complex (*19*), and expression of the protein in the rat red cells and renal tubules does not occur until birth (*13*); however, the total absence of CHIP has not previously been described in prenatal or postnatal humans (*2*, *12*). It is surprising that none of the Co(a−b−) patients suffers from any apparent anemia, and the biological need for water channels in red cell membranes remains unexplained (*1*).

Our studies also dictate the need to rethink the postulated roles of CHIP in nonerythroid tissues (*7*). An average adult human produces ~200 liters of glomerular filtrate daily, of which ~90% was thought to be reabsorbed through the abundant CHIP water channels in the proximal tubules and descending thin limbs of Henle (*5*). CHIP was also thought to be essential for secretion of cerebrospinal fluid, aqueous humor, reproductive fluids, and bile, as well as for maintenance of interstitial fluid and prevention of perialveolar edema (*5*, *20*), processes that appear to be normal in the Co(a−b−) individuals. Further clinical evaluations may reveal other physiological roles for CHIP and elucidate mechanisms that compensate for the absence of CHIP. The existence of subclinical defects in renal concentration, neurological status, or age-dependent problems in the Co(a−b−) individuals must be considered.

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16. Genomic DNA was isolated from peripheral blood lymphocytes of normal healthy volunteers and from Co(a−b−) individuals with the use of the QIAamp blood kit (Qiagen, Chatsworth, CA). Southern analysis of genomic DNA was performed as described (*6*), except that the blot was probed with denatured *aquaporin-1* cDNA (10^6 dpm/ml) corresponding to nucleotides 15 to 822 (*3*). The *aquaporin-1* exons were amplified with primers complementary to the 5′ and 3′ untranslated sequences and intronic sequences flanking each of the four exons (*10*). PCR samples (50 μl) containing 100 to 200 ng of genomic DNA, 25 pmol of each primer, and 0.7 to 1.0 mM $MgCl_2$ were denatured for 5 min at 95°C. Two units of AmpliTaq DNA polymerase (Perkin-Elmer) were added, and 35 cycles of amplification were performed (1 min at 94°C, 1 min at 50° to 60°C, and 1 min at 72°C). PCR products were gel-purified for sequencing or restriction analysis.
17. The single-base insertion in exon I of proband 2 corresponded to the loss of a Pfl MI restriction site and the appearance of a Bst XI site; the single-base substitution in exon I of proband 3 corresponded to the loss of a Msp I site. Similar results were obtained with different sets of nonoverlapping amplification primers.
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21. We thank M. Collins, M. Goldman, J. Carrieres, J. P. Cartron, and Y. P. Le Pennec for samples, and D. J. Anstee for discussions that initiated this study. Supported in part by NIH grants HL33991, HL48268, DK32753, and DK43955, and by NATO grant 880556. M.L.Z. was supported by a Clinical Investigator Career Development Award from the Department of Veterans Affairs.

10 May 1994; accepted 20 July 1994

Reduced Rate of Disease Development After HIV-2 Infection as Compared to HIV-1

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Human immunodeficiency virus type–2 (HIV-2) is a close relative of the prototype acquired immunodeficiency syndrome (AIDS) virus, HIV-1. HIV-2 is biologically similar to HIV-1, but information is lacking concerning clinical outcomes of HIV-2–infected individuals. From 1985 to 1993, a prospective clinical study was conducted in women with HIV-2 and HIV-1 infection to determine and compare rates of disease development. HIV-1–infected women had a 67% probability of AIDS-free survival 5 years after seroconversion in contrast with 100% for HIV-2–infected women. In addition to having significantly less HIV-related disease outcome in HIV-2 enrollees compared to HIV-1 enrollees, the rate of developing abnormal $CD4^+$ lymphocyte counts with HIV-2 infection was also significantly reduced. This natural history study demonstrates that HIV-2 has a reduced virulence compared to HIV-1.

