 1990s and has been transmitted within the cohort in concert with the CRF A/G (Ibng) virus. 37 sub subtype A3 viruses (9%) have been detected in the 17 year study period In addition, we have found novel recombinant viruses demonstrating A/G sequences with A3 sequences in the V3 region of the envelope indicative of a fitness advantage for this novel subtype cluster. Demographic, behavioral and clinical features have been analyzed to determine if biological differences between these viruses can be further demonstrated [51] .

1D. HIV-1 And HIV-2 Incidence Trends in Senegal

The most common modes of HIV transmission in HIV-2 endemic areas are perinatal and heterosexual transmission; since most West African countries have been afflicted with both HIV-1 and HIV-2 infections, direct comparison of transmission rates between the two viruses has been possible. In Senegalese female commercial sex workers followed over an 11 year period, the annual incidence of HIV-1 dramatically increased, with a 1.18 fold increased risk per year and a 13 fold increase in risk over the entire study period. The incidence of HIV-2 remained stable, despite higher HIV-2 prevalence [33, 52] . In this high risk group the heterosexual transmission of HIV-2 was significantly slower than

that of HIV-1, which strongly suggests differences in the infectivity potential of these two related immunodeficiency viruses [33, 52] . Using mathematical modeling techniques, the efficiency of heterosexual transmission of HIV-2 has been estimated to range from 5 to 9 times less than that of HIV-1 per sexual act with an infected partner [42]

Figure 3: Annual Incidence (IR per 100 PYO) by HIV Type



We have collaborated with biostatisticians, Peter Gilbert and Ian McKeague to analyze the comparative infectivity of HIV-1 and HIV-2 from our sex worker cohort data. Data from the study 1948 initially HIV negative sex workers were evaluated spanning the 1985-1999 time period. 196 seroconversions were documented during this period (127 HIV-1, 66 HIV-2 and 6 dual). We have developed new nonparametric competing risks failure time methods (McKeague, Gilbert and Kanki, 2001), which minimize modeling assumptions and control for risk factors for HIV. Probabilities of becoming infected with either type, both or neither were expressed in terms of the estimated numbers of sexual contacts and the parameters $r_1, r_2, p_1, p_2, p_{12}$. The resulting parametric likelihood was maximized under an independent competing risks assumption using standard methods to obtain point estimates and variance estimates r_1 and r_2. Rather than estimating r_1 and r_2 separately and then comparing the estimates to evaluate differential infectivity, our approach estimated the ratio r_1/r_2 directly and assessed if it significantly differed from one. We have submitted this manuscript for publication[53] .

The HIV-1/HIV-2 infectivity ratio over time was estimated by the HIV-1 /HIV-2 infection hazard ratio in sex workers adjusted by an estimate of the relative HIV-1 versus HIV-2 prevalence in the partner population. The estimated HIV-1/HIV-2 infectivity ratio was approximately constant over time, with

estimated common value 3.55. The finding of greater HIV-1 infectivity persisted in sensitivity analyses and in covariate adjusted analyses, with adjusted infectivity ratio estimates ranging between 3.40 and 3.86 [53] .

ID1. Perinatal Transmission of HIV-2 and HIV-1

Maternal or neonatal transmission of HIV-2 also occurs [54] , but it appears to be less efficient than for HIV-1 [55-57] . Prospective studies of HIV-2 perinatal transmission have been conducted in Guinea Bissau, Ivory Coast, France and Senegal, all demonstrating extremely low rates of perinatal transmission of HIV-2 (0-3.7% transmission) in contrast to that of HIV-1 (15-45% transmission) [34-36, 58, 59] . In studies that measured perinatal transmission of both viruses, the rate of HIV-1 transmission was 10-20 fold higher than that of HIV-2.

1E. HIV Dual Infections

For HIV investigators working in Africa where HIV-1 and HIV-2 infections are prevalent, it has been critical to identify cost-efficient alternative antibody testing strategies for screening, confirmation and discrimination of HIV-1 and HIV-2 infections. These strategies have included rapid simple tests (RST) as well as enzyme-linked immunosorbent assays (ELISA). In a study of 1110 consecutive sera from Bissau, Guinea Bissau, 198 (17.8%) were found HIV-seropositive: 52 (4.7%) HIV-1, 120 (10.8%) HIV-2, and 26 (2.3%) HIV-1/HIV-2 dually reactive. Western blot was used as a gold standard for confirming the reactivity of the specimens. The sensitivities of all assays was 100%; the specificities of the screening assays at initial and repeated testing was 98.0 and 99.7%, respectively, for Enzygnost and for Capillus, 99.8 and 99.9%, respectively. The various combinations of two or three assays showed specificities of 99.2-100%. Serodiagnostic strategies for HIV can be based on RST alone and differentiation between HIV-1 and HIV-2 can be achieved as part of these strategies. However, there is considerable variability in the capacity of individual assays to discriminate between HIV-1 and HIV-2, which needs to be considered in design and interpretation of such data.

When human serum samples have been tested in places such as Ivory Coast, Senegal, and Burkina Faso, a disproportionately large fraction of the samples often test as "dual positive" because they appeared reactive on both HIV-1 and HIV-2 confirmatory tests [19, 22, 50, 60] . These sites have significant rates of both HIV-1 and HIV-2, and distinction of viruses and designation of dual reactivity remains a diagnostic challenge for the typical HIV laboratory. The HIV dual antibody profile is characterized by antibodies with strong reactivity to the env antigens of both HIV-1 and HIV-2 by immunoblot and/or radioimmunoprecipitation analysis (RIPA) [1, 61-65] . Several possible biologic

explanations for this phenomenon can be entertained including: extensive cross reactivity by either of the HIV's, dual infection or infection with a recombinant virus. Isolation of both HIV-1 and HIV-2 has been reported from selected HIV-dual cases and PCR evidence of both viruses has ranged from 30-80%, in serologically defined dual reactives reported from similar populations [66-68] . It is unclear whether the low and variable rate of concordance between serology and PCR is due to extensive HIV-1 cross reactivity as suggested, misclassification of samples based on serodiagnosis or insensitivity of the PCR assays. Improvement of PCR assays with Southern blot detection of the amplified product has maximized the sensitivity and specificity for HIV-2 provirus detection [69, 70] . The further development of such assays will be critical to studies that seek to characterize HIV-2 biology and its interaction with HIV-1 in vivo. Largely due to such problems, it has been difficult to predict from the published literature what the interactions of these two distinct HIV type viruses would be in human populations. It seems clear that reports of dual infection in certain parts of Africa as well as the rest of the world, in the absence of HIV-2 infection alone, were more likely misinterpreted laboratory data than a true biological entity.

1F. HIV-2 PROTECTION from HIV-1

1F1. Population Studies in Senegal

Demonstrated differences in the infectivity and disease potential of HIV-2 compared to HIV-1 support the notion that the mechanism for such protection might be analogous to the attenuated virus vaccine model. In our studies of the female sex worker cohort in Dakar, Senegal we tested the hypothesis that the attenuated phenotype of HIV-2 infection might protect from subsequent HIV-1 infection [71] . HIV-1 infection in previous HIV negatives along with superinfection of HIV-2 infected was documented over the study period with both serology and PCR assays. A Poisson regression model was used to estimate the independent effect of demographic, behavioral, and biologic variables on the risk of HIV-1 infection. Despite higher incidence of other sexually transmitted diseases (STDs), HIV-2 infected women had lower incidence of HIV-1 than seronegatives, with a incidence rate ratio (IRR) of 0.32 ($p=0.008$). This analysis led to the conclusion that HIV-2 infection conferred a significant reduction in the subsequent risk of HIV-1 infection. Continued analysis of the Dakar cohort has extended the observation period from the first published report to over 13 years, HIV-2 protection ranges from 52-74% depending on the method of analysis [6, 71, 72] .

The generalizability of these findings have been questioned by studies from other West African sites. In Ivory Coast, Guinea Bissau and the Gambia, studies originally designed as cross-sectional surveys were analyzed for short periods of longitudinal observation [73] , as a result of their design they failed to possess sufficient statistical power, capable only of detecting an extremely high protected fraction

(>99%) of HIV-1 infection due to HIV-2 infection [72] . In addition, a mixture of serologic methods were employed by these studies, failing to meet the PCR based, gold standard for diagnosis of dual infection, critical to the objective evaluation of HIV-2 protection in vivo [[74, 75] . In a longitudinal study conducted in police officers in Guinea Bissau, Norrgren et al. failed to report a statistically significant result; and only a portion of the samples were diagnosed by PCR technology [76] . Unbiased, powerful studies, using sensitive and specific classification methods, will effectively address the generalizability of the observation of HIV-2's protective efficacy against subsequent HIV-1 infection. Despite these findings, the observation of the Dakar cohort continues to document a protective effect. The long person-time of observation with few losses to follow-up and rigorous PCR testing has supported these important findings in people [50] .

1F2. In vitro correlates of HIV-2 Protection

Studies from other research groups including our own have described in vitro interactions of HIV-1 and HIV-2 that support our in vivo observations, these range from virus-virus interactions to potential immune mediated mechanisms for HIV-2 protection. Arya et al have reported that HIV-2 inhibits the replication of HIV-1 at the molecular level. This inhibition was selective, dose-dependent, and nonreciprocal. Though the exact mechanism remains to be defined, the inhibition appeared to be mainly due to an intracellular molecular event because it could not be explained solely on the basis of cell surface receptor mediated interference. The results support the notion that the inhibition likely occurred at the level of viral RNA, possibly involving competition between viral RNAs for some transcriptional factor essential for virus replication [77, 78] .

We also suspected that viral determinants might play a role in HIV-2 protection, whereby one might hypothesize that HIV-2 would differentially protect from certain HIV-1 subtypes better than others. To investigate the HIV-1 subtypes involved in dual HIV-1 and HIV-2 infections, we sequenced the env region from 29 dually infected female commercial sex workers from Senegal [14, 79] . The majority of women (23 of 29) were infected by HIV-1 subtype A; within the HIV-1 subtype A sequences, 14 of 23 (60.8%) clustered with the West African associated A/G recombinant form (IbNG), and 9 of 23 (39.2%) formed a separate cluster distinct from the A/G IbNG. In contrast, in HIV-1 singly infected individuals, non-IbNG subtype A was found in only 13 of 98 (13.3%). Therefore, the lack of protection and/or interaction with HIV-2 was associated with a distinct HIV-1 A genotype. These results suggest differences in the biological properties of HIV-1 genotypes and their in vivo interaction with HIV-2 [79] .

Beta chemokines have now been identified as potent soluble suppressors of macrophage-tropic HIV infection, in vitro. Studies of multiply exposed uninfected individuals have implicated the role of

elevated □eta-chemokines in HIV resistance, in many cases, independent of genetic mutations in the chemokine receptor [80-82] . Macaque studies have also suggested a role for □eta-chemokines in vaccine induced protective immunity using a variety of vaccine candidates and live virus challenge [83] . We used an HIV-1 in vitro challenge system to determine if PBMCs from HIV-2 infected individuals showed altered susceptibility to HIV-1 infection [84] . Peripheral blood mononuclear cells were stimulated and infected with either R5 or X4 HIV-1 viruses. 14 of 28 (50%) HIV-2 PBMCs demonstrated over 90% inhibition of R5/HIV-1 infection compared to 0 of 19 HIV negative controls (Fisher exact test, p value =.0002). In contrast, HIV-2 positive and HIV negative control cells were equally susceptible to HIV-1/X4 infection. RANTES, MIP1□, MIP1□, the natural ligands of the CCR5 receptor, were measured in culture supernatant by ELISA, and supernatant levels of MIP-1□ (r=-0.56, p=.03) and MIP-1□ (r=-0.69, p=.004) were inversely correlated with HIV-1 replication. Using polyclonal antibodies to RANTES, MIP1□ and MIP1□, resistance was neutralized. A significant proportion of HIV-2 infected PBMCs demonstrate HIV-1 resistance in vitro; this resistance was □eta-chemokine dependent [84] . Studies are further needed to characterize this potent anti-viral activity and determine its potential contribution to in vivo protection.

