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Mitochondrial DNA and retroviral RNA analyses of archival oral polio vaccine (OPV CHAT) materials: evidence of macaque nuclear sequences confirms substrate identity

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Abstract

Inoculation of live experimental oral poliovirus vaccines (OPV CHAT) during the 1950s in central Africa has been proposed to account for the introduction of HIV into human populations. For this to have occurred, it would have been necessary for chimpanzee rather than macaque kidney epithelial cells to have been included in the preparation of early OPV materials. Theoretically, this could have led to contamination with a progenitor of HIV-1 derived from a related simian immunodeficiency virus of chimpanzees (SIV_{CPZ}). In this article we present further detailed analyses of two samples of OPV, CHAT 10A-11 and CHAT 6039/Yugo, which were used in early human trials of poliovirus vaccination. Recovery of poliovirus by culture techniques confirmed the biological viability of the vaccines and sequence analysis of poliovirus RNA specifically identified the presence of the CHAT strain. Independent nested sets of oligonucleotide primers specific for HIV-1/SIV_{CPZ} and HIV-2/SIV_{MAC}/SIV_{SM} phylogenetic lineages, respectively, indicated no evidence of HIV/SIV RNA in either vaccine preparation, at a sensitivity of 100 RNA equivalents/ml. Analysis of cellular substrate by the amplification of two distinct regions of mitochondrial DNA (D-loop control region and 12S ribosomal sequences) revealed no evidence of chimpanzee cellular sequences. However, this approach positively identified rhesus and cynomolgus macaque DNA for the CHAT 10A-11 and CHAT 6039/Yugo vaccine preparations, respectively. Analysis of multiple clones of mtDNA 12S rDNA indicated a relatively high number of nuclear mitochondrial DNA sequences (numts) in the CHAT 10A-11 material, but confirmed the macaque origin of cellular substrate used in vaccine preparation. These data re-inforce earlier findings on this topic providing no evidence to support the contention that poliovirus vaccination was responsible for the introduction of HIV into humans and sparking the AIDS pandemic.

Keywords: Oral poliovirus vaccine; CHAT; HIV/SIV; Contamination; Mitochondrial DNA; Numts

1. Introduction

Oral poliovirus vaccines (OPV) are live-attenuated virus vaccines which have been employed in international programmes designed to lead to the eradication of poliovirus infections from the world [1]. Early experimental vaccines developed to combat poliomyelitis first developed in the late

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1950s were used in widespread poliovirus vaccination campaigns at this time, particularly in Central Africa and Europe [2]. The appearance of HIV/AIDS during the latter part of the 20th century in the same geographical regions as some early poliovirus vaccination programmes, however, led to the proposal that certain experimental poliovaccine materials may have been responsible for the introduction of HIV-1 into human populations [2,3]. A wide range of African non-human primates are naturally infected with simian immunodeficiency virus (SIV), and the common chimpanzee (*Pan troglodytes*) harbours SIV_{CPZ} which is the closest animal counterpart to HIV-1 leading to the conclusion that AIDS in humans is the result of a zoonosis with transfer occurring from chimpanzees to man [4]. Contamination of early poliovirus vaccine materials with SIV_{CPZ}/HIV-1, by the use of chimpanzee rather than macaque kidney epithelial cells as part of the manufacture process, could therefore have inadvertently led to a single or multiple zoonotic events and HIV-1 becoming established in humans. Alternatively, the use of macaque monkeys in vaccine preparation could not have led to the introduction of SIV/HIV into humans since Asian macaques are not naturally infected with a simian counterpart to HIV-1. The experimental vaccines known as OPV CHAT developed at the Wistar Institute in the USA have been particularly implicated, with different lots or pools of vaccine having been prepared and used in clinical trials in central Africa during the 1950s [2].

