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Reflections on the origin of human immunodeficiency viruses

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AIDS is a late 20th Century disease that is new to humankind. We may enquire into the origins of HIV-1 and HIV-2 by posing three separate though inter-related questions: Where or what hosts did these viruses come from? When did the cross-species transfer occur? How did the viruses make the leap?

Owing to the gravity of the AIDS pandemic and our apparent impotence in controlling it, myths of denial or conspiracy easily gain currency. How tempting it is, particularly in the regions most affected by AIDS, to heed siren voices that a sexually transmitted virus is not the root cause of the epidemic[1] or when that becomes undeniable to blame human actions, deliberate or unwitting, rather than natural processes for HIV's origin. For example, there is quite a widespread belief in Africa that HIV originated as a laboratory, recombinant virus, 'made in USA' and 'planted' in Africa after being tested on gay or drug-injecting prisoners in America. Such a notion identifies a convenient villain as well as removing blame from Africa and its fauna. But this theory does not fit the scientific data to hand. Neither, to my mind, does the theory that HIV emerged from contaminated polio vaccines remain at all likely, although this notion was by no means ridiculous.

Cross-species transfers

The most telling answers to the whence and when questions come from phylogenetic analyses of HIV and SIV genomes. HIV-1 and HIV-2 appear to be derived each on several separate occasions, from two quite distinct animal sources, the chimpanzee and the sooty managbey monkey. HIV-1 is most closely related in genome sequence to SIVcpz isolated from *Pan troglodytes troglodytes*, one of four subspecies of the common chimpanzee. In fact, the three major groups of HIV-1, Groups M, N and O, genetically differ from each other as much as from different SIVcpz genomes, strongly indicating that they each derive from separate events of transfer to humans[2]. Likewise, HIV-2 is very closely related to SIVsm of sooty mangabeys from which there may have been six or more separate cross-over events to humans[3]. The evidence, however, that chimpanzees are commonly infected by SIVcpz remains scant and requires further study. Could there be another animal species that represents the true reservoir?

At first sight, it appears odd that distinct strains of HIV should have colonized humans from different animal species on different occasions. But natural cross-species transfer is a frequent event for many retroviruses, not just HIV. There is also a precedent with the flaviviruses, yellow fever and dengue, and with malaria. *Plasmodium falciparum* malaria, arising in Africa, is closely related to the parasite of chimpanzees, whereas *P. vivax* came from Asian monkeys.

Yet why has HIV apparently colonized humans several times during the 20th Century but not before? The answer, I suspect, is that there have been many earlier introductions, but that like Ebola or Lassa fever outbreaks, they may only have flowered locally and temporarily if at all, and soon petered out. What helped HIV to become endemic, and Group M to become epidemic, might have been the huge expansion of needle and syringe use in the mid-20th Century during periods of mass vaccination and injecting antibiotic use, as suggested by Marx *et al*[4]. In other words, it is not the cross-species transfer event that is peculiarly modern, but the social conditions and medical practices that allowed HIV eventually to adapt to sexual transmission after its accidental introduction in to humans.

The spread of HIV following its introduction may have a parallel with hepatitis C virus (HCV) epidemiology. No-one knows the ancient provenance of HCV, or whether it was once transmitted by insects, like related flaviviruses and pestiviruses. But the syringe and needle became its major route of transmission[5], and the more frequently non-sterile injecting tools were used, eg, to control bilharzia in Egypt following the building of the Aswan dam, the more prevalent HCV became.

Timing the cross-over

Pinning exact time points to the various cross-over events of the three HIV-1 Groups and the six HIV-2 Groups is imprecise, because it involves retrospective extrapolation based on our knowledge of modern HIV strains. Neither HIV-1 in Central Africa nor HIV-2 in West Africa became epidemic until the late 1970s but it is evident that virus infection was present much earlier. The earliest well documented blood sample containing HIV-1 was taken from a man in Leopoldville (Kinshasa) in 1959[6]. It seems entirely rational to assume that a common ancestor to a particular HIV Group coincides with or post-dates the cross-species transfer date[7]. However, reasonably accurate extrapolation is only possible for HIV-1 Group M, (comprising the clades or subtypes A-K) as insufficient genotypic data exist for the other HIV-1 Groups or for HIV-2.