HIV-2 infection might dramatically influence □eta-chemokine production by enhancing it in magnitude and duration, thus enabling HIV-2-infected individuals to cope favorably with subsequent exposure to HIV-1. This is supported by the studies demonstrating that binding of the HIV-2 envelope to the alpha chain of CD8 stimulates dramatic levels of □eta-chemokine production in comparison to HIV-1 gp120 activity [85] . Not only does this implicate a novel viral suppressive mechanism but one that may be adapted for immunoprophylaxis. Antiretroviral vaccine strategies that incorporate □eta-chemokine induction or other receptor-blocking functions raise some encouraging possibilities for vaccine design and development.

HIV-2 is primarily in West Africa where it infects an estimated 1–2 million people. It is also spread less efficiently than HIV-1, making projections for future infections much lower than for HIV-1. For these reasons, development of an HIV-2 vaccine has not been a high research priority. However, HIV-2 infects monkeys whereas HIV-1 does not, and the SIV vaccine model uses viruses that are closer to HIV-2 than HIV-1. The development and testing of an HIV-2 vaccine might therefore be simpler than the development of an HIV-1 vaccine. A few reports with HIV-2 have described experimental studies with limited vaccine protection [86, 87] , immune correlates of protection have not been identified [88-90] . Nonetheless, the data from human studies, suggests that HIV-2 may afford protection from HIV-1 is suggestive that a candidate vaccine based on HIV-2, might provide necessary cross-immunity for HIV-1 protection, and is worthy of further consideration.

1G. PATHOGENESIS OF HIV-2

During the late 1980s and early 1990s, natural history studies of HIV-1 infection conducted in the developed world provided important data on the pathogenesis of HIV-1 infection in vivo. Although numerous cross sectional studies of HIV-2 infection were conducted in the late 1980s, they were intrinsically limited in their ability to describe the natural history of HIV-2 infection, which required prospective studies [24] . Studies concerning the natural history of chronic infections such as HIV are difficult to achieve particularly with minimal loss to follow-up; not surprisingly such studies have been rare in developing countries, where viruses such as HIV-2 can be studied. Very few studies have evaluated deterioration of the immune system and/or alternatives in the virus load over time in HIV-2-infected people.

In cross-sectional studies, T4 lymphocyte counts and T4:T8 ratios appear reduced in HIV-2-infected healthy carriers, but less dramatically than for HIV-1-infected carriers [91-94] . Alterations in T-cell subsets evaluated prospectively have shown similar results, where immunosuppression in HIV-2 infected people was significantly slower than HIV-1 and could not be demonstrated in all followed subjects [95, 96] . Skin test anergy to various antigens is also less pronounced in HIV-2 infection [91, 94] .

The degree of immune activation and apoptosis in lymphocytes were compared in healthy West African patients infected with HIV-1 or -2. The lower decline of CD4 T cells in HIV-2- compared with HIV-1-infected donors was associated with lower levels of immune activation, evaluated by HLA-DR expression on lymphocytes and sera concentrations of IgG and beta2 microglobulin [40, 93, 94] . Ex vivo apoptosis was found in both infections in all lymphocyte subsets, including CD4 and CD8 T cells, as well as B cells, but was lower in HIV-2 than in HIV-1 infection [97] . These observations support the hypothesis that long-term activation of the immune system, weaker in HIV-2 infection, significantly contributes to T cell deletion and disease evolution[97] . The HIV-2 envelope has also been shown to have an inhibitory effect on T cell proliferation and the upregulation of CD40L and OX40, which are co-stimulatory molecules important in the activation and differentiation of the T cell response; this effect was accompanied by a reduced level of apoptosis [98] .

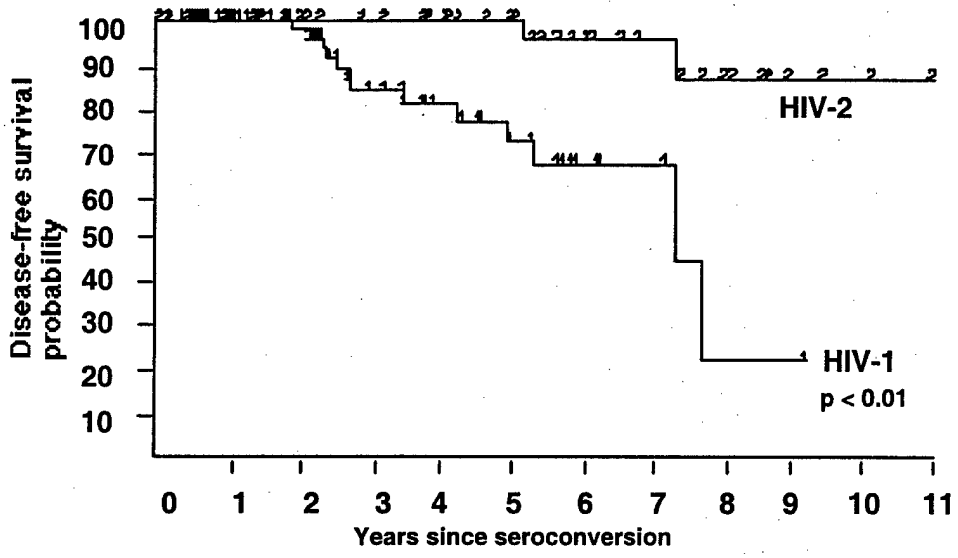


Figure 4 : Kaplan-Meier AIDS-free survival probability comparing incident HIV-1 and HIV-2 infected individuals. Wilcoxon-Gehan, p value <0.01

1H. HIV-2 Replication and Genetic Diversity

It is now well established that new genotypes of HIV-1 evolve over time in the same individual after infection. The earliest isolates generally grow in macrophages as well as lymphocytes. They do not cause syncytia in established T cell lines and are associated with a "slow-low" phase where the load of virus in blood and blood cells is very low. As disease develops, HIV-1s that have changes in the envelope V3 loop evolve. These viruses are often associated with higher virus loads in blood and syncytia induction in T cell lines and they are called "rapid-high" viruses [99, 100] . The ability of HIV-2s to induce syncytia may be lower than for HIV-1s [101] . HIV-2 is more difficult to isolate from asymptomatic people than is HIV-1; but when it is isolated, it appears to show the "slow-low" pattern [102, 103] . Similarly, the "rapid-high" isolates were more likely to be from HIV-2-infected people with disease. The determinants of cell tropism and replication capacity for HIV-2 have not yet been mapped. However, a correlation between the number of charged residues in the V3 loop, the nature of the residues at positions 18 and 19 of the V3 loop and the phenotype of HIV-2 isolates have been described [104, 105] .

1H1 HIV-2 Viral Diversity and Viral Burden

As with HIV-1, the polymerase activity of HIV-2 is error prone, and as a result extensive variation occurs between isolates [106] . This is primarily exhibited in the envelope gene against which much of the selective pressure of the immune system is exerted. This results in differences in env of as much as 1% per year for evolutionary selection [10, 107] . Limited studies on the inter-patient variability of HIV-2 have shown that the range of variability in the envelope V3 sequence is similar to the inter-

patient variability of HIV-1 [10, 13] . Tissue-specific quasi species has been identified in HIV-1 infection in vivo, and this has also been demonstrated in the analysis of blood and brain viral sequences from an HIV-2 infected individual [105, 108] . Evaluation of intra-patient variation in the V3 envelope region of HIV-2 in asymptomatic and symptomatic individuals followed over time has shown a lower variation when compared to HIV-1 [13] . This lower intra-patient variation appears to be a distinct feature of HIV-2 infection that may result from decreased viral burden and also contribute to lower rates of transmission and disease development.

In evaluation of paired blood and cervical lavage samples the rate of viral shedding was 36.4% for HIV-1 and 16% for HIV-2, after repeat PCRs [108] . In most cases, phylogenetic analysis showed that the viral sequences from blood and genital secretions were intermingled and subclusters did not segregate according to sample site. In rare cases, however, tissue-specific sequences were observed, suggesting a complex relationship between quasispecies in the two sites where preferential transmission of HIV variants may be due to multiple factors.

Evidence for a lower viral burden in HIV-2 infected individuals has been reported from both virus isolation and PCR studies [69, 70, 109-111] . The isolation rate of HIV-2 from peripheral blood mononuclear cells or plasma of asymptomatic HIV-2 infected individuals was lower than the isolation rate for HIV-1 [109] . At lower CD4+ lymphocyte counts, virus isolation was equally efficient in both infections. Studies in the Gambia and Senegal, suggest that proviral HIV-2 copies increase with disease development and the drop of CD4+ lymphocytes [70, 110] .

In our studies that compared the HIV-2 proviral loads in HIV-2 singly and dually infected individuals we found that the median proviral loads differed significantly, with those in the HIV-2 group ranging from 63.2 to 669.8 copies/10(5) CD4+ cells and demonstrating an inverse correlation with CD4+ lymphocyte count. The HIV dually infected persons showed less variation in viral load, ranging from 9.9 to 43.3 copies/10^5 CD4+ cells. In contrast, among the HIV dually infected persons, low HIV-2 proviral load was correlated with low CD4+ lymphocyte counts. The HIV-2 proviral loads in HIV dually infected persons were significantly lower than those in HIV-2 monotypically infected individuals ($P < .0001$), despite comparable CD4+ lymphocyte counts. These results suggest that different HIV-2 proviral dynamics prevail in HIV dual infection [70] .

HIV-2 is less pathogenic than HIV-1, but the mechanisms underlying this difference have not been defined. Levels of virus in the plasma are closely related to the pathogenicity of HIV-1. HIV-2 is much less pathogenic than HIV-1, and infection with HIV-2 leads to significantly lower plasma viral load [112,

113] . We developed an internally-controlled quantitative RT-PCR for this purpose, and determined plasma viral load in individuals from the cohort of registered commercial sex workers in Dakar, Senegal [111] . The assay has a lower limit of detection of 100 copies/ml, and is linear over 4 logs. HIV-2 viral RNA was detectable in 56% of all samples tested; the median load was 141 copies/ml. Levels of viral RNA in the plasma were inversely related to CD4+ cell counts. HIV-2 and HIV-1 viral loads were compared among the seroincident women in the cohort.

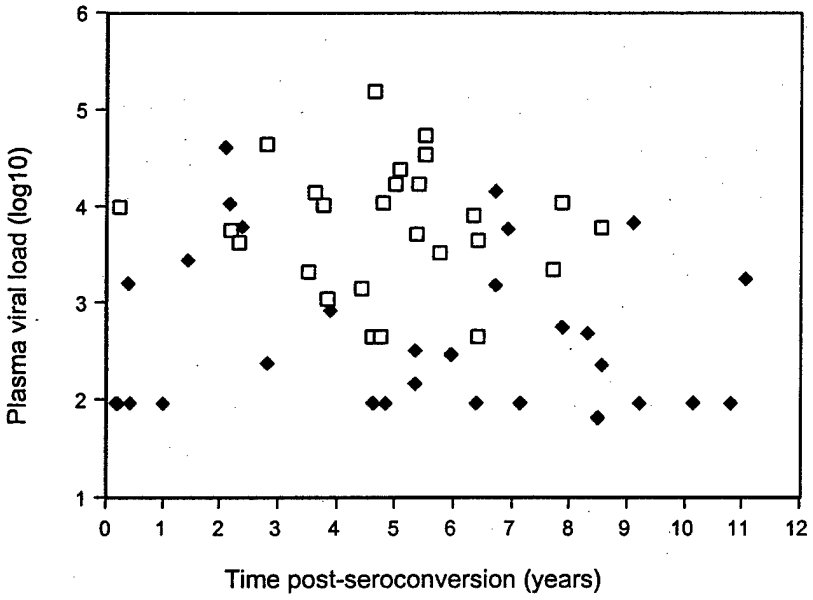


Figure 5: Time since seroconversion versus plasma viral load. HIV-1 infected subjects are represented by open squares (□); HIV-2 infected subjects by filled diamonds (◆). The p values correspond to the probability that HIV-1 and HIV-2 viral load differ within the time interval defined by the dotted lines. N.D. indicates statistical comparison was not done.

