Hot News

Pandemic HIV-1: Its Old Origin and Overlooked Mysteries

The oldest pandemic HIV-1 (group M) sequence known was, up to recently, the ZR59, isolated from an adult male from Kinshasa, Democratic Republic of Congo (DRC), in 1959 (Zhu, et al. Nature. 1998;391:594-7). This sequence branches from within the subtype D lineage after the latter splits from B. The genetic distance between ZR59 and the M-root is about half of that of modern sequences. This fact alone suggested that the MRCA existed decades prior to 1959, in line with molecular clock dating studies which placed it around 1920-1930 (e.g. Salemi, et al. FASEB J. 2001;15:276-8; Korber, et al. Science. 2000;288:1789-96).

In October 2008, Worobey, et al. (Nature 2008;455:661-5) reported the analysis of a new partial sequence isolated from paraffin-embedded lymph nodes of a woman from Kinshasa, collected and stored in 1960. The sequence, which they designated "DRC60", clusters with subtype A and, like ZR59, is much closer to the root than modern strains. For a small *env* region, both DRC60 and ZR59 fragments are available. So now we have two sequences which argue for an origin of pandemic HIV-1 decades before 1960. The authors re-estimated the MRCA timing, applying a relaxed clock coalescent framework. Without the ZR59 and DRC60 sequences included, they obtained a time around 1930, as in previous studies, but when they included these two strains, which provided early calibration points, and therefore improved the reliability of the estimates, the best-fit results, and their 95% CI, became 1921 (1908-33) under a constant population size model, 1902 (1873-1922) under an expansion model, and 1908 (1884-1924) under a Bayesian skyline plot model.

The new study suggests that HIV-1-M has been around for longer than previously thought. An origin in the period 1930-35 is now less likely, occupying the upper end of the probability distribution, in one of the models only. The fact that the two donors of ZR59 and DRC60 were opposite-sex adults may suggest that the epidemic was already predominantly heterosexual by that time. The clustering of these strains with different subtypes suggests that in Kinshasa, by 1960, a wide genetic variation of HIV-1-M already existed, and this broadly reinforces the view that this city was the epicenter for HIV-1-M emergence and spreading.

It is unknown if viral adaptation was necessary for epidemic emergence, and if it was, what process drove it. Some proposed parenteral serial transmission as the key factor (Drucker, et al. Lancet. 2001;358:1989-92), while others suggested urbanization and social changes. These theories leave several loose ends unexplained. One is that the dating of all epidemic HIV groups (HIV-1 groups M and O, and HIV-2 groups A and B) point to early 20th century, and injection intensity peaked after mid-20th century, therefore raising the question of why no more groups emerged after mid-20th century if injections were the key factor. Such potential new groups would have had time to spread enough to be noticed (Lemey, et al. PNAS. 2003;100:6588-92; Lemey, et al. Genetics. 2004;167:1059-68). Cities also grew exponentially, attracted many more potentially SIV-infected rural migrants after the mid century, raising the same question. The existing theories also fail to explain the biogeography of epidemic HIV groups, and why they are so few, despite bushmeat-related human SIV infections being not uncommon (Kalish, et al. Emerg Inf Dis. 2005;11:1928-30), and injections, urbanization, and migration, so ubiquitous. Thus, the enigma about the origin of HIV is still not solved, but with more data on early HIV emerging, we are coming closer to a general picture of the circumstances that permitted it.

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Is HIV Eradication Feasible?

Bone marrow stem cells may have cured one man of HIV (Hütter, et al. N Engl J Med. 2009;360:692-8). The patient had been infected with HIV for a while before developing acute myeloid leukemia. He received a stem-cell transplant from a donor who was homozygote for Δ32 CCR5. Interestingly, the patient has remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy. Moreover, a search for HIV sequences in proviral DNA has also failed to prove that the virus could still be hidden in some reservoirs. In addition, the CD4$^+$ T-cell count has returned to a normal range.

The German physicians in charge of this case essentially did what they would do for any leukemia patient who was not responding adequately to chemotherapy. They searched the registries for bone marrow donors who were a match for their patient's HLA, and prepared to perform a transplant. But,

hematologist Gero Hütter took the search for a donor one step further. He searched for potential donors who carried a short deletion in the *CCR5* gene. This gene encodes a receptor that HIV uses to enter $CD4^+$ T-cells. About 1% of the European population carries the CCR5 mutation in both copies of the *CCR5* gene, making such people much less likely to contract the virus (Liu, et al. Cell. 1996;86:367-77). If the patient's original immune cells could be replaced by new cells lacking the *CCR5* coreceptor, they might be less susceptible, or resistant, to HIV infection.