Three independent groups of viral phylogeneticists have analysed the origin of HIV-1 Group M using different methods and calculations[7,8,9]. There is remarkable agreement that HIV-1 Group M has radiated from a common ancestor around 1931, with 95% confidence limits of 15 years, although if recombination frequency between HIV subtypes was underestimated, the confidence limits might widen a little. Besides this consensus, what convinces me that the timing extrapolations are reasonably accurate is that certain HIV-1 genomes with known dates not used for the analysis fit precisely on the extrapolation curve. Thus Korber *et al*[8] found that the 1959 Leopoldville HIV

sequence, and a 1986 subtype E genome close to the founder sequence for the Thai epidemic each coincide with the median line placing 1931 as the date of origin of HIV-1 Group M.

The oral polio vaccine hypothesis

Oral polio vaccines (OPV), based on the CHAT attenuated lots of polio type 1 developed at the Wistar Institute in Philadelphia, were administered in large scale clinical trials in the Belgian Congo, Rwanda and Burundi from 1957 to early 1959. Broadly speaking, this is the region in which HIV-1 Group M first began to spread and to become manifest by causing AIDS. The OPV hypothesis postulates that HIV Group M entered humans by the administration of contaminated OPV through the propagation of polio vaccine in kidney cells derived from SIV-positive animals.

Like HIV itself, the OPV hypothesis has undergone mutation and adaptation as scientific advances disproved the initial theory. The OPV hypothesis was first presented in academic journals by Curtis[10] and Elswood and Stricker[11]. Early polio vaccines were initially prepared in Rhesus or Cynomolgus macaque kidney cultures. When these were found to contain SV40 virus in 1960, manufacture switched African green monkeys (AGM) as these animals were not contaminated by SV40. In 1986, however, a version of SIV was found to be widely endemic in AGMs. Thus SIVagm-infected animals very likely were used during 25 years' propagation of OPV.

Two observations soon discounted the OPV hypothesis that HIV came from African green monkeys. First, the Leopoldville blood sample[6] positive for HIV-1 predated the use of AGMs; second, the viral genome sequence of SIVagm is only distantly related to HIV-1, whereas the SIVcpz of chimpanzees is much closer. To rescue the OPV hypothesis, two new facets to the theory needed to be invoked, namely, that chimpanzee kidney cells were used for at least one lot of OPV, and that this happened before 1959. Such proposals were powerfully advocated by Hooper in his book *The River*[12]. He suggested that chimpanzee kidney tissue was sent from the Congo to the Wistar Institute, and that contaminated OPV was then returned to the Congo.

On account of Hooper's challenge to the scientific community, new tests have recently been performed on CHAT vaccine lots stored since the 1950s[13,14,15]. Using PCR detection methods it was found that the cell substrate for these early OPV vaccines was Rhesus monkey and not chimpanzee or AGM. Furthermore, no trace of HIV or SIVcpz could be detected by PCR or RT-PCB amplification[13,14]. The analyses included a vial of CHAT 10A11[13], the vaccine lot most implicated in *The River.*

More recently Hooper has changed his view, as I had previously suggested in my review of *The River*[16], namely that the Wistar Institute can be omitted from the loop, by postulating that CHAT vaccine was expanded and propagated locally in the Congo. At a meeting in Rome in September 2001, Hooper held that this was done by Dr Paul Osterrieth. However, this hypothesis contradicts Osterrieth's statement that this never happened[17] as well as the testimony of Plotkin *et al*[18].

Conclusions

OPV has prevented millions of children and adults from developing a fatal or crippling disease, and no-one disputes that today's OPV is as safe as any 'live' agent can be. The World Health Organisation aims to eradicate polio within the next few years. AIDS, however, could possibly interfere with this plan if immunocompromised hosts sustain chronic polio virus infection[19].

The OPV hypothesis on the origin of AIDS was thoroughly by protagonists and antagonists debated, together with the emerging phylogenetics of HIV and SIV, at a conference held at the Royal Society in London in September 2000. As can be gathered from the published proceedings[20], among virologists and geneticists there is profound scepticism that the OPV hypothesis continues to hold merit, owing to the estimated timing of the host species cross-over event, and the lack of evidence that chimpanzee kidneys were ever used. Moreover, some disturbing general lessons might be lost by clinging on to an untenable specific theory. First, iatrogenic transmission by injection of HIV may well have played as important a role in first establishing HIV in the population *after* its transfer from chimpanzees as it did for spreading HIV and hepatitis viruses among haemophiliacs decades later. Second, live vaccines were indeed made with our 'eyes wide shut'; we should regard the fact that the SIVagm of African green monkeys did not infect OPV vaccinees as a very close shave. One needs to be ever vigilant over vaccines and other biologicals put to medical use. So we can thank investigative writers like Curtis and Hooper for shaking the medical establishment's complacency, but they should recognise when to call it a day.

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