Polio vaccine samples not linked to AIDS

A search through the archives clears early vaccines of starting the AIDS pandemic.

It has been suggested that chimpanzee kidney cultures may have been used in the preparation of oral polio vaccine stocks used in Africa during the late 1950s, and so could have introduced the primate precursor of the immunodeficiency virus HIV-1 into humans[1,2]. Here we analyse frozen samples of the suspect vaccine by using the polymerase chain reaction (PCR) to amplify any HIV-1-related nucleic acids or chimpanzee mitochondrial DNA that might be present, but we have failed to detect either. Our findings do not support the hypothesis that HIV-1 was introduced by oral vaccination against poliovirus .

A short description of five samples of oral polio vaccine (OPV) held by the Wistar Institute is given in Table 1. CHAT pool 13 was widely used to vaccinate thousands of people (mainly children) in Léopoldville[3] in the former Belgian Congo (now Kinshasa, Democratic Republic of Congo) and some in Poland. Three further samples of CHAT vaccine held at the Centers for Disease Control (CDC) for 20–40 years were included in the study. The combinations of oligonucleotide primers and probes used can detect a wide variety of HIVs, including HIV-1 M, O and N isolates, as well as chimpanzee isolates Gab1, US and ANT70. The PCR assays have a sensitivity of about 50–80 RNA copies per ml. No sample proved positive for HIV-1-related nucleic acids by any of four primer pairs we used. By contrast, all samples were positive for poliovirus using PCR with reverse transcription, demonstrating that there was not extensive degradation of viral RNA (Table 1).

To identify the source of the kidney epithelial monolayers used to culture OPV, we analysed a small region (140 base pairs) within the mitochondrial 12S ribosomal RNA gene[4]. The primers we used amplify DNA from animals, including the African green monkey, chimpanzee, rhesus monkey and sooty mangabey (see supplementary information). All but one of the OPV samples were positive for mitochondrial (mt) DNA. With the exception of CHAT 1FL (see below), most sequences (86%) were highly related to those of the rhesus (*Macaca mulatta*) or cynomolgus monkey (*Macaca fascicularis*), and differed by only 0–2 bases from a known haplotype (Fig. 1).

A few sequences were nuclear paralogues of mtDNA. The amplification of bona fide mtDNA sequences also picks up these nuclear insertions of mitochondrial sequences (NUMTs). They behave as pseudogenes and fix mutations more slowly than mtDNA itself[5]. A few of these NUMTs, three out of more than 250 sequences, were related to mtDNA sequences of a variety of *Cercopithecus* monkeys. These *Cercopithecus*-like sequences never exactly corresponded with a known sequence in the databases, but differed by about 4 base pairs. Most rhesus monkey mtDNA sequences corresponded to known mtDNA haplotypes. Some of the *Cercopithecus*-like sequences were also derived from single rhesus monkey samples (not shown). We conclude that these really are NUMTs and not evidence that some African monkey