The median viral load was 30 times lower in the HIV-2 infected women ($p<0.001$, Wilcoxon rank-sum), irrespective of the length of time infected. This suggests plasma viremia is linked to the differences in pathogenicity of the two viruses.

We measured both viral RNA and proviral DNA in matched samples from 34 HIV-2 infected individuals [114] . Nearly half had undetectable viral RNA loads (<100 copies/ml), but levels of proviral DNA were relatively high, and confirmed that quantities of provirus were similar in HIV-1 and HIV-2 infection. Overall, HIV-2 proviral DNA did not correlate with viral RNA, and ratios of plasma virus to proviral template increased significantly with viral load. These results suggest that low viral load in HIV-2 infection is due to decreased rates of viral production, rather than differences in target cell infectivity.

Using an LTR-based RT-PCR assay, Berry et al. reported a cross-sectional study of HIV infected patients stratified according to percent CD4 T- lymphocytes (CD4%) [112] . HIV-2 infected individuals had lower plasma RNA levels than HIV-1 infected persons at lower CD4% levels, while at higher CD4% levels the plasma RNA levels were similar. Similarly, in studies of HIV-2 patients in Portugal,

plasma viremia could be detected in only 10 of 30 subjects(33%) , a rate much lower than that seen in HIV-1 infection. Virus was isolated from 16 of 30 patients(53.3%) . A significant correlation was found between CD4+ counts, clinical status, rate of virus isolation, and plasma viral load [115] . In a study of known seroconverters, HIV-2 infected individuals demonstrated a viral setpoint ~28-fold lower than HIV-1 infected individuals in the same study [116] .

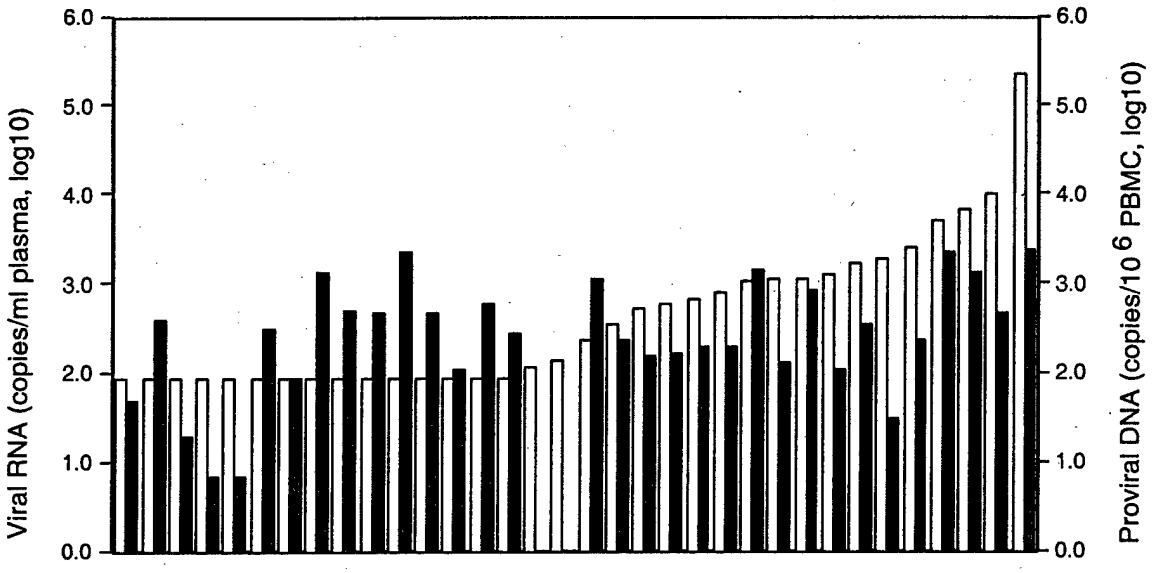


Figure 6: Levels of plasma viral RNA and proviral DNA in matched samples.
Light bars represent RNA measurements; dark bars represent proviral DNA.

Levels of virus in the plasma are closely related to the pathogenicity of HIV-1and infection with HIV-2 leads to significantly lower plasma viral load. To further identify the source of this difference, we measured both viral RNA and proviral DNA in matched samples from 34 HIV-2 infected individuals. The median level of HIV-2 RNA for the group was 189 copies/ml. Levels of HIV-2 RNA were below the limit of detection in nearly half the women, consistent with what we have previously reported in this population [114] . Levels of HIV-2 proviral DNA were similar to that of HIV-1 but failed to correlate with levels of viral RNA. Thus, it appears that significant differences occur upon expression, release and/or maintenance of virions in the bloodstream. It is formally possible that shifts in splicing patterns could be responsible for the differences in virion production, but further studies would be required to verify such an explanation for the current data. Comparative studies of both viral and host factors that may affect expression will be useful for understanding the differences between HIV-1 and HIV-2 pathogenesis

1I. HIV-2 CLINICAL OUTCOME

Early surveys included various case reports with AIDS in HIV-2-infected people [24, 117, 118] . The disease characteristics, including tuberculosis, chronic diarrhea, and *Candida* infections, seemed similar to diseases seen in HIV-1-associated AIDS [24, 119, 120] . Central nervous system involvement was also occasionally seen in HIV-2-infected people [121, 122] . However, classical African AIDS-associated diseases such as tuberculosis often had only a weak epidemiological association with HIV-2, even in HIV-2 endemic areas [6, 91, 123] .

Our prospective studies conducted in a registered female sex worker cohort in Dakar, Senegal has provided the unique opportunity of measuring the infection and progression rates of both HIV-1 and HIV-2 infections [91, 96, 123] . Importantly, these prospective studies have compared disease progression in individuals with known time of infection, and the cohort has been now observed for over 17 years, representing one of the longest HIV natural history studies in the field. The Kaplan-Meier analysis of HIV-2 incident infected individuals indicate that 85% (95%CI = 50 - 96%) remain AIDS-free after 8 years of HIV-2 infection (Figure 4) [50] . These differences in survival probabilities between HIV-2 and HIV-1, were also seen for CDC IV disease and CD4+ lymphocyte counts below 400 cells/mm^3 and CD4+ lymphocyte counts below 200 cells/mm^3, as outcomes.

In our prospective study of HIV-2 infected individuals, we have also identified individuals that fit a definition of long-term non-progression and can determine a rate of this phenotype in the study population [91, 96] , unpublished data. Using a definition of long-term non-progression of $\geq$8 years infection in the absence of AIDS or related symptoms, and stable CD4+ lymphocytes > 500 cells/mm^3, we have found 39 of 41 (95%) of our women would be classified as long-term non-progressors. This dramatic difference in pathogenicity provides a unique opportunity to identify viral and host immune mechanisms involved in a closely related and relevant virus system that is predicted to have a significantly slower course of progression.

Since slower disease course appears to be common in HIV-2 infection, we reasoned that certain subsets of the population would possess host characteristics that might predispose to a more rapid disease course. We conducted a case-control study investigating possible associations between HLA and the risk of disease progression in HIV-2 [124] . The HLA class I status was molecularly typed in 62 female sex workers from the Dakar, Senegal cohort; lack of antibodies to the HIV-2 antigen p26 was used as the surrogate marker for risk of disease progression [125] . Statistical analysis showed that HLA B35 was associated with lack of p26 antibodies ($p < 0.05$), and higher risk of disease

progression. The same association was found for the class I haplotypes B35-Cw4 and A23-Cw7 ($p < 0.05$), similar to the association with HIV-1 [126] . Our data shows that certain HLA molecules are associated with risk of disease progression in HIV-2, some of the alleles and haplotypes involved in susceptibility to disease are similar for both HIV-1 and HIV-2.

2. Development of an HIV-2 based Vaccine

2A. HIV-2 Immunity

The attenuated phenotype of HIV-2 infection in vivo has sparked considerable interest in understanding the immunopathogenesis of this particular HIV infection. Although certain viral determinants appear to be central to the lower replicative capacity in vivo, the virus appears uniquely immunogenic inducing significant immunity which may be central to the explanation of the weaker more attenuated phenotype of HIV-2 compared with HIV-1. As with HIV-1, the antibody response to most viral structural proteins occurs shortly after infection and is thought to persist. In HIV-2 infection, over 90% of seropositives evaluated in Senegal demonstrated strong antibodies to gag, pol and env-encoded antigens [127, 128] . As already noted, the cross-reactive response to analogous HIV -1 antigens is significant in highly conserved gene products such as gag and pol and less with envelope antigens.

Previously, circulating p24 antigen of HIV-1 has been useful as a marker of virus replication, in vivo, although this has been somewhat replaced with more direct measurements of virion particles using PCR technology in recent times. Analogous studies with HIV-2 have not been described, perhaps because antibodies to p26 are found in the majority of HIV-2 infected sera and therefore it has been assumed that complexing by free circulating p26 is less frequent. In our studies, we have found that the lack of antibodies to p26 is a fixed phenotype, not resultant from complexing of free p26, and predictive of a more rapid disease course [Popper, 1998 #364;[124]].

2A1. Neutralizing Antibody Responses

HIV-2 neutralizing antibodies have been described [129-133] , and in studies with fresh isolates, the broadness of the HIV-2 neutralizing response has been unusual and distinct from the neutralizing antibody response to HIV-1 [134] . Virus neutralizaton studies have shown that a proportion of HIV-2 sera are also capable of cross neutralizing HIV-1 isolates in addition to HIV-2 isolates [132, 135] . The degree to which HIV-1 positive sera can cross-neutralize HIV-2 isolates remains controversial. It is still not known whether some of the conserved domains of env will be capable of eliciting a cross-protective response to both viruses.

Studies of HIV-2 humoral immunity have now been extended to evaluate the highly related mucosal

immune response. Evaluation of HIV-2 antibody responses in the cervicovaginal secretions have shown that only a third of infected women generate IgA responses to HIV-2 envelope antigens in this compartment, suggesting lower levels of viral replication compared to HIV-1. Of interest, the cross-reactivity by IgG and IgA to heterologous envelope antigens was more frequent with HIV-2 infection [136] . Cervicovaginal cross-reactivity was more pronounced for HIV-2- specific antibodies to HIV-1 epitopes than conversely. Such features could be relevant to a differential heterosexual transmission of one type of HIV in an individual infected by the other type, in accordance with epidemiologic studies showing that HIV-2 infection protects from HIV-1 infection, and that HIV-1 infection does not appear to protect significantly from HIV-2 infection.

2A2. HIV-2 Cellular Immunity

Knowledge of immune mechanisms responsible for the cross-protection between highly divergent viruses such as HIV-1 and HIV-2 may contribute to an understanding of whether virus variability may be overcome in the design of vaccine candidates which are broadly protective across the HIV subtypes. In early CTL studies of HIV-2, responses against HIV-2 gag, pol and nef proteins were described; HLA-B53 restricted, HIV-2 gag-specific CTLs did not recognize target cells expressing HIV-1 gag proteins suggesting the absence of a cross-protective cellular response [137] . More recently, Bertoletti and colleagues also working in the Gambia, have shown that the majority of HIV-2-infected individuals with different HLA molecules possess a dominant cytotoxic T-cell response which is able to recognize HIV-1 Gag protein [138] . Furthermore, HLA-B5801-positive subjects have shown broad cross-recognition of HIV-1 subtypes since they mounted a T-cell response that tolerated extensive amino acid substitutions within HLA-B5801-restricted HIV-1 and HIV-2 epitopes. These results suggests that HLA-B5801-positive HIV-2-infected individuals have an enhanced ability to react with HIV-1 that could play a role in cross-protection [138] .

