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ORIGIN OF HIV
AND EMERGING PERSISTENT VIRUSES

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ESTRATTO

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CONCLUDIND REMARKS

EMERGING PERSISTENT INFECTIONS: FAMILY HEIRLOOMS AND NEW ACQUISITIONS

FAMILY HEIRLOOMS AND NEW ACQUISITIONS

In his ecological commentary on human health and disease, McMichael [20] calls the microbes that have co-evolved with the human species our 'family heirlooms', whereas those that have come from animals are 'new acquisitions'. The heirlooms are the parasites and commensal micro-organisms that have colonized the host ever since it diverged from other host species. For humans, most of the co-evolving microbes are persistent infections such as the herpesviruses and papovaviruses, including the skin and genital papilloma viruses, and the SV40-related agents, BK and JC. Different genotypes may diversify with their hosts: for example, we have recently tracked human herpesvirus 8 evolution in Sephardi and Ashkenazi Jews [6], although horizontal exchange of HHV-8 also takes place with host population mixing (T.F. Schulz and A. Gessain, this Symposium). Phylogenetic reconstructions can be used to estimate demographic history of an infection within a host species, eg, HIV (P. Sharp, this Symposium), and host parasite co-speciation [16].

The ultimate in co-evolution is the endogenous retrovirus that is integrated in the germ-line of the host [3]. Thus relatives of porcine endogenous retrovirus (PERV) are present in the DNA of all old world pig species studied, but are absent from new world peccaries, suggesting a 30 million year residence in the *Suidae* [23]. It seems extraordinary that such ancient germ-line proviruses can emerge as potentially infectious viruses, yet such endogenous retroviruses can and do cross host species; that is why concern was raised over the possibility of PERV infecting humans through xenotransplantation (J. Stoye, this Symposium). Recent analyses of endogenous DNA related to the murine leukemia virus genus

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(gamma-retroviruses including PERV) show large discontinuities between host and viral phylogenies [19], indicating that cross-species transfer of retroviruses has occurred on numerous occasions during mammalian evolution.

Animal to human infection is called zoonosis. We may regard these zoonotic infections as 'temporary exhibits', borrowed from the animals that represent the long term reservoir necessary for the microbe's survival. At least 300 different viruses, rickettsia, bacteria, fungi, protozoa and helminth parasites are known to infect humans as zoonoses [22]. They occur in developed or developing countries, come from wild or domestic animals, and transfer via diverse routes. Many zoonoses do not spread further than the index patient. This is usually the case with rabies, and also, one hopes, with vCJD (M. Pocchiari and P.L. Gambetti, this Symposium). Others, like Lassa fever, and Ebola virus can be highly contagious for close contacts and carers but soon disappear again rather than becoming permanently established in the human population. For instance, an outbreak in 1996 of Ebola virus infection in Gabon resulted in the death of 21 among the 37 persons following the butchering of a dead chimpanzee involved in the feast [18]. This unfortunate example shows that the 'cut hunter' route of animal to human virus transmission is not merely a theory.

The 'new acquisitions' are the microbes that we have encountered and held on to during our social evolution of the last 14,000 years or so. In fact, humankind's progress since the last ice age from isolated bands of hunter-gatherers to a global population of over six billion has allowed us to become astonishingly 'nouveaux riches' in respect to infectious micro-organisms [20]. Diamond [7] argues that a large proportion of these microbes have been acquired from the animals we have domesticated, but I consider that he underestimates the contribution from non-domesticated species like rats and monkeys. Many of these newly acquired infections are highly pathogenic, but Bill Hamilton (whose life we commemorated at this Symposium) has argued that most are ill-adapted to cause disease in their new hosts [10].

Examples of human infectious disease of animal origin include microbes such as measles virus which has diverged from its probable morbillivirus progenitor, rindepest, to become established as a distinctive virus circulating exclusively among humans. Other epidemic diseases now specific to humans that originally crossed from domestic herd or flock animals include smallpox (from camels?), influenza (from waterfowl), and tuberculosis (from cattle). Thus infectious microbes, and viruses in particular, evolve in diverse ways [8]. The lesson of acquisition is that the

animal source and route of original cross-over is varied, sometimes uncertain, often unexpected.

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CONTAMINATION OF VACCINES

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Most of the viral vaccines that protect us against infection are propagated in animal cells before preparation for human administration by the oral or intramuscular route. Now that we know much more about persistent infections in animal cultures, it is not surprising with hindsight that some cellular substrates used for vaccines were inadvertently contaminated by viruses. The most famous of these contaminants was the SV40 virus discovered in 1960 in kidney cultures of rhesus macaques used to prepare poliovirus vaccines (Tognon, this Symposium). Live SV40 contaminated batches of both the the Sabin live attenuated vaccine and Salk inactivated vaccine because it was more resistant to inactivation then the polio virus itself. Several million people given polio immunization between 1955 and 1963 were exposed to SV40.

