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A1 The role of virus-antibody co-evolution in the development of a V3-glycan-directed HIV-1 broadly neutralizing antibody lineage

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Broadly neutralizing antibodies (bNAbs) are essential for a preventative HIV-1 vaccine but have not been elicited through vaccination. bNAbs develop in 20–30 per cent of HIV-1 infections and often target the V3-glycan epitope of the HIV envelope protein (Env). In these individuals, virus-antibody co-evolution is thought to drive the maturation of antibody lineages to neutralization breadth. We used deep sequencing of *env* genes and antibody transcripts from fourteen time points spanning the first 3 years of infection to characterize the virus-antibody co-evolution in donor CAP255 who developed V3-glycan-specific bNAbs. Sequencing and cloning of *env* genes, followed by neutralization assays, were used to identify Env mutations associated with neutralization escape from two bNAbs (CAP255.G3 and CAP255.C5) isolated at 149 weeks post-infection (wpi). Sequencing data indicated that CAP255 was co-infected by three related viral variants, all of which had an intact N332 glycan, which persisted in > 90 per cent of later viruses. A recombinant V3-region became fixed from 8 wpi, conferring slight neutralization resistance to CAP255.G3/C5 and other V3-glycan bNAbs. Later, T415R/K substitutions in V4 emerged by 51 wpi and were associated with complete viral escape from CAP255.G3/C5, though not from the polyclonal plasma response. All 93-week and later Envs were resistant to CAP255.G3/C5 and V3-glycan bNAb PGT135. Viral escape by 51 wpi suggested that the CAP255 bNAbs arose earlier, driving escape, but persisted to 149 weeks. This was supported by preliminary deep sequencing of the antibody repertoire that indicated bNAb lineage members were already present in the plasma at 39 wpi. Escape from V3-glycan bNAbs via T415R/K mutations have not previously been shown, suggesting a novel mode of recognition within the V3-glycan supersite. Further work will focus on identifying the bNAb-initiating Env and intermediate bNAb lineage members that were capable of engaging contemporaneous Env neutralization escape mutants. Characterization of Envs that engaged bNAb precursors, as well as those that selected for broader members of the bNAb lineage, will inform the design of immunogens capable of eliciting V3-glycan bNAbs in a novel HIV-1 vaccine regimen.

A2 Phylogenetic investigation of transmitted HIV-1 drug resistance mutations in Denmark, 2009–17

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Transmission of HIV-1 resistance mutations among therapy-naïve patients impairs the efficiency of antiretroviral therapy (ART). Therefore, genotypic resistance testing of patients is recommended at baseline, as this both allows for the selection of the correct ART regimen and for surveillance of transmitted drug resistance mutations (TDRM) among therapy naive HIV-1 patients. In Denmark, the occurrence of TDRM in newly diagnosed and therapy naïve HIV-1 patients is monitored through the SERO project. Here, we investigated if the prevalence of TDRM differed between patients within and outside of phylogenetically identified transmission clusters. Samples from 1,227 newly diagnosed HIV-1 patients were sent along with epidemiological information to the Virological Surveillance and Research group at Statens Serum Institut. HIV-1 RNA extraction, RT-PCR and Sanger sequencing of the *pol* gene was performed using an in-house assay. The sequences were analyzed using BioNumerics v. 6.6 and manually checked for the presence of mixed mutations and analyzed for mutations using the HIVDB 8.4 algorithm implemented at the Stanford database. Sequence alignments were performed in Mafft, and phylogenetic analysis was performed using Mega 6.0 using the Maximum likelihood general time reversible model with 100 bootstrap replicates. Clusters were identified with ClusterPicker at default settings (cluster support = 90%, genetic distance 4.5%). Active clusters contained newly diagnosed patients from the 2015 to 2017 period. HIV-1 sequences from 588 patients belonged to one of 154 clusters, and sequences from 639 patients did not belong to a cluster. Patients in clusters were significantly more likely to be men who have sex with men and subtype B and significantly less likely to be late presenters (Fisher's test $P < 0.05$). The TDRM prevalence was significantly higher for patients outside of clusters than within clusters, 16.6 per cent versus 12.1 per cent, respectively (Fisher's test $P < 0.05$); however, no significant differences were found in the TDRM prevalence between the 75 active and 79 inactive clusters, nor between small (<3 patients) and large (≥3 patients) clusters. E138A, V179D, and K103N were the three most prevalent TDRMs for both patient groups, whereas M41L differed between them. In Denmark, the TDRM prevalence is lower within clusters than outside, indicating that TDRM cases are either imported and/or belong to yet unidentified clusters.

A3 Molecular epidemiology of HIV-1 in Nigeria

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Nigeria has been reported to have the highest number of AIDS-related deaths in the world. In this study, we aimed to use molecular epidemiology to investigate the HIV-1 diversity and phylodynamics in Nigeria. We analyzed 1,442 HIV-1 *pol* sequences collected from 1999 to 2014 from seven geopolitical zones in Nigeria. The main circulating strains, CRF02_AG (44.1% of the analyzed sequences), Subtype G (8.3%), and CRF43_02G (16.4%), were introduced to Nigeria in the 1960s, 1970s, and 1980s, respectively. The number of effective infections decreased in Nigeria after the introduction of free antiretroviral treatment in 2006. We also found a significant number of unique recombinant forms (22.7%), the majority of which were recombinants between the two or three of the main circulating strains described above. In addition, phylogeographic analysis indicates multiple occasions of HIV-1 transmission between Lagos and Abuja (two of the main cities in Nigeria). Our results may be relevant for HIV-1 intervention and contribute in making informed decisions in strategies aiming at reducing further spread of HIV-1 in Nigeria.