Evidence of a second human immunodeficiency virus was first demonstrated in a study of Senegalese women in 1985 (*1*). HIV-2 was subsequently found to be highly prevalent in many countries in West Africa (*2*) and has been present in populations in this region as early as the 1960s (*3*). Nonetheless, fewer AIDS cases have been reported in West African countries, where HIV-2 is the predominant AIDS virus, than in Central or East Africa, where HIV-1 infection predominates (*4*). The population of HIV-2–seropositive Senegalese sex workers, in whom HIV-2 was initially described, was clinically surveyed and found to be relatively free from HIV-associated signs or symptoms when compared with prostitutes surveyed in HIV-1–prevalent areas in Africa (*5*). Given the observed differences in the prevalences of AIDS and in the geographical distribution of HIV-2 as compared to

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HIV-1, a key question that emerges is whether HIV-2 and HIV-1 differ in their disease-causing potential.

Most reports surveying the clinical manifestations of individuals infected with HIV-2 have previously been case-series (*2*) or cross-sectional in nature (*5–7*). These types of studies are important in describing the epidemiological and clinical status of HIV-2–infected individuals. When controls are used, cross-sectional studies may also demonstrate disease association. In addition, two studies in West Africa obtained follow-up data on initially hospitalized AIDS patients seropositive for either HIV-1 or HIV-2 (*8*). When hospital-based surveys are used to identify index cases or subsequent cohorts of infected individuals, however, the apparent pathological effects of exposure to HIV-2 (or HIV-1) may not be fully appreciated or may be amplified by this type of case selection. This prospective study was undertaken to define and compare the natural history of both HIV-2 and HIV-1 infection in a cohort of initially asymptomatic individuals.

Since February 1985, all women registered as commercial sex workers at the Institut d'Hygiène Sociale (IHS) clinic in Dakar, Senegal, have been serologically screened for exposure to HIV-1 and HIV-2. The clinic provides clinical examinations and treatment of sexually transmitted diseases (STDs) during biannual visits, which are required for the registration of sex workers at the clinic. We have augmented the health evaluation and services in the clinic with specially trained study physicians, counseling on HIV and STDs, and free condoms and medications as described (*5, 9*). As in most developing country situations, antiviral therapy was not available in this population. A demographic, behavioral, and health history questionnaire, serologic testing for HIV with informed consent, and a baseline health evaluation were performed by study physicians. When women returned for subsequent clinic visits, all who tested HIV seropositive were asked to enroll in our clinical cohort. The dynamic nature of this ongoing serologic screening for HIV resulted in an open cohort design where new incident and prevalent HIV positives were continuously identified and enrolled over the study period. Patients in the seroprevalent case groups were defined as those who were HIV positive at the initial clinic visit. Patients in the seroincident case groups were defined as those women who were previously HIV negative and subsequently seroconverted to either HIV-1 or HIV-2. The time of infection was estimated as the midpoint between the last seronegative and first seropositive sample. In addition, seronegative comparison women were selected for follow-up comparison by distribution sampling on the basis of age (±2 years), nationality, and years of registered prostitution (±3 years) when each eligible seropositive woman was identified. Study design, recruitment procedures, and various protocols have been described (*5, 9, 10*).

At enrollment and at subsequent biannual visits, complete physical examinations were performed, which included a complete medical history and a review of organ systems that emphasized HIV-related signs and symptoms. All biannual blood samples were tested for antibodies to each virus by routine immunoblots (*11*) for HIV-1 and HIV-2 antibodies and by immunoblot to type-specific peptides (*12*) as described (*5, 9, 10*). Sera difficult to characterize serologically were further evaluated by radioimmunoprecipitation for both viruses (*5*). Additional blood samples were requested on a yearly basis to determine a subject's complete blood count and lymphocyte subsets (Simultest, Becton Dickinson).

Multiple antigen skin testing (Multitest IMC, Merieux Institute, Paris) determined that greater than 85% of the entire clinic population reacted to tuberculin antigen in the multiple antigen skin test. Delayed-type hypersensitivity was assessed annually after enrollment by intradermal tuberculin antigen testing [purified protein derivative (PPD), Sclavo].

