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HIV-2 Genetic Evolution in Patients with Advanced Disease Is Faster than That in Matched HIV-1 Patients▽†

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The objective of this study was to estimate and compare the evolutionary rates of HIV-2 and HIV-1. Two HIV-2 data sets from patients with advanced disease were compared to matched HIV-1 data sets. The estimated mean evolutionary rate of HIV-2 was significantly higher than the estimated rate of HIV-1, both in the gp125 and in the V3 region of the *env* gene. In addition, the rate of synonymous substitutions in gp125 was significantly higher for HIV-2 than for HIV-1, possibly indicating a shorter generation time or higher mutation rate of HIV-2. Thus, the lower virulence of HIV-2 does not appear to translate into a lower rate of evolution.

Human immunodeficiency virus type 2 (HIV-2), the second causative agent of AIDS, is associated with slower disease progression than HIV-1. Thus, $CD4^+$ T-cell quantity and function is better maintained in HIV-2 infection (1, 13, 14). Furthermore, the plasma viral set point in HIV-2 infection is significantly lower than in HIV-1 infection (2, 27), possibly due to more effective host control of the virus. In line with this, virus-specific $CD8^+$ T cells in HIV-2-infected individuals maintain a polyfunctional profile similar to that in HIV-1 long-term nonprogressors (14). Autologous neutralizing antibody responses may also play a role in controlling viral load in HIV-2 infection (4, 31, 34). Finally, the level of immune activation is lower in HIV-2 infection than in HIV-1 infection (18, 24, 30, 36).

The correlation between disease progression and viral evolution has been investigated in detail for HIV-1 (10, 19, 25, 32, 35) but to a lesser extent for HIV-2 (20, 22). MacNeil et al. reported a low evolutionary rate of HIV-2 in chronic infections (22), and Lemey et al. reported that the rate of synonymous substitutions was lower for HIV-2 than for HIV-1 in chronic infections (20). The objective of our study was to estimate the evolutionary rate of HIV-2 and to compare it to that of HIV-1. We analyzed data from patients that were matched according to disease stage and CD4 dynamics and found that the rate of evolution was higher for HIV-2 than for HIV-1.

All sequences included in this study were derived from primary virus isolates (5, 33). Detailed descriptions of the sequencing and data sets can be found in Materials and Methods in the supplemental material. Briefly, four different sequence data sets were studied. The first data set included 20 HIV-2 sequences corresponding to gp125 (1,588 bp), the complete major surface glycoprotein (SU) of HIV-2. The sequences had been obtained by direct population sequencing of viral RNA from longitudinally collected HIV-2 isolates from four Swedish individuals who showed different rates of HIV-2 disease progression. In total, the follow-up time was 9 to 13 years, with 3 to 8 samples from each patient (see Table S1 in the supplemental material). The second HIV-2 data set consisted of clonal V3 sequences from five Portuguese individuals. The sequences were derived from longitudinally collected virus isolates, with the collection times spanning 3 to 9 years, 3 to 7 samples per patient, and an average of 9 clones per sample (see Table S2 in the supplemental material). The third data set served as the HIV-1 control for the Swedish HIV-2 data set. The HIV-1 patients were matched as well as possible with respect to length of follow-up, numbers of samples, antiretroviral therapy, plasma HIV levels, and CD4 counts (see Table S3 in the supplemental material). A fourth data set served as the HIV-1 control for the Portuguese HIV-2 V3 data set. This data set consisted of 370 clonal V3 sequences from longitudinally collected HIV-1 isolates from eight patients, represented by an average of 46 clones, and the genetic region (363 bp) was chosen to match that of the Portuguese HIV-2 V3 data set. All sequences are available in GenBank under the following accession numbers: for Swedish HIV-2 SU, DQ213026 to DQ213040 and GU204944 to GU204948; for Swedish HIV-1 SU, GU204919 to GU204943; for Portuguese HIV-2 V3, EU358229 to EU358270, EU358137 to EU358167, AY513663 to AY513671, EU358168 to EU358205, EU358384 to EU358424, and GU217544 to GU217571; and for Swedish HIV-1 V3, EF184307 to EF184524, DQ516085 to DQ516121, and DQ516124 to DQ516338.