A4 An amplicon-based approach for universal amplification, sequencing, and assembly of full-length HIV-1 samples from the DRC

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Phylogenetic studies have contributed to our understanding of the early epidemic onset of HIV-1 in the Democratic Republic of Congo (DRC); however, the factors driving its early emergence and establishment in human populations still remain unresolved. In order to determine the key aspects of its successful epidemic spread, complete genome data are required from samples representative of the viral diversity in the DRC. In this study, we have established a universal PCR-assay that uses seven different panels of primers to produce overlapping amplicons covering the complete HIV genome. To circumvent the limitations of purifying these fragments and sequencing them with traditional approaches, we have developed a massive parallel sequencing method and a protocol for efficiently assembling HIV-1 genomes. A total of thirty-six samples, collected between 1997 and 2001 from different locations across the DRC, have been obtained, and, at this stage, we are focusing on complementing our dataset with more archival samples that can be used as HIV 'molecular fossils'. By generating complete genome phylogeographic data from the DRC, we aim to create a genomic window into the past evolutionary and epidemiological dynamics of HIV-1 in Central Africa and understand the natural history of this devastating pandemic.

A5 Near full-length HIV-1 genome sequencing in newly diagnosed individuals in Sweden

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The Swedish HIV-1 epidemic is characterized by a high diversity in HIV subtypes and recombinants as a result of migration. To study the time from infection through viral diversification, transmission patterns, and drug resistance in minor quasispecies, a robust protocol for pan-genotypic near full-length HIV-1 genome (HIV-NFLG) next-generation sequencing (NGS) is key. Our group has established two protocols for HIV-NFLG on the Illumina platform that we aim to compare and, if necessary, modify to find a method with optimized coverage, depth, and subtype inclusivity. Zanini et al. (https://doi.org/10.7554/eLife.11282.001) have developed a method with one-step RT-PCR with six overlapping primer sets, followed by NGS and quality filtering and assembly with in-house methods. Aralaguppe et al. (https://doi.org/10.1016/j.jviromet.2016.07.010) have designed amplification in two fragments, followed by multiplexed NGS and quality control and assembly with Iterative Virus Assembler and VICUNA. Both methods have high coverage per nucleotide and low error rates in amplification and sequencing and can reliably identify SNPs at 1 per cent of the viral population with linkage within the quasispecies. Subtype inclusivity remains a challenge even though both methods show success in amplifying and sequencing subtypes B, C, and the common recombinants 01_AE and 02_AG. Therefore, we aim to evaluate and optimize our NFLG NGS methods on a panel of patient samples that more completely reflects HIV-1 diversity in Sweden. Patient samples from fifty treatment-naïve viremic individuals representing the genotypic HIV-1 panorama in Sweden, including CRFs, are being amplified and sequenced by both protocols. Coverage of the genome, error rate, and possible depth of quasispecies analysis is being evaluated. We will compare number of reads, coverage across the HIV genome, and representation of minor single nucleotide variants as well as subtype inclusivity and impact of plasma RNA levels. To do this we will use an in-house bioinformatic pipeline. The NFLG sequences will also be analyzed with phylogenetic tools for determination of subtypes including CRFs and URFs.

A6 Does treatment cause virulence changes in HIV-1?

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Antiretroviral treatment (ART) has provided substantial benefits for HIV-1-infected patients and has reduced incidence in areas with high uptake since its introduction in the late 1980s. As ART has led to shifts in the worldwide epidemiology of HIV-1, it may also have the potential to cause concomitant selective pressure on the virus population. Evidence for changes in HIV-1 virulence since the introduction of ART appears to be inconsistent. As well as reviewing both empirical and theoretical studies on the likely impact of ART on HIV-1 virulence, we developed a mathematical framework to evaluate the likely impact of ART on virulence selection under the widespread treatment programs and the future impact of recent test-and-treat recommendations. By quantifying both the relationship between virulence changes with the transmissibility through disease progression and the speed of diagnosis and treatment, we reconcile observational studies on virulence changes with the mathematical model predictions. On adoption of new test-and-treat programs—synonymous with early detection and immediate treatment—it is likely that increased virulence will be observed. Our findings highlight the potential public health consequences of mass treatment and the ensuing requirement for greater access and adherence to nullify the public health effect of these virulence changes.

A7 Co-receptor tropism determined by genotypic assay in HIV-1 non-B subtypes circulating in Cuba: Implications for pathogenesis and Maraviroc resistance

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The V3 loop of the HIV-1 envelope (*env*) gene is involved in binding to the chemokine receptors CCR5 and CXCR4, thus determining viral tropism. With the aim of genetically characterizing the C2V3 *env* region of HIV-1 samples from Cuban patients, naive to Maraviroc (MVC) therapy, 115 plasma samples were taken in the period of 2014–6 and analyzed by sequencing of the C2V3 region. HIV-1 subtyping was performed using COMET V.2 and Rega subtyping toolV.3 software. Subtypes were confirmed by phylogenetic analyses using Mega-6. Prediction of co-receptor tropism was performed using the geno2pheno algorithm. The viral