In collaboration with Dr. Huyen Cao, now at the California State Laboratories, we evaluated CTL responses in 13 HIV-2 infected women from Senegal using IFN-□ Elispot and flow based cytokine release assays using overlapping peptides from HIV-2 (ST) Gag, Env and Nef. We found that most (12/13) have reproducible CTL activity to one or more peptides with spot forming cells/millions ($SFC/10^6$ cells) averaging 200. However immunodominant activity to specific regions in Gag and Env was observed and correlated with intracellular production of cytokines (IL2, IFN-□) and chemokines (Mip-1□ and Mip-1□). Our findings suggest that HIV-2 infection actively induces a virus-specific cellular immune responses and release of soluble mediators that are known to inhibit HIV replication in most of the individuals studied. This further corroborates our results with in vitro challenge of HIV-2 PBMCs with HIV-1 virus, where beta chemokines were found to be a specific inhibitor of R5 virus infection [139]

In depth examination of this response might provide further insight to the lower pathogenesis of HIV-2 infection.

2B. Development of the LFn-HIV-2 constructs

Global statistics of the HIV epidemic continue to underscore the urgency for an effective HIV vaccine. The developing world, particularly regions of Africa, continue to bear a significant portion of the global HIV burden, and they are unlikely to benefit from recent advances in therapeutic regimes in the foreseeable future. Important to vaccine design is the understanding of pathogenic mechanisms of HIV infection and the potential immunologic responses or correlates necessary for HIV containment. The identification of such correlates has been hampered by relatively rare instances of natural immunity that have withstood the challenge of viral exposure. In the early 1800s, Jenner's identification of the protected milkmaids provided the characterization of cowpox, later utilized for vaccination against the antigenically related smallpox for its ultimate eradication.

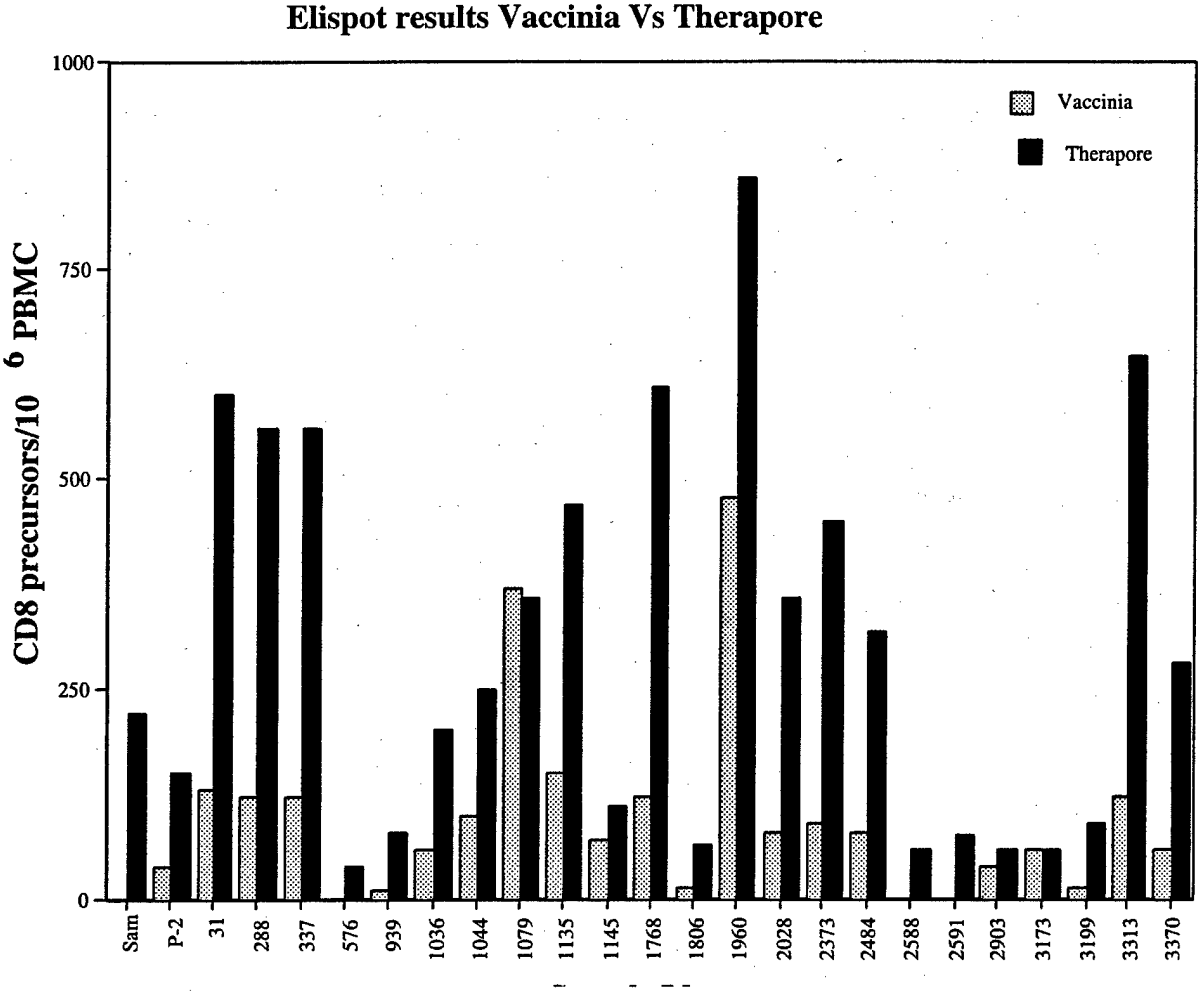
In our efforts to characterize the HIV-2 cellular immune response, we have developed novel methods that demonstrate high sensitivity and specificity. In collaboration with Dr. Huyen Cao at Massachusetts General Hospital, Dr. Abdoulaye Dieng-Sarr DSc., a Research Associate in my group has been trained in various methodologies for CTL detection and quantification. Upon initial assays with the Elispot technique, we were faced with abnormally low levels of reactivity that suggested poor antigen production by the vaccinia delivery system. Dr. Dieng-Sarr then collaborated with Dr. Yichen Lu, a research scientist in our department to develop the modified anthrax antigen for HIV-2 and to evaluate it in comparison to recombinant vaccina virus.

We have applied the sensitive Elispot technique to quantify the frequency of HIV-2 specific CTL precursors in uncultured PBMC from HIV-2 infected individuals. To assess the antigen delivery system using the modified non toxic form of the anthrax toxin, we fused the HIV-2 gag (P-26) to the terminal domain of Lethal Factor (LFn; 255 aa). The LFn-HIV-2 recombinant proteins was expressed and used as antigens to stimulate CTL in a ELISPOT assay in comparison to the classic delivery system using recombinant vaccinia Virus (rVV) expressing HIV-2 gag. HIV-2 specific Cytotoxic T-Lymphocyte responses against the structural protein Gag were investigated in unstimulated PBMC from HIV-2 infected individuals. CD8+ CTL's responsiveness to HIV-2 was measured by IFN-□ ELISPOT using two antigen delivery systems (rVV, LFn-P26). The assays with rVV and LFn-P-26 were performed in parallel on PBMCs from the same blood sample. Using the validation criteria, specific response to HIV-2 rVV gag were seen on 14/24 (58.3%). The frequencies of responsive HIV-

2 rVV CD8+ ranged from 0-150 precursors/10^6 PBMC with a mean number of precursors 58.9±47.7/10^6 PBMC. When cells were primed with the LFn-P-26 fusion protein, 21/24 (87.5%) demonstrated a specific CTL response with a higher mean number of precursors (235.2±214.59/10^6 PBMC); the frequencies of responsive LFn-P-26 CD8+ ranged from 0-650 precursors/10^6 PBMC. All three samples negative with the LFn-P-26 ELISPOT were also negative with the HIV-2 rVV assay. We also have found that samples that demonstrated a low frequency of precursors below 100 precursors/ 10^6 PBMC using the LFn-P-26 priming system were rarely positive with the rVV priming system (1/7). These results showed that the LFn-P-26 fusion protein prime better when samples have a low frequency of precursors [140].

We tested the level of HIV-2 plasma viral load in samples collected the same day among the 24 subjects included in this study. The plasma load varied from O - 60136 copies/ml with a mean of 7094.2±18711.15 copies/ml. We did not found a correlation between HIV-2 plasma viral load and and CTL precursors. However, when we stratified the individuals in three groups based on the cellular immmune response as low (CTL precursors < 100 precursors/ 10^6 PBMC), moderate (CTL precursors 100-200 precursors/10^6 PBMC) and strong (CTL precursors >300 precursors/10^6 PBMC). Interestingly we found that the group with strong cellular immune response had no detectable HIV-2 plasma load. We did not found a significant difference in viral load between the two groups with low and moderate cellular immune responses.

Figure 7: IFN-gamma production from CD8 precursors to Vaccina versus LFn- HIV-2 antigens



2B1. Construction, Expression and Purification of fusion proteins: LFn-gag(P-26) HIV-2.

The DNA fragment encoding the HIV-2 gag (P-26) was generated by PCR from the molecular clone pGh-123 (kindly provided by A. Adachi). The amplification was done using an upstream primer including at the 5'end the last two amino acid of the LFn coding sequence followed by a *SmaI* restriction site and sequence encoding HIV-2 gag P26. The downstream primer included at the 5' end two stop codons followed by a *BglII* restriction site and HIV-2 gag P26 specific sequence. The HIV-2 pGh123 was used as template; the purified PCR product was ligated and cloned using TOP-10 (In Vitrogen, San Diego,CA). Clones containing an insert after Eco RI digestion were sequenced in both strands to verify the reading frame and any mutations that could be introduced during PCR. The DNA fragment with the correct sequence was double digested with *SmaI* and *BglII* and ligated into the LFn

expression plasmid pET15bLFn (Novagen, Madison, WI). The pET15bLFn plasmid contains a T7 promoter, Histidine Tag(His6),the terminal domain of LF (LFn; 255 aa) and multiple cloning sites. The HIV-2 gag coding sequence ligated into the pET15b was used to transform E. coli BLR(DE3) for expression of the HIV-2 fusion protein (LFn-P-26). Clones containing an insert were sequenced to confirm that the fusion was correct.

The E. coli BLR(DE3) transformed with the LFn-P-26 fusion protein was grown in Luria Broth containing carbenicillin (50 µg/ml) at 37°C with shaking at 225 rpm. When culture cells density reached an OD_{600} of 0.6-0.8 unit, protein expression was induced by the addition of IPTG at a final concentration of 1mM for approximately 2.5 hours. Cells were pelleted by centrifugation at 4°C then resuspended in imidazole (1 mM) binding buffer (Novagen, Madison, WI), in presence of Proteases inhibitors cocktail EDTA free (Boehringer Mannheim, Framingham,MA). After homogenization cells were disrupted by sonication, the sonicate was centrifuged at 4°C, and the supernatant was loaded in a equilibrated nickel charged column. The His_6 tag present in the amino terminal of the fusion proteins allowed one step affinity purification of the expressed proteins using a Ni+ resin. The bound protein was eluted with 200 mM imidazole as specified by manufacturer (Novagen, Madison, WI). The eluted protein was desalted with a PD-10 Sephadex G-25 M column (Amersham pharmacia biotech, Piscataway, N.J.) and eluted in PBS. The Protein was concentrated with microconcentrator centricon-10 (Amicron, Danvers, MA), protein concentration was determined and samples were stored at -70°C.