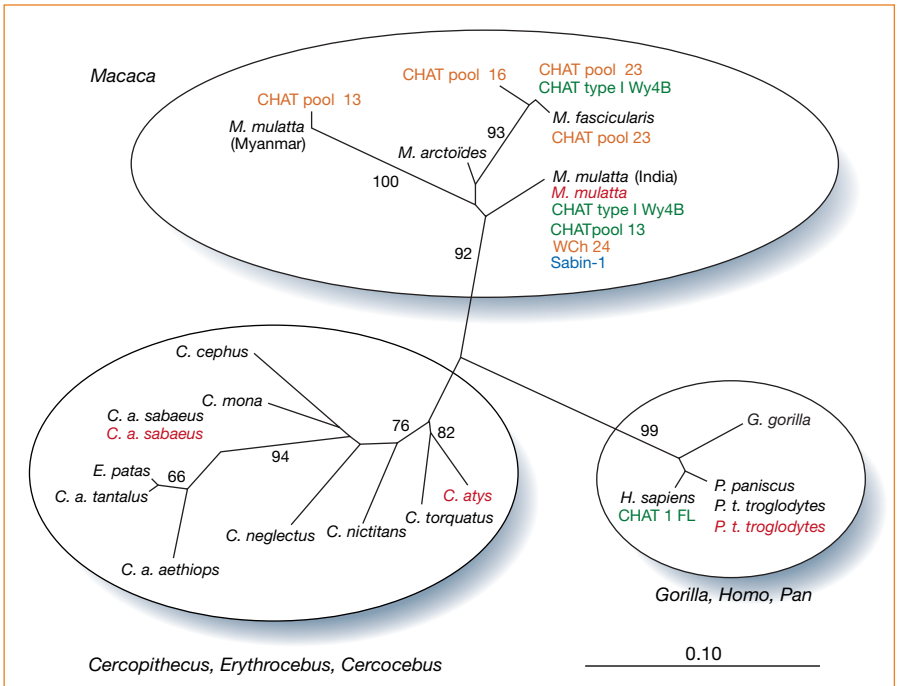

Figure 1 Neighbour-joining tree of 12S mtDNA sequences (see ftp.pasteur.fr/pub/retromol/wistar01). The three groups correspond to the *Macaca*, *Gorilla/Homo/Pan* and *Cercopithicus/Erythrocebus/Cercocebus* lineages. Of the latter, *C. atys* and *C. torquatus* are *Cercocebus* lineage. Wistar and CDC OPV samples and mtDNA controls are in yellow, green and red, respectively; the Sabin-1 positive control is in blue. Other sequences are from databases. The phylogenetic tree was derived by the neighbour-joining method applied to pairwise sequence distances calculated using the Kimura two-parameter method transition/transversion ratio set to 10. Branch lengths are drawn to scale: bar, 0.1 nucleotide replacements per site. Numbers indicate the percentage of bootstrap samples in which the cluster is supported (only bootstrap values over 60% are given). Rhesus macaques are split into two groups, corresponding to the Chinese and Indian subspecies. Remaining sequences are nuclear insertions of mtDNA sequences (see supplementary information).

Table 1 Oral poliovirus vaccine samples tested

Wistar samples		SIV_{CPZ}/HIV-1	Polio
CHAT pool 13	75,000 vaccinated in Léopoldville	–	+
CHAT pool 16 A-5	Closest passage to pool 13 available	–	+
CHAT pool 23 7.7. logs	Possibly used in other large trials; protocol on file	–	+
W Ch 24 57C-40 137-71	Possibly used in other large trials	–	+
W Ch 25	Late-passage virus	–	+
CDC samples			
CHAT pool 13	Monkey-kidney passage of CHAT pool 13 (29 Aug 1960)	–	+
CHAT type I Wy4B-5	Obtained from Wistar, made at Wyeth	–	+
CHAT 1FL	Late passage of CHAT 13 in human FL cells (15 Oct 1979)	–	+

SIV_{CPZ} is the isogenic chimpanzee counterpart of HIV-1 strains. For detection of SIV_{CPZ}/HIV-1 sequences, we used four primer pairs and two for poliovirus. A committee set up by the Wistar Institute identified frozen OPV samples that had been used in Central Africa or had been prepared during that era. Samples and controls were coded and hand-delivered to Roche Molecular Systems and the Institut Pasteur, each ignorant of the other's identity. Choice of amplification primers was left to the individual investigators to increase the chances of detection by at least one group. Small fragments were amplified in case there had been degradation of nucleic acids, particularly RNA, after being frozen for 40 years. Samples were also tested for poliovirus sequences (because if an OPV sample tested negative, then a negative result for SIV/HIV-1 and mtDNA would be meaningless). Infectious poliovirus was recovered for CHAT pool 16 and all three CDC-held samples (V. Racaniello, personal communication). A recent sample of Sabin-1 poliovirus grown on a rhesus monkey kidney cell line[11] was included as a positive control. mtDNA from four animals and a titration of HIV-1 isolate VQA, SIV_{CPZ} Gab1, HIV-2 NIH-Z stocks served as controls.

kidneys, along with those of macaques, had been used to grow OPV.

Given that OPV was prepared from pools of culture supernatants derived from individual pairs of kidneys, the presence of multiple mitochondrial haplotypes in the same sample is not unexpected. This would also explain the 3:2 mix of *M. mulatta* and *M. fascicularis* haplotypes in CHAT type 1 Wy4B. The detection of *Homo* sequences in CHAT 1FL stems from the fact that the virus was grown on the FL human diploid cell line.

To confirm these findings, we studied a mitochondrial 12S-rRNA locus (101 base pairs) just 5′ to the previous segment. This too was highly informative, with ten point substitutions and one insertion/deletion distinguishing macaque and chimpanzee sequences. All of our findings were confirmed. As the Wistar CHAT pool-13 lot was widely used in Léopoldville, we increased the resolution of the analysis. No chimpanzee sequences were found at a resolution of 1.7%. As this frequency is below the reciprocal of the number of animals generally used to make a lot of OPV, we conclude that none of these OPVs were prepared using chimpanzee kidneys.

In addition, none of the samples was positive when amplified with primers specific for chimpanzee nuclear DNA (data not shown). The extensive use of rhesus and cynomolgus monkeys in the preparation of all the Wistar samples described here not only confirms earlier claims[6–8], as well as those denying the use of chimpanzee kidneys in the preparation of OPV[9], but also reflects the procedures used in the late 1950s by Salk and Sabin.

CHAT pool 13 was used in Léopoldville between August 1958 and April 1960. The earliest bona fide HIV-1 sequence was derived from a 1959 serum sample taken from an adult living in the suburbs of Léopoldville[10]. According to the OPV/AIDS hypothesis[1,2], the CHAT pool-13 sample should contain chimpanzee DNA, but as only macaque sequences were found, this tight geographical and temporal association would seem to have been a coincidence.