CHAT pool 13, for example, was used to vaccinate 75,000 people in Leopoldville between 1958 and 1960, coinciding with the retrospective identification of the earliest known sequence of HIV-1 dating to 1959 and obtained from an adult living in Leopoldville at this time [5]. Materials from the period, including CHAT pool 16A-5, CHAT pool 23, W Ch 24, CHAT type 1 Wy4B, CHAT 10A-11 and a vial known as CHAT 6039/Yugo, have been independently analysed by ourselves and others [6–9]. CHAT pool 10A-11 and CHAT pool 13, were identified as being particularly relevant to the OPV/AIDS hypothesis yet these studies were unable to provide scientific evidence to support the notion that these materials contributed to the AIDS pandemic, based on molecular investigations of retroviral contamination and cellular substrate composition.

Mitochondrial DNA (mtDNA) sequence analysis represents a powerful tool to the evolutionary biologist [10] and such an approach was used to evaluate the origin of cellular components in OPV materials, all of which proved to be macaque and not chimpanzee in origin. However, one of the potentially confounding issues in such exercises is the existence of nuclear mitochondrial pseudogenes or numts. Numts are nuclear copies of mitochondrial sequences present in genomic DNA representing non-functional copies of a normal gene, slightly modified such that they are no longer expressed and which can potentially obscure the true phylogenetic relationship between different species [11–13]. Indeed, the ability for numt sequences to complicate a genetic analysis of substrate composition of OPV materials has been investigated by comparing mtDNA haplotypes from archival oral polio materials and Chinese rhesus macaque DNA sequences [8]. Wide sequence variation was identified, including a relatively high proportion of divergent numt sequences, though all were of macaque origin. The locus studied, 12S rDNA, was selected due to the relatively large number of sequences available in the database [14]. In our previous study, sequences from the displacement loop (D-loop) region, indicated the species of origin used to prepare CHAT 10A-11 and CHAT 6039/Yugo to be *M. mulatta* (Rhesus macaque) and *M. fascicularis* (Cynomolgus macaque), respectively [6]. However, these investigations did not fully investigate the presence of numts.

To address this, we have re-analysed materials from both vaccine pools using 12S rDNA sequences and compared cloned sequences with the relatively larger database now available for this locus [14,8]. We also present a more detailed analysis of the two vaccine materials and confirm their authenticity by sequence identity of poliovirus RNA. Details of the reverse transcriptase (RT)-PCR assays for detection of viral RNA with multiple primer sets for genetically diverse HIV-1/SIV_{CPZ} and HIV-2/SIV_{SM} phylogenetic groups are presented which indicate no evidence of contaminating retroviral RNA. The additional mitochondrial DNA sequence studies identified a significant number of numt sequences, particularly in the CHAT 10A-11 material partly reflecting the relatively more complex genetic lineage of Asian rhesus macaques compared with cynomolgus macaques.

Moreover, the subsequent data confirm the original findings, now performed on two distinct gene loci, which indicate that only macaque cells and not those from chimpanzees were used to prepare these materials. These data further indicate that an analysis of numt sequences should be taken into account when embarking on analyses of archived OPV vaccine materials when elucidating substrate composition.

2. Materials and methods

2.1. Vaccine stocks and poliovirus isolates

Archival samples of OPV CHAT, an oral poliovirus type 1 vaccine, were identified at NIBSC. The first was a vial of frozen liquid labelled 19. CHAT 10A-11, received by NIBSC in 1981 from the Karolinska Institute, Stockholm, and represents an original vial prepared at the Wistar Institute, Philadelphia, PA and forwarded to Sweden in 1958 for use in oral poliovirus vaccine trials in humans [15]. The CHAT 10A-11 pool was also extensively used in OPV campaigns conducted in Central Africa in 1957–1958 in the former Belgian Congo (Democratic Republic of Congo), Burundi and Rwanda. Around 215,000 individuals living along the Ruzizi Valley and Lake Tanganyika reportedly received CHAT 10A-11 [2,16]. Outside Africa, apart from the Swedish trials CHAT 10A-11 was used in Switzerland [17] and sites in New Jersey, USA [18,19]. The second sample, CHAT 6039 (also