3. METHODS:

The clinic provides clinical examinations and treatment of sexually transmitted disease (STDs) during biannual visits, which are required for the legal registration of prostitutes. All women that give informed consent will be enrolled in the study and a baseline questionnaire and health evaluation administered by study physicians. At the time of enrollment, and at subsequent visits, a complete physical examination was performed which emphasized HIV-related signs and symptoms. All biannual blood samples were tested for antibodies to each virus by immunoblot for HIV-1 and HIV-2 antibodies [141] . Samples showing dual reactivity will be confirmed with specific recombinant peptides from the envelope glycoproteins of HIV-1 and HIV-2 . All samples fitting the serologic criteria for a dual profile will be then subjected to HIV-1 and HIV-2 proviral DNA PCR using nested primer pairs for *gag* and *env* of both HIV-1 and HIV-2 viruses, as previously described [69] . T-cell subset determinations will be performed and DNA was extracted from the peripheral blood mononuclear cells (PBMCs) that remain after T-cell subset analysis. Clinical blood counts and chemistries will be

performed at regular intervals at Bio-24, the commercial clinical laboratory, that will perform automated analyses within 24 hours of sampling [91] .

STD Diagnosis

Specimens for gonorrhea culture will be collected, and chocolate medium supplemented with isovitalex, colistin, nystatin, and with and without vancomycin will be inoculated and incubated in CO2 atmosphere for the diagnosis of *Neisseria gonorrhoeae*. *Chlamydia trachomatis* diagnosis will be performed with an antigen ELISA assay (Syva, Palo Alto, CA). An alternative assay for chlamydia, based on PCR, has also been developed collaboratively between the Dakar research group and HSPH. Briefly, cervical swabs are collected and stored in PBS; these are subsequently processed using standard DNA extraction protocol. A 500 bp fragment of the human betaglobin gene and a 300 bp fragment of the endogenous plasmid of *Chlamydia trachomatis* are amplified by PCR, and visualized by EtBr gel electrophoresis. Results are confirmed using a colorimetric Southern blot, using a labeled internal oligonucleotide probe. This assay has been shown to be more sensitive than the assays currently in use [142] .

Treatment of Opportunistic Infections

An adequate management of opportunistic infections including a regular supply of drug for treatment and prophylaxis, is a key component of the success of HIV therapy level of care.

The most common opportunistic infections in AIDS found in Senegal include:

- Pulmonary TB, Herpes zoster, Cryptoccocus meningitis
- Toxoplasmosis, Candidiasis, Pneumocystis carinii
- Isosporosis- microsporidiosis

The following drugs are the standard care for treatment of these opportunistic infections:

- Rifampicin- Isoniazid- Pyrazinamide- Ethambutol
- Acyclovir
- Fluconazole
- Amphotericin B
- Trimethoprim- sulfamethoxazole

All HIV-related infections will be diagnosed with complete laboratory and clinical methods prior to the prescription of drugs. All laboratory diagnoses will be conducted at the clinical laboratory at Fann Hospital.

Data Analysis

Cases will be censored at their last clinic examination date or date of death, not caused by AIDS. Kaplan-Meier AIDS-free survival analysis and Cox proportional hazards models for AIDS defining events will be performed, stratified by treatment arm, as described (STATA, Stata Corporation, College Station, TX).

Logarithmic transformation of the viral load samples will yield a distribution that is approximately normally distributed. The mean HIV-1 viral load will be determined and used as a cutoff point to categorize new HIV-1 infection outcomes as either low or high viral load. Poisson regression modelling of the relative risk of HIV-1 infection with high viral load (as the dependant variable) associated with HIV-2 infection will be analyzed using Poisson regression modelling as previously described.

HIV-1 Viral Load Determination

Plasma will be immediately separated from the blood samples, aliquoted in two tubes, and stored at -80°C in Dakar prior to viral load determination. The Quantiplex bDNA assay 3.0 assay (Chiron) will be performed according to manufacturer's instructions. The Dakar laboratory has been successfully performing this assay for the past 3 years.

Quantitative HIV-2 DNA and RNA Viral Loads:

Plasma samples for detection of viral RNA have been collected beginning in 1996; blood was collected in EDTA-containing Vacutainer tubes, and separated using Ficoll-Hypaque (Organon Teknika, Durham, NC, USA), after which the plasma was stored at -70C within 6 hours of collection. Peripheral blood mononuclear cells (PBMCs) from the same separation are lysed immediately at 56C using 100ug/ml proteinase K in 1x Sodium Chloride -Tris -EDTA with 1% SDS, or stored in liquid nitrogen for later processing. DNA will be extracted using phenol/chloroform/isoamyl alcohol, followed by ethanol precipitation. The concentration of the purified DNA will be calculated by measuring the optical density at 260nm, and the purity by comparison with the optical density at 280nm; only samples with a ratio of 1.6 or greater will be further analyzed for this study.

HIV-2 viral load will be measured as described previously [111] . Briefly, virions will be pelleted from plasma, and lysed using a guanidinium isothiocyanate solution. An internal control RNA (IC) has been prepared by in vitro transcription, and will be added to samples during the purification process. The IC contains the same conserved primer binding sites as the HIV-2 samples, but is 25 nt longer, enabling us to distinguish the sample and IC amplicons by size. The purified RNA will be amplified

using a one-step RT-PCR kit (rTth EZ kit, Perkin-Elmer, Roche Molecular Systems, Brandenburg, NJ, USA) and primers designed to amplify a 200bp fragment of HIV-2 *gag*. One of the primers (OG63) will be labeled with a fluorescent dye, and the reaction product will be denatured and processed on an ABI 377 Automated Sequencer. The intensity of the fluorescence from each of the two products (sample and IC) will be recorded using Gene-Scan software (ABI, Foster City, CA, USA). The sample copy number is calculated as the ratio of fluorescence of the two products, multiplied by the number of copies of the internal control RNA per RT-PCR reaction (1000) and adjusted for the volume of sample processed (200µl). Previous studies have shown that the assay is linear over a 3-log range, and the limit of detection is <100 copies/ml .

HIV-2 proviral DNA

A quantitative assay for HIV-2 proviral DNA was established in our laboratory, and we have previously reported results using this technique [69] . The assay used a nested PCR to amplify the same portion of the *gag* gene of HIV-2 as in the RNA assay; results were obtained by comparison of the signal strength of the products from the sample and an internal control DNA template amplified in the same tube. For the proposed studies, we will use a non-radioactive format, replacing the 32P label for the OG63 primer in the second round with the fluorescent dye 6-FAM, processed the samples using an ABI 377 Sequencer, and analyzing the results using Gene-Scan software (ABI, Foster City, CA, USA).

HIV viral sequence analysis

HIV-1 viral subtype will be determined by PCR-based env sequencing. The HIV-1 C2-V3 env region will be amplified by nested PCR using two sets of primers: WT1-WT2 for the first round and KK30-KK40 for the second round. The conditions for the nested PCR and the set of primers for PCR amplification have been described elsewhere [143] . The PCR product will be purified by agarose gel electrophoresis and purification columns (Qiaquick gel extraction kit, Quiagen Inc., Chatsworth, CA). The purified product will be cloned in PCR2.1 vector (T/A cloning, Invitrogen, San-Diego, CA). Positive colonies chosen based on X-Gal metabolism or after PCR screening. Plasmid preparation for double-stranded DNA sequencing will be performed by alkaline lysis, using DEAE columns (Qiagen plasmid minikit, Quiagen Inc., Chatsworth, CA). Sequences will be determined for both DNA strands by dye terminator cycle sequencing using Taq polymerase (Perkin-Elmer, Applied Biosystem Division, Foster City, CA) and an automatic sequencer ABI 377 (Perkin-Elmer, Applied Biosystem Division, Foster City, CA). In all cases, two clones or more will be sequenced per samples.

Multiple alignment of all generated sequence and reference sequences from the HIV database will be performed with the Clustal package (Clustal X) with minor manual adjustment when necessary and complete removal of positions that contain gaps [144] . Phylogenetic analysis will be performed by the neighbor-joining method and reliability estimated by 1000 bootstrap resamplings. Subtype assignment was established based on phylogenetic clustering with reference sequences supported by a bootstrap value above 75%. Distance analysis (p distance) will be performed using MEGA (Pennsylvania State University).

4. CONCLUSIONS

Since the discovery of the second human immunodeficiency virus in 1985, considerable progress has been made in understanding the virology and epidemiology of HIV-2. The data suggests differences between HIV-2 and HIV-1 in geographic distribution, distinct epidemic trends, differences in perinatal transmission rates and incubation periods to the development of AIDS. The virologic determinants and mechanisms for these apparent biological differences are still unknown. However, an understanding of how HIV-2 differs from HIV-1 is essential for interpretations of comparative virologic studies. We are hopeful that such comparative studies will yield important information on the pathogenic mechanisms employed by HIV viruses and lead the way to the development of effective interventions for the prevention of AIDS. This is best exemplified in the studies that indicate that this close relative of HIV-1 infection, via its attenuated phenotype, may confer significant protection from subsequent HIV-1 infection. This further suggests that understanding HIV-2 immunity and cross-immunity may be useful for HIV vaccine design and development.

References:

1. Barin F, Mboup S, Denis F, Kanki P, Allan JS, Lee TH, Essex M. Serological Evidence for Virus Related to Simian T-Lymphotropic Retrovirus III in Residents of West Africa. Lancet (1985); **ii**:1387-1390
2. Essex M, Kanki P. The Origins of the AIDS Virus. Sci. Amer. (1988);**259**:64-71
3. Gao F, Yue L, White A, Pappas PG, Barchue J, Hanson AP, Greene BM, Sharp PM, Shaw GM, Hahn BH. Human infection by genetically diverse SIVSM-related HIV-2 in West Africa. Nature (1992);**358**:495-9
4. Essex M, Kanki P. Human Immunodeficiency Virus Type 2 (HIV-2). In: Broder S, Merigan T, Bologenesi D, eds. Textbook of AIDS Medicine. Baltimore: Williams & Wilkins, 1994:873-886
5. Markovitz DM. Infection with the human immunodeficiency virus type - 2. Ann Intern Med (1993);**118**:211-18
6. Kanki PJ. Human Immunodeficiency Virus Type 2, (HIV-2). AIDS Reviews (1999);**1 (20)**:101-108
7. Kanki P, Barin F, Mboup S, Essex M. Relationship of Simian T-Lymphotropic Virus Type III to Human Retroviruses in Africa. Antibiot. Chemother (1987);**38**:21-27
8. Biberfeld G, Brown F, Esparza J, Essex M, Gallo R, Montagnier L, Najera R, Risser R, Schild G. Meeting Report, WHO working group on characterization of HIV-related, retroviruses: Criteria for characterization and proposal for a nomenclature system. AIDS (1987);**1**:189-90
9. Franchini G, Collalti E, Arya SK, Fenyö EM, Biberfeld G, Zagury JF, Kanki P, Wong-Staal F, Gallo RC. Genetic Analysis of a New Subgroup of Human and Simian T-Lymphotropic Retroviruses: HTLV-IV, LAV-2, SBL6669, and STLV-III$_{AGM}$. AIDS Res Hum Retroviruses (1987);**3**:11-17
10. Boeri E, Giri A, Lillo F, Ferrari G, Varnier O, Ferro A, Sabbatani S, Saxinger WC, Franchini G. In vivo genetic variability of the human immunodeficiency virus type 2 V3 region. J Virol (1992);**66**:4546-50
11. Kirchhoff F, Jentsch KD, Bachmann B, Stuke A, Laloux C, Luke W, Stahl Hennig C, Schneider J, Nieselt K, Eigen M. A novel proviral clone of HIV-2: biological and phylogenetic relationship to other primate immunodeficiency viruses. Virology. (1990);**177**:305-311
12. Gao F, Yue L, Robertson DL, Hill SC, Hui H, Biggar RJ, al. e. Genetic diversity of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology. J Virol (1994);**68**:7433-7447
13. Sankalé JL, Sallier de la Tour R, Renjifo B, Siby T, Mboup S, Marlink R, Essex M, Kanki P. Intra-patient Variability of the Human Immunodeficiency Virus Type-2 (HIV-2) Envelope V3 Loop. AIDS Research Human Retroviruses (1995);**11**:617-23
14. Dieng-Sarr A, Sankalé J-L, Hamel DJ, Guèye-NDiaye A, Essex M, MBoup S, Kanki PJ. Genetic Analysis of HIV Type 2 in Monotypic and Dual HIV Infections. AIDS Research and Human Retroviruses (2000);**16(3)**:297-300
15. Peeters M, Gueye A, MBoup S, Bibollet-Ruche F, Ekaza E, Mulanga C, al. e. Geographical distribution of HIV-1 group O viruses in Africa. AIDS (1997);**11**:493-498
16. Dietrich U, Adamski M, Kreutz R, Seipp A, Kuhnel H, Rubsamen Waigmann H. A highly divergent HIV-2-related isolate. Nature (1989);**342**:948-950
17. Kawamura M, Katahira J, Fukasawa M, Sakuragi J, Ishikawa K, Nakai M, Mingle JA, Osei-Kwasi M, Netty VB, Akari H. Isolation and characterization of a highly divergent HIV-2[GH-2]: generation of an infectious molecular clone and functional analysis of its rev-responsive element in response to primate retrovirus transactivators (Rev and Rex). Virology (1992);**188**:850-3
18. Pieniazek D, Ellenberger D, Janini L, Ramos A, Nkengasong J, al. e. Predominance of Human Immunodeficiency Virus Type 2 Subtype B in Abidjan, Ivory Coast. AIDS Research Human Retroviruses (1999);**15**