From February 1985 to December 1993, 78 HIV-1 seropositive women were enrolled, of whom 32 were seroincident and 46 were seroprevalent. In addition, 136 HIV-2 seropositive women were enrolled, of whom 33 were seroincident and 103 were seroprevalent. Twelve women dually reactive to HIV-1 and HIV-2 by all serologic criteria and 348 seronegative comparison women were also followed. The mean age of the enrolled cohort was 37 ± 7 years, with no significant difference between HIV-1 and HIV-2 seroincident women. HIV-1 seroprevalent women were slightly younger than the other groups, with a mean age of 34 ± 6 years. In general, women were officially registered as prostitutes 4 to 8 years before enrollment and had been sexually active since 16 to 17 years of age. As a result of the distribution sampling of the seronegative comparison group, HIV-1 and HIV-2 seropositive women were similar to seronegative women in terms of age, years of registered prostitution, and nationality.

At the beginning of each calendar year, study physicians and social workers actively investigated enrolled women who had not attended the clinic for more than 12 months. A separate data instrument was completed for these women each year. During the time of yearly follow-up investigations from 1986 until the end of 1993, of the 574 seropositive and seronegative women followed, the clinic staff determined that 85 women had

Table 1. Incidence rates of disease development in study enrollees according to HIV-1 and HIV-2 infection. We determined person-years of observation (PYO) for seronegative and seroprevalent women by calculating the time from the initial basic health evaluation and serostatus determination until the most recent clinical consultation or the noted disease outcome. For seroincident women, the midpoint between the last negative and the first positive serostatus determination was used as the estimated seroconversion date and as the start date for HIV-positive observation time. Incidence rates (IR) per 100 PYO and 95% confidence intervals (95% CI) for the diagnosis of AIDS or for the onset of CDC IV disease (both AIDS defining and not AIDS defining) were determined by serostatus. Seroincident and seroprevalent women were evaluated separately for all analyses. Women found to be dually reactive to HIV-1 and HIV-2 by our strict serologic criteria (n = 12) were excluded from the present comparison because their numbers were too small to statistically analyze and because temporally the classification of seroprevalent and seroincident status in this category is difficult. We can note that the 12 dually seropositive women were followed for a total of 24.25 PYO with an AIDS incidence rate of 8.25 [95% CI 1.31–21.0]. We further evaluated early signs of immune compromise in seroincident women by determining the IR of developing an abnormal $CD4^+$ lymphocyte count. Person years of observation for this evaluation was the time of seroconversion until the time of the most recent $CD4^+$ lymphocyte determination. Confidence intervals for small numbers of outcomes were calculated according to a Poisson distribution (*23*).

	HIV-1		HIV-2	
	Seroincident subjects	Seroprevalent subjects	Seroincident subjects	Seroprevalent subjects
Number of women	32	46	33	103
Total person years	82.50	106.50	111.91	436.17
Number of AIDS cases	4	1	0	1
AIDS IR	4.85	0.94	0	0.23
95% CI	[1.32–12.4]	[0.02–5.23]	[0–3.30]	[0.01–1.28]
Number of CDC IV cases	8	9	2	12
CDC IV IR	9.70	8.45	1.79	2.75
95% CI	[4.85–19.39]	[4.40–16.24]	[0.22–6.46]	[1.56–4.86]
Number of seroconverters developing abnormal $CD4^+$ lymphocyte count (<400 cells/mm^3)	7	–	1	–
Abnormal $CD4^+$ IR	10.1	–	1.0	–
95% CI	[4.8–21.2]	–	[0.02–4.98]	–

moved from Dakar without health information follow-up and had no definitive information in their follow-up record despite active attempts. Of these 85 women, 10 were HIV-1 seropositive (2 seroincident), 21 HIV-2 seropositive (2 seroincident), and 54 seronegative. Eight seropositive women and three seronegative comparison women had died. Furthermore, neither HIV-1 nor HIV-2 seropositive women were significantly more likely to have missed a clinic visit for more than 12 months compared with seronegative women in a logistic regression model controlling for nationality. As might be expected, being of non-Senegalese nationality was associated with missing a recent clinic visit.