The patient had 80 matches in the bone-marrow registries of the German Bone Marrow Donor Center, and Hütter reasoned that one of those matches might also carry CCR5 mutations. Donor number 61 turned out to be the one, and in February 2007 the transplant was performed. Since then, the patient has remained without viral rebound even after discontinuation of antiretroviral therapy. This is a tremendous proof of principle that if you can make the majority of the cells resistant to HIV infection, you can really halt virus replication. However, was the patient cured? That remains unclear. As pointed out in the editorial accompanying the report (Levy. N Engl J Med. 2009;360:724-5), although the patient has gone about two years without a relapse of either HIV or leukemia, it is still possible that the virus will make a comeback. The virus could be lurking in cells that have not been tested such as cells in the brain or heart. In addition, HIV strains with tropism for the CXCR4 coreceptor could make its way and reestablish the infection. While X4 strains of HIV do not typically show up in patients with preserved immune systems, they could eventually proliferate in this patient, as shown occasionally in subjects homozygote for Δ32 CCR5 who became infected with X4 HIV-1 variants (Michael, et al. J Virol. 1998;72:6040-7).

It is clear that stem cell transplantation is not a treatment that most HIV-positive people would want to receive. The risks involved with a bone marrow transplant far outweigh those that come with years of antiretroviral therapy, even considering the troublesome side effects of these drugs. Before receiving the transplant, recipients have to receive ablative immune suppressors and radiation to destroy their own blood-producing stem cells. The procedure leaves them vulnerable to infections, and there is also the possibility that their bodies will eventually reject the transplant. Moving a step forward, instead of risking a transplant, could we not just use a drug to block CCR5? Only one CCR5 inhibitor, maraviroc, has already been approved for the treatment of HIV infection. More enthusiasm for this class of drugs is warranted. However, HIV resistance to maraviroc may occur, given that the CCR5 molecule remains expressed on cells. Alternatively, gene therapy approaches to prevent CCR5 from being expressed might be successful. Development of such technologies could include injecting into the bloodstream vectors carrying small interfering RNA (siRNA), antisense RNA, or ribozymes, which may reduce CCR5 cellular expression.

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Multicentre Inter-cohort Studies - Reliable Responses for HIV Disease Management?

The most recent evidences on HIV infection were obtained through large multicentre trials/inter-cohort analyses including patients enrolled by hundreds of investigators and co-investigators around the world. While extensively pooled data are needed to assess infrequent events (rare toxicities), long-term endpoints, or safety (e.g. mortality or lipodystrophy syndrome), or to perform comparative studies between different treatments, relevant biases/distortions descending from the extremely elevated number of enrolling centers/investigators are expected, while the reports among quoted co-authors of all investigators/co-investigators supports an unacceptable number of presumptive authors. As is known, a relevant number of outstanding multicentre/inter-cohort studies signed by thousands of co-authors, comprehensively quoted in all bibliographic databases, have been published in the last months by leading medical journals (starting just from 2008), especially in the attempt to focus on some infrequent HIV disease complications, and regarding efficacy and tolerability of novel antiretroviral therapies (ART). Some representative studies are summarized in the table.

Some studies address relevant, but proportionally infrequent, complications of HIV disease and/or antiretroviral therapy, like cardiovascular disease (Sabin, et al. Clin Infect Dis. 2008;46:1101-10; Sabin, et al. Lancet. 2008;371:1417-26), or lipodystrophy (Zanone-Poma, et al. AIDS. 2008;22:1769-78). Other cooperative studies focused on strong but infrequent and long-term endpoints, like the overall mortality of HIV-infected individuals compared with that of the general population in both adults (Bhaskaran, et al. JAMA. 2008;300:51-9) and infants (Violari, et al. N Engl J Med. 2008;359:2233-44), as well as the malignancy-related mortality rate (Monforte, et al. AIDS. 2008;22:2143-53).

Other studies assessed as endpoints the appearance of sentinel clinical events in virologically-failed patients at their first antiretroviral regimen (Mugavero, et al. AIDS. 2008;22:2481-92), or evaluated the efficacy/safety of newly introduced antiretroviral

Table 1. Major 2008 studies on the management of HIV infection, its complications, and treatment regimens recorded in PubMed

Reference	Comprehensive patient sample (n)	Main outcome or major endpoint(s)	Years of study (n)	Main authors (n)	Co-authors fully indexed in PubMed as authors (n)
Sabin C, et al. Clin Infect Dis. 2008;46:1101	33,389	Risk of cardiovascular disease, myocardial infarction	8	13	559
Sabin C, et al. Lancet. 2008;371:1417-26	33,347	Myocardial infarction	N.A.	15	578
Zanone-Poma B, et al. AIDS. 2008;22:1769-78	255	Genetic basis of the lipodystrophy syndrome	N.A.	12	94
Bhaskaran K, et al. JAMA. 2008;300:51-9	16,534	Mortality rate in HIV vs. general population	2	7	87
Violari A, et al. N Engl J Med. 2008;359:2233-44	377	Mortality among HIV infants on early cART	< 1	8	83
Monforte A, et al. AIDS. 2008;22:2143-53	23,437	Mortality associated with malignancies	N.A.	13	588
Mugavero MJ, et al. AIDS. 2008;22:2481-92	13,546	Clinical event(s) occurring in virologically failing patients	6	19	1,037
Gulick R, et al. N Engl J Med. 2008;359:1429-41	1,049	Drug efficacy (as for surrogate virologic and immunological markers), and safety	3	18	301

agents and combinations with superiority or non-inferiority study designs (Gulick, et al. N Engl J Med. 2008;359:1429-41).