The sequences were aligned and putative recombinants

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TABLE 1. Comparison of evolutionary rates in HIV-1 and HIV-2

Genetic region[a]	HIV type	Substitution rate at 10^{-3} substitutions site^{-1} year^{-1} (95% HPD[b])
SU	HIV-2	10.15 (7.56, 12.87)
SU	HIV-1	6.36 (5.18, 7.59)
V3	HIV-2	29.37 (23.25, 35.84)
V3	HIV-1	12.26 (10.24, 14.50)

[a] SU, the complete major surface glycoprotein (gp125/gp120); V3, the third variable loop of the *env* gene.
[b] HPD, highest posterior density.

TABLE 2. Rates of synonymous and nonsynonymous substitutions of the SU region

Genetic region[a]	HIV type	Substitution rate at 10^{-3} substitutions site^{-1} year^{-1} (95% HPD[b])	
		Synonymous	Nonsynonymous
SU	HIV-2	13.38 (9.51, 17.43)	8.54 (6.27, 10.90)
SU	HIV-1	7.06 (5.40, 8.82)	6.01 (4.85, 7.32)

[a] SU, the complete major surface glycoprotein (gp125/gp120).
[b] HPD, highest posterior density.

identified and removed using Recco (23). The evolutionary rates were estimated using the Bayesian program BEAST (12). Details of the recombination analyses and evolutionary rate estimations are presented in Materials and Methods in the supplemental material. In comparing two different rates, we computed the posterior probability (PP) that one rate exceeded the other. This probability was determined numerically by randomly sampling from the empirical posterior distributions obtained from BEAST. These distributions typically had an effective sample size between 800 and 7,000 and were roughly Gaussian in shape. Scripting and statistical analyses were done with the statistical package R (28).

Using the Swedish HIV-2 data set and the matched HIV-1 data set, we compared the evolutionary rates of the SU region. These analyses showed that the evolutionary rate of the HIV-2 SU region was significantly higher than that of the HIV-1 SU region, i.e., 10.15×10^{-3} compared to 6.36×10^{-3} substitutions site^{-1} year^{-1} (Table 1) (PP $> 99\%$). The V3 region from the HIV-2 patients showed a similar result: the evolutionary rate of HIV-2 was 29.37×10^{-3} substitutions site^{-1} year^{-1}, compared to the evolutionary rate of HIV-1 at 12.26×10^{-3} substitutions site^{-1} year^{-1} (Table 1). The empirical posterior distributions were nonoverlapping, and thus, HIV-2 also evolved significantly faster than HIV-1 in the V3 region, again with a PP value of $>99\%$. To try to understand whether the higher evolutionary rate in HIV-2 could be due to differences in selection pressure on the amino acid level, we determined the rates of nonsynonymous and synonymous substitution separately. The analyses were only carried out for the long SU sequences. In our data sets, the rate of synonymous substitutions in SU was significantly higher for HIV-2 than for HIV-1, i.e., 13.38×10^{-3} versus 7.06×10^{-3} substitutions site^{-1} year^{-1} (PP $> 99\%$), possibly indicating a shorter generation time or higher mutation rate of HIV-2 than of HIV-1 (Table 2). The nonsynonymous sites also evolved faster in HIV-2 than in HIV-1, i.e., 8.54×10^{-3} versus 6.01×10^{-3} substitutions site^{-1} year^{-1} (PP $> 97\%$). Interestingly, while the synonymous rate was only slightly greater than the nonsynonymous rate in HIV-1 (15) (PP = 84%), we found that the nonsynonymous sites evolved at a significantly lower rate than the synonymous sites in the Swedish HIV-2 data sets (PP $> 98\%$) (Table 2). In addition, we used the single-likelihood ancestor counting (SLAC) method (26), which confirmed a global negative (purifying) selection (ratio of nonsynonymous to synonymous nucleotide substitutions [dN/dS] = 0.49) on the HIV-2 SU region, while the HIV-1 SU region had an almost neutral signal (dN/dS = 0.93). Collectively, these analyses indicate that the SU protein of HIV-2 evolves faster than the SU protein of HIV-1 but that this difference is not likely to be due to a stronger positive selection pressure for amino acid change.