19. Kanki P. West African Human Retroviruses Related to STLV-III. AIDS (1987);**1**:141-145
20. Harrison LH, José da Silva AP, Gayle HD, Albino P, George R, Lee-Thomas S, Rayfield MA, Castillo FD, Heyward WL. Risk factors for HIV-2 infection in Guinea-Bissau. JAIDS (1991);**4**:1155-1160
21. Naucler A, Albino P, Da Silva AP, Andreasson P, Andersson S, Biberfeld G. HIV-2 infection in hospitalized patients in Bissau, Guinea-Bissau. AIDS (1990);**5**:301-304
22. Denis F, Barin F, Gershy-Damet G, Rey JL, Lhuillier M, Mournier M, Leonard G, Sangaré A, Goudeau A, Mboup S, Kanki P, Essex M. Prevalence of Human T-Lymphotropic Retroviruses Type III (HIV) and Type IV in Ivory Coast. Lancet (1987); **i**:408-411
23. Pepin J, Dunn D, Gaye I, Alonso P, Egboga A, Tedder R, Piot P, Berry N, Schellenberg D, Whittle H. HIV-2 infection among prostitutes working in The Gambia: association with serological evidence of genital ulcer diseases and generalized lymphadenopathy. AIDS (1991);**5**:69
24. Romieu I, Marlink R, Kanki P, M'Boup S, Essex M. HIV-2 link to AIDS in West Africa. JAIDS (1990);**3**:220-230
25. Naucler A, Andreasson PA, Costa CM, Thorstensson R, Biberfeld G. HIV-2-associated AIDS and HIV-2 seroprevalence in Bissau, Guinea-Bissau. JAIDS (1989);**2**:88-93
26. Kanki P, Marlink R, Siby T, Essex M, Mboup S. Biology of HIV-2 Infection in West Africa. In: Papas TS, ed. Gene Regulation and AIDS. Houston: Portfolio Publishing Company of Texas, 1990:255-271
27. Kanki P, DeCock KM. Epidemiology and Natural History of HIV-2. AIDS (1994);**8**:S1-S9
28. Ankrah T, Roberts M, Antwi P, Atubrah MP, Bawuah PP, Kotoh S, Acheampong JW, Wood JG, Boateng KA, Mingle JA. The African AIDS case definition and HIV serology in medical in-patients at Komfo Anokye Teaching Hospital, Kumasi, Ghana. West Afr J Med (1994);**13**:98
29. Olaleye O, Bernstein L, Ekweozor C, Sheng Z, Omilabu SA, Li XY, Sullivan-Halley J, Rasheed S. Prevalence of human immunodeficiency virus types 1 and 2 infections in Nigeria. J Infect Dis (1993);**167**:710
30. Maiga Y, Sissoko Z, Maiga M. Etude de la seroprevalence de l'Infection a VIH dans les 7 regions economiques du Mali. In: VIIIth International Conference on AIDS in Africa/VIIIth African Conference on Sexually Transmitted Diseases. Marrakech:, 1993
31. Peeters M, Koumare B, Mulanga C, al. e. Genetic subtypes of HIV type 1 and HIV type 2 strains in commercial sex workers from Bamako, Mali. AIDS Research & Human Retroviruses (1998);**14**:51-58
32. Anderson RM, May RM. The population biology of the interaction between HIV-1 and HIV-2: coexistence or competitive exclusion? AIDS (1996);**10**:1663-73
33. Kanki P, Travers K, Hernandez-Avila M, Marlink R, Mboup S, Gueye-Ndiaye A, Siby T, Thior I, Sankalé JL, Ndoye I, Hsieh CC, Essex M. Slower Heterosexual Spread of HIV-2 Compared with HIV-1. Lancet (1994);**343**:943-946
34. Andreasson P, Dias F, Naucler A, Anderson S, Biberfeld G. A prospective study of vertical transmission of HIV-2 in Bissau, Guinea-Bissau. AIDS (1993);**7**:989
35. Ngagne M, Diouf A, Kebe F, et al. Histoire Naturelle de la transmission verticale VIH1 et VIH 2 a Dakar. In: IXth International Conference on AIDS and Associated Cancers in Africa. Kampala, Uganda:, 1995
36. Adjorlolo-Johnson G, DeCock K, Ekpini E, Vetter KM, Sibailly T, Brattegaard K, Yavo D, Doorly R, Whitaker JP, Kestens L. Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. JAMA (1994);**272**:462-66
37. Smallman-Raynor M, Cliff A. The Spread of Human Immunodeficiency Virus Type 2 into Europe: A Geographical Analysis. Intl J of Epi. (1991);**20**:480
38. Santos-Ferreira MO, Cohen T, Lourenco MH, Almeida MJ, Chamaret S, Montagnier L. A study of seroprevalence of HIV-1 and HIV-2 in six provinces of People's Republic of Angola: clues to the spread of HIV infection. Journal of Acquired Immune Deficiency Syndromes (1990);**3**:780-6

39. Rubsamen-Waigmann H, Briesen H, Maniar J, Rao P, Scholz C, Pfutzner A. Spread of HIV-2 in India. Lancet (1991);**337**:550-551
40. Victorino RM, Guerreiro D, Lourenco MH, Ferreira S, Teles LC. Prevalence of HIV-2 infection in a family planning clinic in Lisbon. International Journal of STD & AIDS (1992);**3**:281-4
41. Rubsamen-Waigmann H, Maniar J, Gerte S, Brede HD, Dietrich U, Mahambre G, Pfutzner A. High proportion of HIV-2 and HIV-1/2 double-reactive sera in two Indian states, Maharashtra and Goa: first appearance of an HIV-2 epidemic along with an HIV-1 epidemic outside of Africa. Int J Med Microbiol Virol Parasitol Infect Dis. (1994);**280**:398-402
42. Donnelly C, Leisenring W, Kanki P, Awerbuch T, Sandberg S. Comparison of transmission rates of HIV-1 and HIV-2 in a cohort of prostitutes in Senegal. Bulletin of Mathematical Biology (1993);**55**:731-43
43. Hendry R, Parks D, Campos Mello D, Quinnan G, Balao Castro B. Lack of evidence for HIV-2 infection among at-risk individuals in Brazil. JAIDS (1991); **4**:623
44. Kvinesdal BB, Worm AM, Lindhardt BO, Jensen BL, Nielsen CM, Poulsen AG. HIV-2 infection in Denmark. Scandinavian J Inf Dis (1992);**24**:419-21
45. Georgoulias V, Agelakis A, Fountouli P, Darivianakis K, Bibakis K, Nikoloudis E, Ammar A, Bertoli AM, Malliarakis-Pinetidou E. Seroprevalence of HIV-2 infection in Greece (Crete). JAIDS (1990);**3**:1188-92
46. Costigliola P, Ricchi E, Manfredi R, Tumietto F, Barelli A, Borri A, De Rosa V, Chiodo F. No evidence of HIV-2 infection amongst HIV-1 Ab positive people in the largest cities of north-eastern Italy. European J Epid (1992);**8**:140-1
47. Estebanez P, Sarasqueta C, Rua-Figueroa M, Fitch K, Eugenia PV, Varela JM, Contreras G, Casado C, Perez L, Najera R. Absence of HIV-2 in Spanish groups at risk for HIV-1 infection. AIDS Research & Human Retroviruses (1992);**8**:423-4
48. Downing RG, Biryahwaho B. No evidence for HIV-2 infection in Uganda. Lancet (1990);**336**:1514-5
49. O'Brien TR, George JR, Epstein JS, Holmberg SD, Schochetman G. Testing for antibodies to human immunodeficiency virus type 2 in the United States. MMWR - Morbidity & Mortality Weekly Report (1992);**41**:1-9
50. Kanki PJ, Hamel DJ, Sankalé J-L, Hsieh C-C, Thior I, Barin F, Woodcock SA, Guèye-NDiaye A, Zhang E, Montano M, NDoye I, Essex ME, MBoup S. Human Immunodeficiency Virus Type 1 Subtypes Differ in Disease Progression. Journal of Infectious Diseases (1999);**179**:68-73
51. Thakore S, Sankalé, J-L, Hamel, D., Eisen, G., Obasanjo, O., MBoup, S., Kanki, P.J. Unique HIV-1 variant emerging in West Africa: HIV-1 Sub-subtype A3. submitted (2002)
52. Kanki P. Epidemiology and Natural History of HIV-2. In: DeVita VJ, Hellman S, Rosenberg S, eds. In AIDS: Etiology, Diagnosis, Treatment ,and Prevention. 4th Edition ed. Philadelphia, PA: J. B. Lippincott Co, 1997:127-135
53. Gilbert P, McKeague I, Eisen G, Mulllins C, Guéye-Ndiaye A, MBoup S, Kanki P. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. Statistics in Medicine (2002);**in press.**
54. Morgan G, Wilkins HA, Pepin J, Jobe O, Brewster D, Whittle H. AIDS following mother-to-child transmission of HIV-2. AIDS (1990);**4**:879-82
55. Gayle H, Gnaore E, Adjorlolo G, Ekpini E, Coulibaly R, Porter A, Braun M, Zabban M, Andou J, Timite A. HIV-1 and HIV-2 infection in children in Abidjan, Cote D'Ivoire. JAIDS (1992);**5**:513
56. Del Mistro A, Chotard J, Mali A, Whittle H, De Rossi A, Chieco-Bianchi L. HIV-1 and HIV-2 seroprevalence rates in mother-child pairs living in The Gambia, West Africa. JAIDS (1992);**5**:19-24
57. Poulsen AG, Kvinesdal BB, Aaby P, Lisse IM, Gottschau A, K. M, Dias F, Lauritzen E. Lack of evidence of vertical transmission of human immunodeficiency virus type 2 in a sample of the general population in Bissau. JAIDS (1992);**5**:25-30

