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Recent Evolutionary History of HIV-1 Subtype B—Rebuttal

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Abstract. The history of the HIV-1 B epidemic is the subject of a continuing debate. Did the epidemic start in the 1970s, as it was established based on the epidemiological data, or decades earlier, as it was suggested based on the analysis of nucleotide distances in the *env* gene? Our study [Lukashov and Goudsmit, *J Mol Evol* (2002) 54: 680-691] found that the overestimation of the age of the epidemic in the analysis of *env* sequences was a bias resulting from the non-clock-like evolution at nonsynonymous sites, while the estimates based on synonymous substitutions agreed with the results of epidemiological studies. Besides the principal difference between the evolution of synonymous and nonsynonymous sites, several issues have to be addressed: (i) the onset of the HIV/AIDS epidemic, and not the circulation of the preepidemic viruses, should be taken as the gold standard for the timeline of HIV diversification; (ii) the circulation of ancient, preepidemic, viruses, whether long- or short-term, does not influence the increase of HIV divergence during the epidemic; and (iii) application of the same random latency probability for all viruses, irrespective to their age and distance from the common ancestor, biases the estimation of the age of the epidemic.

Key words: HIV-1, evolution, molecular history — AIDS — Epidemic — Molecular clock — Most recent common ancestor

Did the HIV-1 Subtype B Epidemic Originate in the 1970s or Decades Earlier?

Our study of HIV-1 epidemics in the United States (US) and The Netherlands, presented in 1997 (Lukashov and Goudsmit 1997a), was recently published (Lukashov and Goudsmit 2002). Its analysis of nucleotide distances among *env* sequences pointed to onset dates in the 1950s–1960s for these epidemics (1953 or 1967 for the US epidemic), while analysis of *pol* sequences indicated dates in the 1970s, in accord with established epidemiological estimates. We demonstrated that the overestimation of the age of the epidemics in the analysis of *env* sequences was a bias resulting from the non-clock-like evolution at nonsynonymous sites (Lukashov and Goudsmit 1997b, 2002). Our estimates based on synonymous substitutions in the *env* region (1974 or 1976 for the US epidemic) agreed with the results of the analysis of the slow-evolving *pol* region. Based on epidemiological estimates for the onset of the epidemics and our demonstration that evolution at nonsynonymous sites is not clock-like, we concluded that 1974–1976 is the most accurate estimate for the onset of the US epidemic. Our conclusions are in complete agreement with the results of Salemi et al. (2000) showing that nonsynonymous evolution of HIV is not clock-like and pointing to 1970s as the onset date for subtype B diversification.

As in our study, the onset date of the US epidemic was used by Korber et al. (2000) to validate their method. Their analysis based on nucleotide distances among *env* sequences pointed to the same early dates in the 1950s–1960s as our "biased" analysis. While

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acknowledging that their method pointed to "earlier than previously thought" dates, Korber et al. nevertheless concluded that their method—and not the established onset date of the US epidemic—is correct. To justify their 1950s–1960s estimates, Korber et al. speculated about a long preepidemic period.

Data Presentation by Us vs Smith et al. (2003)

Korber et al. reported 14 dates for the onset of the US epidemic, with confidence intervals (CIs) covering a period of up to 50 years. We presented their earliest (1954), latest (1972), and "best" (1967) estimates in our paper. In both our Introduction and our Discussion, we specified that these dates were estimated within wide CIs and referred to the paper by Korber et al. We therefore strongly believe that our presentation of the results of Korber et al. could not have misled the readers in any way.

In contrast, Smith et al. (2003; preceding Letter) presented just a single date for the US epidemic as estimated by us (1976), giving the impression that this was our only estimate and that the 1950s–1960s estimates of Korber et al. were obtained by more sophisticated methods. We would like to stress once again that the analysis by Korber et al. of nucleotide distances in the *env* region resulted in the same estimates as our analysis of nucleotide distances in the *env* region, and that we argued these estimates based on the arguments provided in our paper and this response.

Accepting the Onset of the HIV/AIDS Epidemic as the Gold Standard for the Timeline of HIV Diversification

To grasp fully the methodology used by us and by Korber et al., we suggest looking at a well-described HIV-1 epidemic, such as the epidemic in the former Soviet Union (FSU), which took off with several thousand HIV-1 cases in 1994. This does not mean that there were no earlier HIV-1 cases in the FSU: hundreds of viruses have been described in the FSU during the preepidemic period from 1982 to 1994. The point is that accurate reconstruction of the increasing virus divergence during the epidemic should set 1994 as the year when the founder virus—the most recent common ancestor (MRCA) of the epidemic—existed and started to expand and diverge. We consider nonviable any approach by which the reconstruction and timing of the MRCA of the FSU epidemic set a date earlier than 1994. The methods used by us and by Korber et al. analyze the growing virus divergence during the epidemic, which is in no way influenced by the circulation of preepidemic HIV-1 strains, whether long- or short-term. Consequently, the circulation of any earlier, preepidemic, viruses cannot be reconstructed by the analysis of a growing virus divergence during an epidemic. Therefore, viruses such as the 1959 sequence used by Korber et al. can play no role in the validation of a reconstruction method for an epidemic, and any reference to preepidemic HIV-1 strains is irrelevant to a discussion of the onset of the epidemic. From the many regression lines that can be drawn through a virus population based on various assumptions, one can select a line that goes through any randomly chosen sequence point, such as the 1959 sequence. Since preepidemic HIV-1 strains do not influence the increase in HIV-1 divergence during an epidemic, this selection gives no grounds for justifying the conclusion that this is the epidemic's "best" line and that its particular assumptions are correct.