Although SV40 does not cause disease in macaques, it can cause tumors of the central nervous system and mesothelioma when inoculated into neonatal rodents. Epidemiological studies of SV40 exposed human populations have not revealed an increase in these types of tumor. However, as Tognon described at this Symposium, several laboratories have detected SV40 DNA sequences in these tumors in humans, including children born long after the SV40 contamination of polio vaccines had ceased. It has therefore become an important question to determine whether SV40 was introduced into humans via the polio virus vaccines and whether it is now transmitted down the generations as a human to human infection.

The data remain controversial, and the field has polarised into those who are utterly convinced that SV40 plays a role in human carcinogenesis and those who are sceptical. Further uncertainties are why SV40 is not clonally integrated into the tumors, for which Tognon provided a plausible model, and whether the T-antigen positive tumors could be expressing antigen not of SV40 but of the human relatives, BK and JC viruses. It is also odd that some laboratories regularly detect SV40 DNA sequences in human tumors whereas others using the same primers and probes do not. Is it, then, the tumors or the laboratory reagents that are contaminated by SV40 [4]? And could humans naturally harbor SV40 as an heirloom predating polio vaccination, while macaques are, perhaps, sensitive indicator species who picked it up from humans? The human SV40 story is far from resolved, and represents an important question

concerning vaccines. Since the Symposium, further reports have been published linking SV40 to human lymphoma [28, 30].

Other human vaccines known to have been contaminated by persistent animal virus infections are those grown in chick embryo fibroblast culture and in eggs. Avian substrates are used in the manufacture of killed influenza virus vaccines, and in live attenuated measles, mumps and yellow fever vaccines. Avian leukosis viruses are retroviruses that pass vertically in the egg from hen to chicks; they are known to have contaminated some of these vaccines in the days before screening was introduced, but they do not appear to infect humans or human cells in culture, because human cells do not express compatible receptors for the avian retrovirus envelope [32]. Suppose, however, that the retrovirus became pseudotyped with the envelope of the vaccine virus? More recently, chick embryo cells free of avian leukosis virus have been found to release endogenous particles containing reverse transcriptase activity. These are not known to be infectious, but their recent detection indicates how important it is to probe vaccines for possible contamination.

For the foregoing reasons, it is pertinent to enquire whether HIV might also have been introduced to humans via contaminated vaccines, before its emergence as an epidemic. This was discussed in detail during the Symposium, together with data on HIV evolution and phylogeny.

ORIGIN OF HUMAN IMMUNODEFICIENCY VIRUSES

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?

The most telling answers to the questions of provenance and timing 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 simian immunodeficiency virus (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, differ from in genetic sequence as much from each other as from different SIVcpz genomes, which strongly indicates that they each derive from separate events of transfer to humans [12]. Likewise, HIV-2 is very closely related to SIVsm of sooty mangabeys from which there may have been six or more sepa-

rate cross-over events to humans [13]. 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. Humans have become infected with other chimpanzee retroviruses, such as T-cell leukemia viruses and foamy viruses [14, 31]. There is also a precedent for simian sources 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.

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 Drucker *et al.* [9]. In other words, it may not be 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 origin of HCV, and whether it was once transmitted by insects, like related flaviviruses and pestiviruses. But the syringe and needle became its major route of transmission [29], 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.

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 [36]. 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 (Sharp, this Symposium). However, 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 [17, 26, 27]. 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 (M. Schierup, this Symposium). 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.* [17] 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 FOR HIV

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, Burundi and the neighbouring border of Tanzania from 1957 to early 1959 (Hooper, this Symposium). 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 [5] and Elswood and Stricker [11]. Early polio vaccines were 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 strain of SIV was found to be widely endemic in AGMs. Thus SIVagm-infected animals almost certainly 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 [36]

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* [15]. 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.

Following Hooper's suggestion in *The River* new tests have recently been performed on CHAT vaccine lots stored since the 1950s [1, 2, 25]. 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-PCR genome amplification [1, 2]. The analyses included a vial of CHAT 10A11 [1], 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* [34], 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 this Symposium, Hooper postulated that this was done by Dr Paul Osterrieth. However, this hypothesis contradicts Osterrieth's own statement that he never used chimpanzee kidneys [21] as well as the testimony of Plotkin *et al.* [24]

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 children sustain chronic polio virus infection [33].

The OPV hypothesis on the origin of AIDS was thoroughly debated by protagonists and antagonists, 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 [35], 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. We must thank investigative writers like Curtis and Hooper for raising an issue that the medical establish-

ment did not wish to contemplate. Now, however, I invite Hooper himself to contemplate more deeply whether the OPV hypothesis really remains plausible, rather than attribute base motives to those who disagree with his views.

Furthermore, by clinging on to an untenable special theory some disturbing general lessons might be lost, lessons that were not overlooked by Bill Hamilton (M. Bozzi, this Symposium). 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 hemophiliacs decades later. Second, live vaccines were indeed made with our 'eyes wide shut'; as T.F. Schulz remarked at this Symposium, we should regard the fact that the SIVagm of African green monkeys did not infect OPV vaccinees as a very close shave. Third, infection hazards may accompany novel medical advances like xenotransplantation. Fourth, the BSE/vCJD crisis shows how we contaminated the very sustenance of humans and livestock alike. We need to be ever vigilant, microbiologically, over foodstuffs, vaccines and other biologicals such as xenografts.

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