58. Anonymous. Comparison of vertical human immunodeficiency virus type 2 and human immunodeficiency virus type 1 transmission in the French prospective cohort. The HIV Infection in Newborns French Collaborative Study Group. Pediatr Infect Dis J. (1994);**13**:502
59. Abbott RC, Ndour-Sarr A, Diouf A, Ndoye T, Ba D, Tall N, Mbaye N, Kébé F, Diadhiou F, Essex M, Marlink R, Mboup S, Kanki P. Risk Determinants for HIV Infection and Adverse Obstetrical Outcomes in Pregnant Women in Dakar, Senegal. JAIDS (1994);**7**:711-717
60. Zuber M, Samuel KP, Lautenberger JA, Kanki PJ, Papas TS. Bacterially produced HIV-2 env polypeptides specific for distinguishing HIV-2 from HIV-1 infections. AIDS Research & Human Retroviruses (1990);**6**:525-534
61. Kanki PJ, Barin F, M'Boup S, Allan JS, Romet-Lemonne JL, Marlink R, McLane MF, Lee TH, Arbeille B, Denis F, et al. New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III (STLV-IIIAGM). Science (1986);**232**:238-43
62. Holzer T, Allen R, Heynen C, Kennedy M, Knigge M, Paul D, Dawson G. Discrimination of HIV-2 infection from HIV-1 infection by western blot and radioimmuno-precipitation analysis. AIDS Res Hum Retroviruses (1990);**6**:515-24
63. Tedder R, O'Connor T, Hughs A, N'Hjie H, Corrah T, Whittle H. Envelope cross-reactivity in western blot for HIV-1 and HIV-2 may not indicate dual infection. Lancet (1988); **ii**:927-30
64. Kanki P, Mboup S, Barin F, Denis F, Marlink R, Romet-Lemonne JL, Essex M. The Biology of HIV-1 and HIV-2 in Africa. In: Giraldo G, Beth-Giraldo E, Klumeck N, Gharbi MDR, Kyalwazi SK, de Thé G, eds. AIDS and Associated Cancers in Africa. Basel: S. Karger, 1988:230-236
65. Gueye-Ndiaye A, Clark R, Samuel K, NDour-Sarr AN, Ouangre A, Sangare L, Mboup S, Marlink R, Papas R, Child R, Coll-Seck A, Essex M, Kanki P. Cost-Effective Diagnosis of HIV-1 and HIV-2 By Recombinant-Expressed env Peptide (566/966) Dot Blot Analysis. AIDS (1993);**7**:481-95
66. Evans L, Moreau J, Odehouri K, Seto D, Thomson-Honnebier G, Legg H, Barboza A, Cheng-Mayer C, Levy J. Simultaneous isolation of HIV-1 and HIV-2 from an AIDS patient. Lancet (1988); **ii**:1389-91
67. Peeters M, Gershy-Damet GM, Fransen K, Koffi K, Coulibaly M, Delaporte E, Piot P, van der Groen G. Virological and polymerase chain reaction studies of HIV-1/HIV-2 dual infection in Cote d'Ivoire. Lancet (1992);**340**:339-40
68. Rayfield M, De Cock K, Heyward W, Goldstein L, Krebs J, Kwok S, Lee S, McCormick J, Moreau JM, Odehouri K. Mixed human immunodeficiency virus (HIV) infection in an individual: demonstration of both HIV type 1 and type 2 proviral sequences by using polymerase chain reaction. Journal of Infectious Disease (1988);**158**:1170-1176
69. Dieng-Sarr A HD, Thior I, Kokkotou E, Sankale JL, Marlink R, Coll-Seck EM, Essex M, Siby T, NDoye I, Mboup S, Kanki P. HIV-1 and HIV-2 dual infection: lack of HIV-2 provirus correlates with low CD4+ lymphocyte counts. AIDS (1998);**12**:131-137
70. Dieng-Sarr A, Popper S, Thior I, Hamel DJ, Sankalé J-L, Siby T, Marlink R, Essex M, MBoup S, Kanki P. Relationship between HIV-2 proviral load and CD4+ lymphocytes differs in HIV monotypic and dual infection. Journal of Human VIrology (1999);**2**:45-51
71. Travers K, MBoup S, Marlink R, Gueye-NDiaye A, Siby T, Thior I, Traore I, Dieng-Sarr A, J.L. S, Mullins C, NDoye I, Hsieh CC, Essex M, Kanki P. Natural Protection Against HIV-1 Infection Provided by HIV-2. Science (1995);**268**:1612-1615
72. Travers K, Eisen G, Marlink, R., Kanki, P., MBoup, S. Protection from HIV-1 infection by HIV-2. AIDS (1998);**12**:224-225
73. Ariyoshi K, van der Loeff M, Sabally S, al. e. Does HIV-2 infection provide cross-protection against HIV-1 infection? (letter). AIDS (1997);**11**
74. Wiktor SZ, Ekpini E, Sibailly T, Diaby L, Teurquetil MJ, Tossou O, DeCock KM, Greenberg AE. Seroconversion to dual reactivity among HIV-1 and HIV-2 seropositive women in Abidjan, Cote d'Ivoire. Int Conf AIDS (1994);**10**:316 (abstract no. PC0195)
75. Aaby P, Poulsen AG, Larsen O, Christiansen CB, Jensen H, Melbye M, Naucler A, Dias F. Does HIV-2 protect against HIV-1 infection? [letter] [see comments]. Comment in: AIDS 1998 Jan 22;12(2):224-5. AIDS (1997);**11**:939-40

76. Norrgren H, Andersson S, Biague AJ, da Silva ZJ, Dias F, Naucler A, Biberfeld G. Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, West Africa: no protection of HIV-2 against HIV-1 infection. AIDS (1999);**13**:701-707
77. Arya SK, Gallo RC. Human immunodeficiency virus (HIV) type 2-mediated inhibition of HIV type 1: a new approach to gene therapy of HIV-infection. Proceedings of the National Academy of Sciences of the United States of America (1996);**93**:4486-91
78. Rappaport J, Arya SK, Richardson MW, Baier-Bitterlich G, Klotman PE. Inhibition of HIV-1 expression by HIV-2. Journal of Molecular Medicine (1995);**73**:583-9
79. Dieng-Sarr A, Sankalé J-L, Hamel DJ, Travers K, Guèye-NDiaye A, Essex M, MBoup S, Kanki PJ. Interaction with Human Immunodeficiency Virus (HIV) Type 2 Predicts HIV Type 1 Genotype. Virology (2000);**268**:402-410
80. Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, VanDevanter NL, Padian N, Braun JF, Kotler DP, Wolinsky SM, Koup RA. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. Nature Medicine (1996);**2**:412-7
81. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science (1995);**270**:1811-5
82. Zagury D LA, Chams V, Fall L, Bernard J, Zagury J-F, Bizzini B, Gringeri A. C-C chemokines, pivotal in protection against HIV type 1 infection. Proc. Natl. Acad. Sci. USA (1998);**95**:3857-3861
83. Lehner T, Wang Y, Cranage M, Bergmeier LA, Mitchell E, Tao L, Hall G, Dennis M, Cook N, Brookes R, Klavinskis L, Jones I, Doyle C, Ward R. Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques. Nature Medicine (1996);**2**:767-75
84. Kokkotou EG, Sankalé J-L, Mani I, MBoup S, Guèye-Ndiaye A, Schwartz D, Essex M, Kanki PJ. In vitro correlates of HIV-2 mediated HIV-1 protection. Proceedings of the National Academy of Sciences of the United States of America (2000);**97(12)**:6797-6802
85. Neoh LP, Akimoto H, Kaneko H, Hishikawa T, Hashimoto H, Hirose S, Kaneko Y, Yamamoto N, Sekigawa I. The production of beta-chemokines induced by HIV-2 envelope glycoprotein [letter]. Aids (1997);**11**:1062-3
86. Putkonen P, Thorstensson R, Albert J, Hild K, Norrby E, Biberfeld P, Biberfeld G. Infection of cynomolgus monkeys with HIV-2 protects against pathogenic consequences of a subsequent simian immunodeficiency virus infection. AIDS (1990);**4**:783-9
87. Putkonen P, Thorstensson R, Walther L, Albert J, Akerblom L, Granquist O, Wadell G, Norrby E, Biberfeld G. Vaccine protection against HIV-2 infection in cynomolgus monkeys. AIDS Research & Human Retroviruses (1991);**7**:271-7
88. Andersson S, Makitalo B, Thorstensson R, Franchini G, Tartaglia J, Limbach K, Paoletti E, Putkonen P, Biberfeld G. Immunogenicity and protective efficacy of a human immunodeficiency virus type 2 recombinant canarypox (ALVAC) vaccine candidate in cynomolgus monkeys. J Inf Dis (1996);**174**:977-85
89. Biberfeld G, Thorstensson R, Putkonen P. Protection against human immunodeficiency virus type 2 and simian immunodeficiency virus in macaques vaccinated against human immunodeficiency virus type 2. AIDS Research & Human Retroviruses (1996);**12**:443-6
90. Myagkikh M, Alipanah S, Markham PD, Tartaglia J, Paoletti E, Gallo RC, Franchini G, Robert-Guroff M. Multiple immunizations with attenuated poxvirus HIV type 2 recombinants and subunit boosts required for protection of rhesus macaques. AIDS Research & Human Retroviruses (1996);**12**:985-92
91. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, Traore I, C.C. H, MC. D, Gueye EH, Hellinger J, Gueye -NA, Sankalé JL, Ndoye I, Mboup S, Essex M. Reduced Rate of Disease Development with HIV-2 Compared to HIV-1. Science (1994);**265**:1587-1590

