Preferential detection of HIV subtype C′ over subtype A in cervical cells from a dually infected woman

Although over a dozen HIV subtypes exist, HIV subtype C is the most prevalent subtype worldwide, accounting for half of HIV infections in sub-Saharan Africa (UNAIDS/WHO-2002). Studies indicate that subtype C originated in Ethiopia, and later spread to South Africa, where it established a distinct genotype (C′) [1]. During the past two decades, HIV subtype C has spread through south and eastern Africa, invading areas formerly dominated by subtypes A and D [2] (BioAfrica, www.Bioafrica.net). The two C genotypes are currently co-circulating in Ethiopia, and the relative prevalence of C′ is increasing [1]. The strain of subtype C rapidly

spreading in India is phylogeneticly linked to C′. The spread of subtype C′ could simply be the result of the increased mobility of the population in the affected areas. However, the increasing dominance of subtype C′ in sub-Saharan Africa strongly suggests that there are biological factors favouring the transmission of this subtype.

Here we present evidence of the selective replication of subtype C′ in the cervix from a Kenyan woman infected with both subtypes A and C′ (Fig. 1). The woman moved

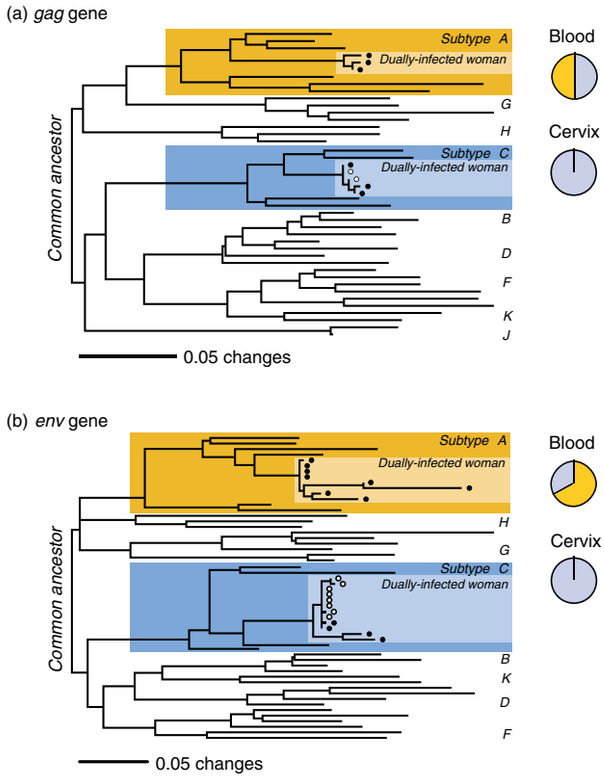

Fig. 1. Phylogenetic trees of gag and env sequences and pie charts of subtypes A (orange) and C (blue) sequence distribution in blood and cervix cells. (a) *Gag* (p17/24, HXB2 coordinates: 889–1242) and (b) *env* (C2-C3, HXB2 coordinates: 7086–7397) sequences from the dually infected woman and the HIV-1 group M reference sequences for subtypes A–K (http://www.hiv.lanl.gov/). Open circle, cervix-derived sequences; filled circles, peripheral blood mononuclear cell (PBMC)-derived sequences. The scale bars show the branch length equal to five nucleotide changes per 100 bases (0.05). Subtype C is blue, A is orange and the other subtypes are uncoloured. The pie charts show the distribution in the blood and cervix of subtypes A and C *gag* and *env* sequences, respectively. The number of sequences belonging to each subtype were: blood, *gag*: three A, three C; *env*: eight A, four C; cervix, *gag*: no A, two C; *env*: no A, eight C. The distribution of subtypes in PBMC and cervix DNA were significantly different (Fisher's exact test (two-tailed), *env* sequences only ($n = 20$), $P = 0.005$; all sequences ($n = 28$), $P = 0.002$). The sequences have been submitted to GenBank (accession numbers AY721629–AY721656).

to Denmark in 1992 and was found to be HIV-1 positive in 1994, but the time of infection is unknown. Viral transmission was through heterosexual contact. We obtained peripheral blood mononuclear cells (PBMC) and cervical cell samples from her in 1996.