In our study, AIDS as an outcome was defined according to the Centers for Disease Control (CDC) revised surveillance case definition for AIDS (*13*). As shown in Table 1, the incidence rates (IRs) for AIDS development were markedly different between HIV-2 and HIV-1 seropositive women. Incidence rate ratios for AIDS as the outcome were not meaningful because the rate for HIV-2 seroincident women, which would be the denominator in such a comparison, was zero despite 112 person-years of observation. A meaningful analysis is possible when AIDS-free survival is plotted by means of Kaplan-Meier product limit estimates (*14*) in the seroincident women. In this comparison, the likelihood of developing AIDS was significantly greater in the HIV-1 seroincident women versus HIV-2 seroincident women (Fig. 1; log-rank test, $P = 0.01$ and Gehan's Wilcoxon test, $P = 0.02$). In seroincident HIV-1–infected women, the probability of AIDS-free time was 99% (SD, 1.0%) at 2 years, 88.3% (SD, 7.8%) at 3 years, 70.5% (SD, 13.4%) at 4 years, and 66.8% (SD, 14.3%) at 5 years after infection. Although study designs, methods, and geographic location have varied, the range of 65 to 80% AIDS-free survival at 5 years after infection in other seroincident cohort studies is similar to our point estimate of 67% for HIV-1 infection (*15*). In contrast, no HIV-2 seroincident women developed AIDS, thereby exhibiting a 100% (SD inestimable) probability of AIDS-free survival time greater than 5 years after infection.

A second disease outcome category was defined according to the CDC Classification System for HIV Infection (*16*) as CDC IV disease for those HIV-related diseases, both AIDS defining and not AIDS defining, included in group IV of the classification system. Because for CDC IV disease the HIV-2 denominator incidence rate was not zero, a Cox proportional hazards model (*17*) can be used to compare incidence rate of disease outcome with HIV-1 infection over the rate with HIV-2 in seroincident women. This model can control for confounding and also is not based on any assumptions concerning the nature or shape of the underlying disease-free survival distribution for HIV-2. When used to compare CDC IV disease outcome between HIV-1 incidence rate and HIV-2 incidence rate, the hazard ratio (the relative risk of disease outcome over the observation period with HIV-1 over HIV-2) was 6.31 [95% CI 1.23–32.24]. Kaplan-Meier survival analysis of the occurrence of CDC IV HIV-related disease in seroincident enrollees also showed a significantly greater likelihood of disease-free survival in HIV-2 versus HIV-1 enrollees (Fig. 2; log-rank test, $P = 0.01$ and Gehan's Wilcoxon test, $P < 0.01$).

The development of early immune compromise was further studied in seroincident study subjects. We noted the rate of occurrence of abnormal $CD4^+$ lymphocyte counts (<400 cells/mm^3) in seroincident women and the development of skin test anergy in the subset of seroincident women who agreed to multiple PPD skin tests and who were also initially positive to PPD skin testing. These analyses were independent of other disease outcomes. Assuming seroincident women had similar $CD4^+$ lymphocyte counts before infection with HIV-1 or HIV-2, the hazard ratio for developing an abnormal $CD4^+$ lymphocyte count with HIV-1 infection compared to HIV-2 infection was 12.14 [95% CI 1.41–