Hitherto, little was known about HIV-2 evolution (11). This is because (i) the prevalence of HIV-2 is much lower than that of HIV-1, (ii) study recruitment is challenging since most HIV-2 patients live in West Africa, and (iii) the low virus levels in HIV-2 patients make it difficult to grow the virus and obtain sequences. Thus, our study was limited to relatively few HIV-2 patients (from Sweden and Portugal) and we had to study virus isolates since the viral load often was too low to allow direct sequencing. However, we tried to account for potential selection during virus isolation by comparing the HIV-2 results to results from matched HIV-1 samples processed in the same way. Notice also that our estimates of the HIV-1 evolutionary rate, which were based on sequences in both the SU and V3 regions of virus isolates, agree well with previously published estimates based on sequences from patient plasma (17, 19, 29, 32). This indicates that the potential selection during isolation did not adversely affect our evolutionary rate estimates. The strengths of our study include (i) a long time of follow-up of the patients, (ii) analysis of a longer genetic region (SU region) than in earlier studies and, importantly, (iii) comparison of our results for the evolutionary rate of HIV-2 to HIV-1 data that were matched with respect to sampling time, antiretroviral treatment, CD4 count, and viral load. Thus, this avoids the potential artifact from differences in the stage of disease progression between HIV-1 and HIV-2 patients affecting the evolutionary rate estimates, as it is known that the evolutionary rate changes during infection (19, 20). It should also be mentioned that none of the Swedish HIV-1 or HIV-2 patients were on successful combination antiretroviral treatment, which minimized the risk that treatment may have affected the estimated rates of evolution.

In our study, we observed that HIV-2 evolved at a higher rate than HIV-1, which does not agree with the results of earlier studies (20, 22). The reasons for this difference are unclear but could possibly be due to differences between the data sets that were analyzed or differences in the methods that were used. In this context, it is worth mentioning that our study was the first that employed both longitudinal sampling spanning many years and the most recent advances in Bayesian phylogenetic inference. Thus, we feel that our results are likely to be valid for our patients, but the conflicting results in comparison with those of earlier studies stress the need for additional and larger studies.

We investigated the HIV evolutionary rate on the within-patient level. Thus, the observed differences may be due to immune selection or generation-time effects. The higher evo-

lutionary rate in HIV-2 was more pronounced in synonymous sites than in nonsynonymous sites. This finding is in agreement with the results of Choisy et al. and Barroso and Taveira (3, 9), who reported a global negative (purifying) selection in the *env* gene of HIV-2. One possible explanation for this observation could be that the HIV-2 envelope glycoproteins have to accommodate more functions than those of HIV-1, e.g., *vpu*-like activity (6, 21) and immunosuppressive activity (7, 8). Another possibility is that the positive selection is weaker in HIV-2 infection, as indicated by the rare escape from autologous neutralizing antibodies (34). Taken together, our results indicate that the higher evolutionary rate in HIV-2 than in HIV-1 is primarily due to factors that affect the rate of synonymous mutations, e.g., generation times and replication error frequency (16), rather than factors that affect nonsynonymous mutations, e.g., immune escape. It should also be mentioned that a history of a different degree of recombination in HIV-1 than in HIV-2 could lead to the differences seen in our analyses, but to minimize the influence of recombination, we have removed all clearly identified recombinants from the analyses.

In conclusion, our study indicates that the rate of evolution of HIV-2 *env* is higher than that of HIV-1, at least for the patients with advanced disease included in our study. The reasons for this difference are not completely clear, but it is probably not due simply to differences in the immune pressure on the amino acid level. Additional studies are needed to confirm and explain our findings.

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