HIV *gag* and *env* genes were amplified using limited-dilution polymerase chain reaction and were sequenced as described previously [3,4]. The same primers were used to amplify subtypes A and C sequences from both PBMC and cervical cell DNA. The amplifications were carried out at different times by two researchers, who were blinded to the results of each other. Subtypes A and C′ sequences were seen in equal proportions in the blood, but no subtype A virus was ever found in the cervix. No sign of recombination between subtypes was detected in the regions screened.

One could argue that our patient might have been infected with subtype C′ first and that the superinfection with subtype A had not established itself in all body compartments at the time of sampling [5–7]. However, the maximum divergence within the subtype A sequences (0.15) is three times that found among subtype C′ (0.05), strongly suggesting that subtype A has been present in this patient longer than subtype C′. The subtype A sequences clustered with A sequences from Tanzania and Kenya, whereas the subtype C′ sequences clustered with subtype C′ from Botswana, Malawi and South Africa (Fig. 1).

Compartmentalization, the presence of distinct but phylogenetically related HIV genotypes within different anatomical sites, has been documented in many body compartments in various patient groups [8–11]. One functional study found subtype E to replicate better than subtype B in genital tract-derived epithelial Langerhans' cells [12], but the findings have since been disputed [13]. Here we present the first in-vivo evidence for the preferential replication of one subtype over another in a particular body compartment.

HIV tropism and the ability to induce syncytia have been associated with the V3 loop. CCR5/macrophage tropism and the lack of induction of syncytia have been associated with an uncharged or negatively charged V3 and with uncharged or negatively charged amino acids at positions 11, 13, 25 and 32 [14–17]. We found that the total charge of the V3 loop was either the same between subtypes A and C′ sequences (+4), or higher (+5) in two out of 13 subtype C′ sequences, and our analysis of the specific amino acid sites suggested that sequences from both virus subtypes would be CXCR4 or CXCR4/CCR5 tropic and syncytium inducing [position 13; R or H (A and C), position 32; 6/7 R, 1/7 Q (A), 14/15 Q, 1/15 R (C)]. We found no difference in these parameters when comparing subtype C′ sequences from PBMC and cervix.

We next looked at the variation in cytotoxic T-lymphocyte (CTL) epitopes and glycosylation patterns. The human leukocyte antigen (HLA) type of our patient was HLA-A1/30, B39/58 and C 7/12. Subtype-specific changes were observed in Gag in one A1 epitope (GSEELRSLY) and one A30 epitope (RSLYNTVATLY), and in Env in an A30 epitope (QRGPGRAFV). However, all differences between subtype-specific epitopes in *gag* were fixed in the sequence population, only the *env* A30 epitope, encompassing the V3 crown, showed polymorphism in the first amino acid position, possibly indicative of CTL or antibody-induced selective pressure, and no changes in subtype C′ sequences were associated with either compartment. The differences in subtypes A and C′ virus response to CTL immunity thus do not explain why essentially the same C′ virus should comprise only 50% of the virus population in blood but dominate in the genital tract.

The N-linked glycosylation patterns between subtype A and C *env* sequences were identical except for the glycosylation site at position 7239-47 (HXB2 coordinates), which was absent in all cervical-derived subtype C′ sequences, but was present in three out of five PBMC-derived sequences. We are currently amplifying full-length sequences from both PBMC and the cervix to look for compartmental-specific changes in other areas of the genome.

Approximately half the HIV-positive adults worldwide are women, and 90% of new infections are transmitted through heterosexual contact. Therefore, for the vast majority of individuals exposed to HIV, the genital mucosa is the site of initial contact. The hypothesis that subtype C′ strains have a replicative advantage over subtype A in the genital tissue could explain the steep increase in the prevalence of subtype C′. Indirect support for our hypothesis comes from a recent mother-to-child transmission study [18], which found that HIV subtype C was more frequently transmitted *in utero*, but not neonatally, than subtypes A or D.

Recognizing how tissue tropism varies between HIV variants, and how efficiently they replicate and compete in the genital tract, is critical for the understanding of the dynamics of the pandemic, as well as for the development of vaccines, therapies and intervention strategies.

Details of the phylogenetic analysis can be obtained from the corresponding author (A.K.N.I., aiversen@hammer.imm.ox.ac.uk).

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