Moreover, the rate of the increase of HIV-1 divergence during an epidemic is not influenced by the age of evolutionary events, such as the separation among group M, whether it occurred in the 1900s or the 1950s. Therefore, this rate cannot be used to estimate the age of old events, as attempted by Korber et al.

Does Virus Introduction to the US from Haiti or Africa Make the US Epidemic Older?

Smith et al. cited well-known reports on early AIDS/HIV cases in the US, which are also referred to in our paper. These statistics were obtained by epidemiologists who concluded that the US epidemic started in the mid-1970s. Korber et al. did admit that their 1950s–1960s estimates are "earlier than previously thought." To explain this discrepancy, they postulated a hypothetical preepidemic period of up to 15 years. As noted above, speculation about preepidemic viruses is irrelevant in a discussion or calculation of the age of the epidemic. The proposal to revise the onset date of the US epidemic reads like circular reasoning: it is based exclusively on the 1950s–1960s estimates of Korber et al., the accuracy of which is in turn proven by their correct prediction of the onset of the US epidemic—if we revise it according to the estimates by Korber et al. Besides being unjustified, this proposal is also unnecessary: we demonstrated that the established scenario is in complete agreement with the patterns of the synonymous evolution of the HIV-1 *env* gene.

Smith et al. proposed that the Haiti cases should be included in the analysis of the US epidemic, referring to the data showing that "while the first AIDS cases were found in Haiti and the US at around the same time (1976–1978), in 1978 the prevalence of HIV was found to be much higher in Haiti than in the US." This argument cannot serve as evidence for an older epidemic, since HIV-1 prevalence depends upon the dynamics of an epidemic. The introduction of HIV-1 to the US from Africa, possibly through Haiti,

is indeed a likely scenario. However, the further logic of the authors of the preceding Letter is unclear to us, since they did not specify why, in their opinion, the hypothesis that the US epidemic started after virus introduction from Haiti (or Africa) makes it mandatory to use Haitian (or African) samples as an ingroup together with US samples in calculations. "A scenario that is widely considered plausible" is that HIV-1 subtype A entered the FSU from Africa, and subtype B entered The Netherlands from the US. However, it is irrational to include African or US samples as ingroups for the analysis of the resulting FSU and Dutch epidemics. The hypothetical earlier presence of HIV-1 in Haiti does not make the MRCA of the US epidemic older.

Regarding the global subtype B epidemic, the aim of our study was to trace its MRCA. We demonstrated that the synonymous evolution of the *env* region of HIV-1 is in agreement with the unanimous view that the global subtype B epidemic started as the result of virus expansion from the US in the 1970s. We believe that there could—and should—have been earlier preepidemic strains in Africa and maybe in Haiti, which cannot be traced based on the data for the Western subtype B epidemic.

Smith et al. proposed that, if the US epidemic had started in 1976 (indeed, 1974 or 1976 in our paper), it would require "at least several hundred people infected in 1976 alone," which is "very difficult to reconcile with the estimate of an MRCA that same year." This passage contradicts their own logic, which proclaims the simultaneity of the MRCA with the start of an epidemic: "In 1986, no evidence of HIV-1 was found (in Thailand). Yet by 1988, over 5000 Thais were infected.... [This suggests that] a single founder virus seeded the Thai E epidemic a few years before 1990.... A single founder subtype E virus (existed) some time near 1986–87" (Korber et al. 2000). Since Smith et al. did not propose an alternative scenario for the US epidemic, we can only guess what, in their opinion, it might be—hundreds of HIV-1 cases in the US in 1952 or 1967?

Factoring in a Latency Concept to Identify the Origin of an HIV Epidemic

Accurate correction for virus latency requires consideration of two factors: virus distance from the MRCA and virus age. At any given time point, an HIV-1 population contains viruses that are evolutionarily far from or close to the MRCA. One of the causes of this distribution is that viruses far from the MRCA have evolved over the whole epidemic, while viruses close to the MRCA have spent years in latency. Independently of their distance from the MRCA, viruses sampled from an epidemic in 2000 have longer evolutionary history than sequences from 1980 and thus a higher probability of having spent years in latency. Therefore, a "latency parameter" should assign shorter effective evolution times to sequences that are close to the MRCA and, independently, sequences sampled later in the epidemic, shifting them back in time. On the other hand, the positions of sequences sampled in the first years of the epidemic should be intact, since by definition they could not have spent years in latency. Clearly, without the latency, the current divergence of viruses—and the slope of the regression line—would be greater, resulting in a more recent estimate for the onset of the epidemic. The results of our model, which did not include the latency parameter, were already in agreement with the epidemiological data, suggesting that the influence of the latency parameter was too small to add extra power to our calculations. Nevertheless, we specified that our estimates should be considered as the earliest possible.

Instead of correcting for the latency, the method used by Korber et al. additionally biases the estimates. Korber et al. did not consider the different latency probabilities for viruses but applied the same random probability for all viruses, irrespective of their age and their distance from the MRCA. This approach shifts the regression line back to the past and results in an older, instead of a more recent, estimate. Smith et al. wrote that the influence of their approach upon their own results was "enormous." Since the calculations by Korber et al. have nevertheless pointed to the same dates as in our respective analysis—1950s to 1960s—we can only guess that this negative bias in their work was either relatively small or neutralized by another, positive bias.

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