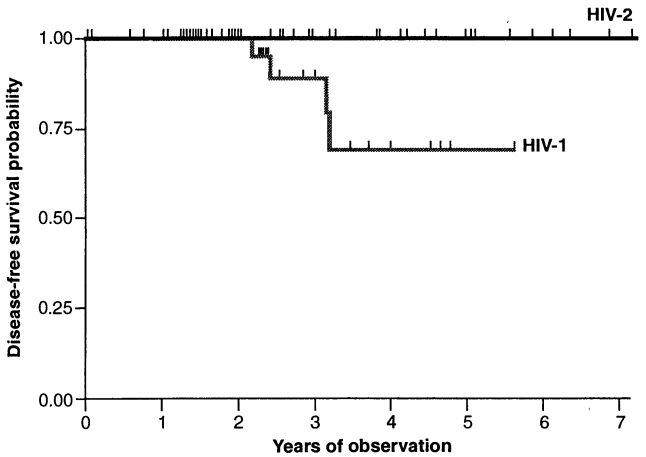

Fig. 1 (top, left). AIDS disease-free survival in seroincident women according to serostatus. AIDS as an outcome was defined according to the CDC revised surveillance definitions for AIDS (*13*). Kaplan-Meier disease-free survival analysis was performed on the seroincident women grouped by serostatus (*14*). Statistical significance was determined by Gehan's Wilcoxon test ($P = 0.02$) and by log-rank sum ($P = 0.01$).

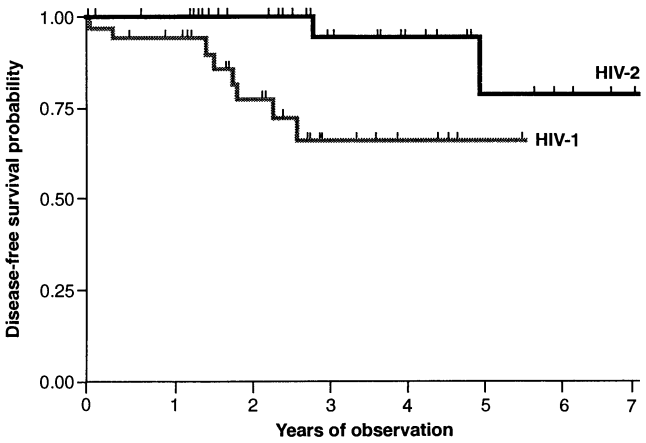

Fig. 2 (top, right). CDC IV disease-free survival in seroincident women, according to serostatus. CDC IV HIV-related disease as an outcome included all those conditions outlined in the CDC Classification System for HIV Infection fulfilling group IV disease criteria for HIV-related outcome, both AIDS defining and not AIDS defining (*16*). (Wilcoxon test, $P < 0.01$ and log-rank sum, $P = 0.01$).

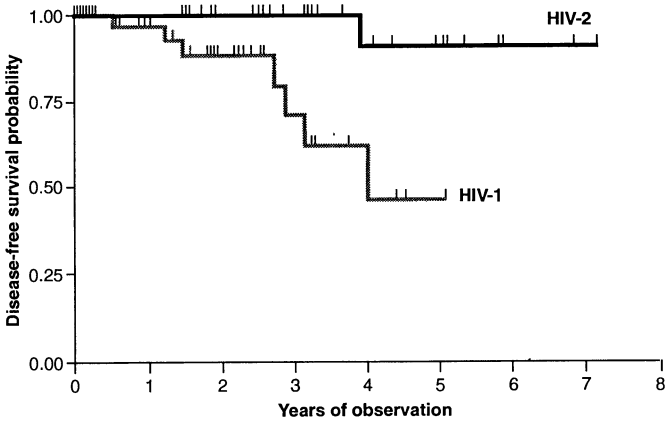

Fig. 3 (bottom, right). Disease-free survival as measured by abnormal $CD4^+$ count in seroincident women according to serostatus. Graph includes 31 of 32 HIV-1 seroincident women and 32 of 33 HIV-2 seroincident women. Disease outcome reflects a lymphocyte count of less than 400 cells/mm^3 (Wilcoxon test, $P < 0.01$ and log-rank sum, $P = 0.01$).

104.39]. The disease-free survival plot indicated that HIV-1 seroincident women were significantly more likely to develop abnormal $CD4^+$ counts over time than HIV-2 seroincident women (Fig. 3; log-rank test, $P < 0.01$ and Gehan's Wilcoxon test, $P < 0.01$). Furthermore, we looked at seroincident women who were routinely skin tested over the period of the study and who had an initially positive PPD result. In those PPD skin test–positive seroincident women with multiple PPD skin test results, 6 of 17 HIV-1 seroincident women became anergic, whereas only 2 of 20 HIV-2 seroincident women became anergic over the study period (Fisher exact test, $P = 0.05$). The reduced likelihood for immune compromise over time will be important in monitoring HIV-2–infected outpatients, in staging this human immunodeficiency virus infection, and perhaps in evaluating therapeutic decisions and responses.