We are aware that the willingness to obtain a statistically different distribution of some events (including drug toxicity, or rare long-term events, and significantly different regimen responses based on virologic/immunologic markers) require a specific and robust statistical design, which has to rely on the sample size. If an event is known to be proportionally rare, or presumably tends to occur late during disease course, or to become apparent after a very long time (for instance, myocardial infarction, patient's death, or lipodystrophy), and if the difference between comparable therapeutic regimens are supposed to be limited or minimal, the only way to try to obtain a statistically significant difference is to recruit extremely large patient samples with multicentre studies, or analyze pooled patients samples with *post hoc* analyses. Therefore, it becomes more and more clear why enormous patient samples are recruited to reach a sufficient statistical potency to show so-called differences (or at least a non-inferiority outcome, when comparative drug regimens are evaluated). The problems related to inflated patient samples become dramatically more and more actual, since pooled data from thousands of cases followed by hundreds of investigators, may "force" the study outcome towards the expected results by creating a "statistical" world as opposed to the "real" world. In some cases, these extremely large studies may lead to unexpected, casual correlations, which are only the consequence of statistical testing applied to enormous population samples, and may lead to distorted interpretations, which usually cannot be extrapolated to the clinical practice.

Unfortunately, it seems that in 2008 the only way to study hundreds or thousands of HIV-infected patients in a proportionally reduced recruitment time is to create joined multicentre studies, or to exploit "*post hoc*" inter-cohort analyses, both of them being increasingly performed in developing countries, where some patients with selected features still live (i.e. antiretroviral-naive subjects), although an elevated number of different genetic, dietary, pharmacogenomic, and life expectancy features, as well as eventual comorbidities, cannot be compared in any way with those regarding the inhabitants of Western countries.

Therefore, we are fully aware that extremely large but heterogeneous patient samples, recruited in different centers worldwide, and followed by hundreds of investigators and sub-investigators, may finally allow us to observe some statistically significant occurrences, which also need careful analysis, in order to demonstrate that bare statistical differences maintain a sense as true, clinically differences. Anyway, we have to take into account that these studies are burdened by relevant potential biases and distortions of recruitment and analysis, which often do not allow to reach reliable, generalizable results (as often claimed by the authors), which are not easy to be extrapolated to the management of the general population of HIV-infected subjects throughout the world. For instance, patients enrolled by hundreds of investigators from different continents necessarily include a case mix of subjects with different age, gender, racial, and body mass index distribution, as well as pharmacogenomic features, which clearly predispose to obtain non-comparable results from one centre to another, although eventual intra-cohort differences become "hindered" by presenting cumulate, mean, or median data extrapolated from thousands of individuals. Moreover, it is virtually impossible that hundreds of investigators and sub-investigators involved in these mega-trials use reproducible laboratory and instrumental assessments, especially when some subjective examinations are of concern (i.e. race-dependent cardiovascular risk calculation, lipodystrophy analysis, ultrasonographic instrumental assessments, patient's adherence, and quality of life measurements). Finally, when large cohort studies are pooled, we have to assume the risk that the same patients may be counted more than once.

Furthermore, starting just with 2008, the renown database PubMed started to recognize the contribution of each co-investigator as that of a true author (see table 1), leading to the uncontrolled and probably unreliable, exponential multiplication of co-authors, listed in the order of hundreds (Sabin, et al. Clin Infect Dis. 2008;46:1101-10; Sabin, et al. Lancet. 2008;371:1417-26; Monforte, et al. AIDS. 2008;22:2143-53; Mugavero, et al. AIDS. 2008;22:2481-92; Gulick, et al. N Engl J Med. 2008;359:1249-51), and all perfectly searchable through the web engine facilities. First of all, this behavior is significantly responsible for an unacceptable decline of the true authorship of literature contributions, which until now required that all individuals listed as authors were responsible for conceiving the study, drafting it, and discussing data in relation with the literature. As easily understandable from some data reported in the table, some lists of co-authors (actually collaborators, although fully quoted in scientific databases), exceeding the number of hundreds are absolutely unacceptable according to the past and present rules to recognize a full authorship of an article: it seems evident that each single "named" co-author and their co-investigators probably did not add anything significant to the study, save including mechanically an elevated number of enrolled patients with some selected characteristics.

We have to increase our vigilance level and our critical appraisal when assessing "giant" multicentre studies conducted probably in a non-uniform way, which essentially aim to recruit a sufficiently large sample size to reach statistically established parameters, and we have also to beware of literature search engines, which introduce among true literature authors, also simple co-investigators who lack of all the necessary characteristics to be considered as full authors. The tendency to inflate patient samples in favor of statistical needs, and the tendency to enormously inflate the authorship of leading studies has been recognized 25 years ago (Moulopoulos, et al. Br Med J. 1983;287:1608-10; Lazar. Acta Paediatr. 2004;93:589-91), but it is wrongly increasing, so that it deserves extensive examination and a critical appraisal by all authorities in this field.

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