There is a corollary to our conclusion that the Wistar OPV samples were prepared using macaque kidneys. Given that simian immunodeficiency viruses (SIVs) are confined to African primates, the kidneys could not have been positive for SIV. In other words, the absence of SIV nucleic acids in all samples do not represent false negatives. We find no evidence to support the OPV/AIDS hypothesis.

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Supplementary information is available on *Nature*'s website at www.nature.com or as paper copy from the London editorial office of *Nature*. All sequences are available at ftp.pasteur.fr/pub/retromol/wistar01.

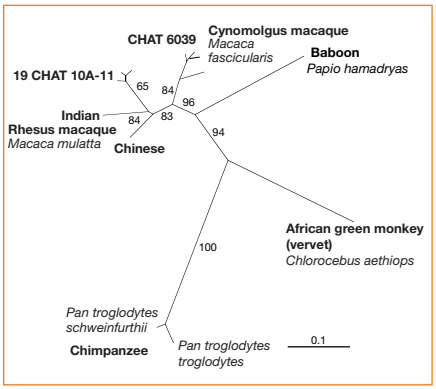

Figure 1 Nucleic-acid sequences were aligned using ClustalW (version 1.5) and evaluated using the Phylip Software package. Evolutionary distances were estimated using DNADIST (default: Kimura 2-parameter method, transition:transversion ratio 2.0) and phylogenetic relationships estimated by the neighbour-joining method using NEIGHBOR. Reproducibility of the branching pattern was assessed with SEQBOOT (boot strap, 100 replicates).

Vaccine safety

Analysis of oral polio vaccine CHAT stocks

Batches of experimental oral vaccines against poliovirus (OPV CHAT) that were administered in Central Africa in the 1950s have been implicated in the origin of the AIDS pandemic because of possible retroviral contamination during the vaccine's manufacture, which allegedly involved chimpanzee kidney cells[1]. Here we use a molecular analysis to show that two CHAT type-1 polio vaccine stocks were prepared from macaque and not chimpanzee cells, and contain neither human nor simian immunodeficiency virus sequences. Our results do not support the hypothesis that these materials were responsible for the transmission of the AIDS virus to humans.

The potential for oral polio vaccines to initiate the AIDS pandemic has been investigated previously[2,3]. Many species of African non-human primates are naturally infected with simian immunodeficiency viruses (SIV) and the common chimpanzee (*Pan troglodytes*) harbours SIV_{CPZ}, the closest relative to modern strains of the human immunodefiency virus HIV-1 (ref. 4). The use of chimpanzee cells to prepare CHAT vaccine may therefore have resulted in the inadvertent transmission of SIV_{CPZ} to humans.

To test this, we analysed two stocks of OPV CHAT. The first, labelled 19 CHAT 10A-11, was received by NIBSC in 1981 from the Karolinska Institute, Stockholm, and represents an original vial sent from the Wistar Institute, Philadelphia in 1958. The second, CHAT 6039 (an internal NIBSC number) made at the Institute of Immunology, Zagreb, was received by NIBSC in 1987. The presence of CHAT virus was confirmed by molecular assay and both samples yielded viable poliovirus in culture as evidence of quality of long-term storage (J. Martin, personal communication).

We tested extracted nucleic acid for HIV-1 RNA using polymerase chain reaction with reverse transcription (RT-PCR) assays based on *gag* (Amplicor HIV-1 Monitor assay, version 1.5) and combinations of long terminal repeat (LTR) sequences[5], which are capable of detecting HIV-1 group M (subtypes A–H)[6], group N and O viruses and SIV_{CPZ}. Additional combinations of LTR sequences used in nested PCR reactions were 507–529 and 524–543 (forward orientation), 622–641 and 628–648 (reverse orientation); the numbering is based on HIV-1 HXB2. HIV-1 primers in *pol* and *env* (gp41; ref. 7) and for HIV-2/SIV_{SM} (ref. 5) were also included. Sensitivity was shown to be less than 400 RNA equivalents per ml for each primer set. Using assays that detect this wide range of genetic variants of HIV-1, including SIV_{CPZ} and the earliest known sequence of HIV-1 present in the Belgian Congo in 1959 (ref. 7), we were unable to find any evidence of HIV/SIV in either CHAT stock.

Substrate cells were identified by targeting the D-loop control region of mitochondrial DNA using PCR. Chimpanzee-specific primers failed to amplify a product from either sample. Furthermore, these primers were capable of detecting limiting amounts of chimpanzee template in the presence of 10^5 macaque-cell equivalents, making it unlikely that chimpanzee cells were used to propagate poliovirus in the two prior passages of vaccine material. However, generic D-loop primers capable of amplifying DNA from baboon, African green monkey