Because of the range of disease progression rates and the extreme rarity of outpatient natural history studies in an African setting for either HIV-1 or HIV-2 (*18*), it is important to report cohort follow-up data for both the seroincident and seroprevalent women. HIV-1–related disease development was greater in seroincident women than seroprevalent women. Four of five HIV-1–related AIDS cases developed in the seroincident group. This most likely represents a selection bias as a result of a "survivor effect" in those women enrolling in the seroprevalent group. Alternatively, it may also represent the movement of HIV-1 viral subtypes with more rapid disease outcome into the region, but this explanation remains to be investigated. Overall, the minimal loss to follow-up and the presence of HIV-1 seropositive subjects as a comparison group in the same cohort in this study allowed for a more relevant examination of the rate of disease development with HIV-2 infection.

We have previously described distinct risk determinants for HIV-2 and HIV-1 infection in both prevalent and incident infection in this cohort (*9*, *10*). Further, mathematical modeling of the HIV-1 and HIV-2 seroincidence data has suggested a five- to ninefold difference in the infectivity of HIV-2 compared with HIV-1 per sexual act (*19*). Perinatal transmission studies have also shown a 15- to 20-fold difference in the rate of HIV-2 transmission compared with HIV-1 transmission (*20*). In this study, we have shown that HIV-2– and HIV-1–associated AIDS and CDC IV disease incidence rates are distinct, and the disease-free survival time for HIV-2 is significantly longer when compared with HIV-1. This disease outcome study supports the hypothesis that these two related HIV viruses have different biologic behaviors. One determinant of these differences may be a lower viral burden in HIV-2 infection, on the basis of polymerase chain reaction and viral isolation studies (*21*). Numerous viral and host etiologies for a reduced virulence have also been proposed (*22*). Further investigations into the basic biologic differences that may explain the distinct rates of disease development with HIV-2 versus HIV-1 may help us understand the pathogenic mechanisms of this class of retroviruses.

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24. Supported in part by grant DAMD 17-87-7072 from the U.S. Army and grants AI 30795 and CA 39805 from the National Institutes of Health.

1 April 1994; accepted 18 July 1994

Analysis of Sequence Transfers Resembling Gene Conversion in a Mouse Antibody Transgene

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The role of gene conversion in murine immunoglobulin gene diversification is unclear. An antibody gene construct designed to provide the homologous donor and acceptor sequences required for conversion mechanisms was produced and used to generate transgenic mice. When these transgenic mice were immunized, DNA sequence transfers between tandem transgene VDJ regions were detectable and resembled gene conversion events. There is a strong link between these conversion-like sequence transfers and transgene somatic hypermutation, suggesting that both processes might occur at the same stage of B cell differentiation.

Gene conversion is an important mechanism for the generation of antibody diversity in chickens and rabbits (*1*–*3*). However, despite many similarities in the organization of chicken, rabbit, and mouse immunoglobulin (Ig) genes, there is currently little evidence that conversion plays any role in murine antibody diversification (*4*–*6*). We have designed a transgene construct (VVC_μ, Fig. 1) that contains tandem homologous heavy chain VDJ segments [variable (V), diversity (D), and joining (J)] to simplify the identification of gene conversion events during mouse B cell development.

The VVC_μ transgene construct is based on the previously described ARS_μ transgene (*7*) which encodes a μ heavy chain that has a VDJ region derived from the R16.7 hybridoma. The ARS_μ heavy chain (H chain) can participate in an antibody response to the phenylarsonate (ARS) hapten and is reactive with the rat antiidiotypic monoclonal antibody (mAb), AD8 (*7*). In VVC_μ, a second 900–base pair VDJ region is inserted 1.5 kb upstream of the R16.7

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