92. Lisse IM, Poulsen A-G, Aaby P, Normark M, Kvinesdal B, Dias F, Molbak K, Knudsen K. Immunodeficiency in HIV-2 infection: a community study from Guinea-Bissau. AIDS (1990);**4**:1263-1266
93. Kestens L, Brattegard K, Adjorlolo G, Ekpini E, Sibailly T, Diallo K, Gigase P, Gayle H, De Cock KM. Immunological comparison of HIV-1, HIV-2 and dually-reactive women delivering in Abidjan, Cote d'Ivoire. AIDS (1992);**6**:803-807
94. Pepin J, Morgan G, Dunn D, Gevao S, Mendy M, Gaye I, Scollen N, Tedder R, Whittle H. HIV-2-induced immunosuppression among asymptomatic West African prostitutes: evidence that HIV-2 is pathogenic, but less so than HIV-1. AIDS (1991);**5**:1165-1172
95. Lisse IM, Poulsen AG, Aaby P, Knudsen K, Dias F. Serial CD4 and CD8 T-lymphocyte counts and associated mortality in an HIV-2-infected population in Guinea-Bissau. JAIDS (1996);**13**:355-62
96. Traore I, Marlink R, Thior I, et al. HIV-2 as a model for long term non-progression. In: XI International Conference on AIDS. Vancouver, Canada:, 1996
97. Michel P, Balde AT, Roussilhon C, Aribot G, Sarthou JL, Gougeon ML. Reduced immune activation and T cell apoptosis in human immunodeficiency virus type 2 compared with type 1: correlation of T cell apoptosis with beta2 microglobulin concentration and disease evolution. Journal of Infectious Diseases (2000);**181**:64-75
98. Cavaleiro R, Sousa A, Loureiro A, Victorino R. Marked immunosuppressive effects of the HIV-2 envelope protein in spite of the lower HIV-2 pathogenicity. AIDS (2000);**14**:2679-2686
99. Asjo B, Albert J, Karlsson A, Morfeldt-Manson L, Biberfeld G, Lidman K, Fenyo EM. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet (1986);**ii**:660-662
100. Fenyo E, Albert J, Asjo B. Replicative capacity, cytopathic effect and cell tropism of HIV. AIDS (1989);**3**:S5-12
101. Mulligan MJ, Kumar P, Hui HX, Owens RJ, Ritter GD, Jr., Hahn BH, Compans RW. The env protein of an infectious noncytopathic HIV-2 is deficient in syncytium formation. AIDS Research & Human Retroviruses (1990);**6**:707-720
102. Albert J, Naucler A, Bottiger B, Broliden P, Albino P, Ouattara S, Bjorkegren C, Valentin A, Biberfeld G, Fenyo E. Replicative capacity of HIV-2, like HIV-1, correlates with severity of immunodeficiency. AIDS (1990);**4**:291-5
103. Schulz T, Whitby D, Hoad J, Corrah T, Whittle H, Weiss RA. Biological and molecular variability of human immunodeficiency virus type 2 isolates from The Gambia. J Virol. (1990);**64**:5177-82
104. Albert J, Stalhandske P, Marquina S, Karis J, Fouchier RA, Norrby E, Chiodi F. Biological phenotype of HIV type 2 isolates correlates with V3 genotype. AIDS Research & Human Retroviruses (1996);**12**:821-8
105. Sankalé JL, De La Tour RS, Marlink RG, Scheib R, Mboup S, Essex ME, Kanki PJ. Distinct quasi-species in the blood and the brain of an HIV-2-infected individual. Virology (1996);**226**:418-23
106. Bakhanashvili M, Hizi A. Fidelity of the RNA-dependent DNA synthesis exhibited by the reverse transcriptases of human immunodeficiency virus types 1 and 2 and of murine leukemia virus: mispair extension frequencies. Biochemistry (1992);**31**:9393-8
107. Myers G, Pavlakis GN. Evolutionary potential of complex retroviruses. In: Levy JA, ed. The retroviridae. New York: Plenum Press, 1992:1-37
108. Sankalé JL, MBoup S, Marlink R, Essex M, Kanki P. HIV-2 and HIV-1 Quasispecies in Cervical Secretions and Blood. AIDS Research Human Retroviruses (1998);**14**:1473-1481
109. Simon F, Matheron S, Tamalet C, Loussert AI, Bartczak S, Pepin J, Dhiver C, Gamba E, Elbim C, Gastaut JA. Cellular and Plasma Viral Load in Patients Infected with HIV-2. AIDS (1993);**7**:1411-7
110. Berry N, Ariyoshi K, Jobe O, Ngum P, Corrah T, Wilkins A, Ngum P, Corrah T, Wilkins A, Whittle H, Tedder R. HIV Type 2 Proviral Load measured by Quantitative Polymerase Chain

Reaction correlates with CD4+ Lymphopenia in HIV Type 2-Infected Individuals. AIDS Res Hum Retroviruses (1994);**10**:1031-7

111. Popper SJ, Dieng-Sarr A, Travers KU, Guèye-NDiaye A, MBoup S, Essex M, Kanki PJ. Lower HIV-2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. Journal of Infectious Disease (1999);**180**:1116-1121
112. Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R, Whittle H. Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. Journal of Human Virology (1998);**1**:457-68
113. Popper SJ, Sarr AD, Gueye-Ndiaye A, Mboup S, Essex ME, Kanki PJ. Low Plasma Human Immunodeficiency Virus Type 2 Viral Load Is Independent of Proviral Load: Low Virus Production In Vivo. J. Virol. (2000);**74**:1554-1557
114. Popper SJ, Dieng-Sarr A, Guèye-NDiaye A, MBoup S, Essex M, Kanki PJ. Low Plasma HIV-2 viral load is independent of proviral load: low virus production in vivo. Journal of Virology (2000);**74(3)**:1554-1557
115. Soriano V, Gutierrez M, Heredia A, Bravo R, Aguilera O, Mas A, Hewlett I, Baquero M, Gonzalez-Lahoz J. Serial dilutions on synthetic peptide-based assays can resolve dual seroreactivity to HIV-1 and HIV-2 [letter]. Vox Sanguinis (1996);**71**:67-8
116. Andersson S, Norrgren H, da Silva Z, Biague A, Bamba S, Kwok S, Christopherson C, Biberfeld G, Albert J. Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. Archives of Internal Medicine (2000);**160**:3286-3293
117. Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C, Rouzioux C. Isolation of a new human retrovirus from West African patients with AIDS. Science (1986);**233**:343-6.
118. Saimot AG, Coulaud JP, Mechali D, Matheron S, M.C. D, Rey MA, Brun-Vezinet F, Leibowitch J. HIV-2/LAV-2 in Portuguese man with AIDS (Paris, 1978) who had served in Angola in 1968-74. Lancet (1987);**i**:688
119. De Cock K, Odehouri K, Colebunders R, Adjorlolo G, Lafontaine M, Porter A, Gnaore E, Diaby L, Moreau J, Heyward W. A comparison of HIV-1 and HIV-2 infections in hospitalized patients in Abidjan, Cote d'Ivoire. AIDS (1990);**4**:443
120. Le Guenno BM, Barabe P, Griffet PA, Guiraud M, Morcillo RJ, Peghini ME, Jean PA, M Baye PS, Diallo A, Sarthou J-L. HIV-2 and HIV-1 AIDS Cases in Senegal: Clinical patterns and immunological perturbations. JAIDS (1991);**4**:421-427
121. Schneider J, Luke W, Kirchhoff F, Jung R, Jurkiewicz E, Stahl-Hennig C, Nick S, Klemm E, Jentsch KD, Hunsmann G. Isolation and characterization of HIV-2 obtained from a patient with predominantly neurological defects. AIDS (1990);**4**:455
122. Dwyer D, Matheron S, Bakchine S, Bechet J, Montagnier L, Vazeux R. Detection of human immunodeficiency virus type 2 in brain tissue. J Infect Dis (1992);**166**:888
123. Marlink RG, Ricard D, M'Boup S, Kanki PJ, Romet-Lemonne J-L, N'Doye I, Diop K, Simpson MA, Greco F, Chou MJ, DeGruttola V, Hsieh C-C, Boye C, Barin F, Denis F, McLane MF, Essex M. Clinical, hematologic, and immunologic cross-sectional evaluation of individuals exposed to human immunodeficiency virus type 2 (HIV-2). AIDS Research & Human Retroviruses (1988);**4**:137-148
124. Diouf K, Dieng-Sarr A, Popper S, Eisen G, Kanki PJ. Associations between MHC Class I and Susceptibility to HIV-2 Disease Progression. Journal of Human Virology (2002);**5**:1-7
125. Popper S, Sankalé JL, Thior I, Siby T, MBoup S, Essex M, Kanki P. Vpx reactivity as a predictor of disease progression in HIV-2 infection. AIDS Research Human Retroviruses (1998);**14**:1157-1162
126. Carrington M, Nelson GW, Martin MP, al. e. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science (1999);**283**:1748-1752

127. Kanki P, Essex M, Barin F. Antigenic Relationships Between HTLV-3/LAV, STLV-3, and HTLV-4. In: Chanock RM, Lerner RA, Brown F, Ginsberg H, eds. Vaccines 87: Modern Approaches to New Vaccines. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1987:185-187
128. Essex M, Kanki P, Barin F, Chou MJ, Lee TH. Immunogenicity of HIV-1 and HIV-2 Antigens and Relationship to Disease Development. In: Girard M, Vallette L, eds. 2e Colloque des «Cent Gardes»: Retroviruses of Human A.I.D.S. and Related Animal Diseases. Paris: Marnes-la-Coquette, 1988:219-220
129. Robert-Guroff M, Aldrich K, Muldoon R, Stern TL, Bansal GP, Matthews TJ, Markham PD, Gallo RC, Franchini G. Cross-neutralization of human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus isolates. Journal of Virology (1992);**66**:3602-3608
130. Bjorling E, Scarlatti G, von Gegerfelt A, Albert J, Biberfeld G, Chiodi F, Norrby E, Fenyo EM. Autologous neutralizing antibodies prevail in HIV-2 but not in HIV-1 infection. Virology (1993);**193**:528-30
131. Bjorling E, Chiodi F, Utter G, Norrby E. Two neutralizing domains in the V3 region of the envelope glycoprotein gp125 of HIV-2 type 2. J Immunol (1994);**152**:1952-9
132. Weiss RA, Clapham PR, Weber JN, Whitby D, Tedder RS, O'Connor T, Chamaret S, Montagnier L. HIV-2 antisera cross-neutralize HIV-1. AIDS (1988);**2**:95-100
133. Bottiger B, Karlsson A, Andreasson PA, Naucler A, Costa CM, Biberfeld G. Cross-neutralizing antibodies against HIV-1 (HTLV-IIIB and HTLV-IIIRF) and HIV-2 (SBL-6669 and a new isolate SBL-K135). AIDS Research & Human Retroviruses (1989);**5**:525-533
134. Fenyo EM, Putkonen P. Broad cross-neutralizing activity in serum is associated with slow progression and low risk of transmission in primate lentivirus infections. Immunology Letters (1996);**51**:95-9
135. Bottiger B, Karlsson A, Andreasson PA, Naucler A, Costa CM, Norrby E, Biberfeld G. Envelope cross-reactivity between human immunodeficiency virus types 1 and 2 detected by different serological methods: correlation between cross-neutralization and reactivity against the main neutralizing site. Journal of Virology (1990);**64**:3492-3499
136. Belec L, Tevi-Benissan C, Dupre T, Mohamed AS, Prazuck T, Gilquin J, Kanga JM, Pillot J. Comparison of cervicovaginal humoral immunity in clinically asymptomatic (CDC A1 and A2 category) patients with HIV-1 and HIV-2 infection. J Clin Immunol (1996);**16**:12-20
137. Gotch F, McAdam SN, Allsopp CE, Gallimore A, Elvin J, Kieny MP, Hill AV, McMichael AJ, Whittle HC. Cytotoxic T cells in HIV2 seropositive Gambians. Identification of a virus-specific MHC-restricted peptide epitope. Journal of Immunology (1993);**151**:3361-9
138. Bertoletti A, Cham F, McAdam S, al e. Cytotoxic T cells from human Immunodeficiency virus type 2 infected patients frequently cross-react with different human immunodeficiency virus type 1 clades. Journal of Virology (1998);**72**:2439-2448
139. Kokkotou E, Philippon V, Wang W-K, MBoup S, Essex M, Kanki PJ. Role of the CCR5D32 allele in resistance to HIV-1 infection in West Africa. Journal of Human Virology (1998);**1(7)**:469-474
140. Dieng Sarr, A., Lu, Y., Sankalé, J-L, Popper, S., Mboup, S., Kanki, P.J., Cao, H. Robust HIV-2 cellular immune response measured by a modified Anthrax toxin-based ELISPOT assay.AIDS Research and Human Retroviruses(2001) **17** (13)263-70
141. Mboup S, Kanki P, Mhalu FS. Laboratory Diagnosis of HIV-1 and HIV-2 in Africa. AIDS (1991);**5**:S93-S101
142. Sturm-Ramirez K, Brumblay H, Diop K, Guèye-Ndiaye, Sankalé J-L, Thior I, NDoye I, MBoup S, Kanki PJ. Molecular epidemiology of genital Chlamydia trachomatis infection in high risk women in Senegal, West Africa. Journal of Clinical Microbiology (2000);**38(1)**:138-145
143. Sankale JL, Hamel D, Woolsey A, Traore T, Dia TC, Gueye-Ndiaye A, Essex M, Mboup T, Kanki P. Molecular evolution of human immunodeficiency virus type 1 subtype A in Senegal: 1988-1997. Journal of Human Virology (2000);**3**:157-64

144. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research (1994);**22**:4673-80

DAMD17-95-C-5005
Interactions of HIV-1 and HIV-2 in West Africa
Personnel List
October 31, 2002

Personnel on Project:

Phyllis J. Kanki DVM DSc, Primary Investigator
Don Hamel, Research Assistant
Geoffrey Eisen, PhD, Data Manager/Analyst