from the outbreak. The present study highlighted the integral role that real-time phylogenetic analyses can play alongside extensive epidemiological investigations, assisting the clarification of epidemiological case definitions in temporal transmission network in a HIV outbreak investigation.

A25 Analysis of non-structural genes subtype A1 HIV-1 circulating in Russia

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The HIV infection epidemiological situation in Russia is characterized by the predominance of a unique genetic variant of subtype A1 HIV-1 named AFSU. This variant has significant differences from other subtype A1 HIV variants in the genes coding for structural proteins Gag, Pol, and Env, but no analysis of the non-structural genes was carried out. These genes may have a significant influence on the rate of viral replication and transmission, playing major role in the pathogenesis of HIV virus and interaction with the human immune system. The aim of this work was to find out if the differences in *vif*, *vpr*, *vpu*, *tat*, *rev*, and nef *genes* of AFSU variant from other subtype A1 variants exist. NGS methodology was used for the analysis of the viruses in blood plasma samples obtained from HIV-infected patients in different regions of Russia, previously identified as AFSU. We received forty-seven complete genome sequences using the MiSeq (Illumina, USA); additionally fifty-four complete genome sequences of subtype A1 HIV-1 were extracted from Genbank. All sequences were divided into fragments corresponding to vif, vpr, vpu, tat, rev, and nef genes. All sequences were subjected to phylogenetic analysis using MEGA 6.0 program. Phylogenetic analysis of vif, vpr, vpu, tat, and rev genes has shown that all AFSU samples formed a sub-cluster inside the subtype A1 cluster formed by other A1 nucleotide sequences. The *nef* gene sequences did not form any clusters irrespectively of the mode of phylogeny estimation. The results of the phylogenetic analysis showed that AFSU HIV-1 non-structural genes *vif*, *vpr*, *vpu*, *tat*, and *rev* have differences from other subtype A1 HIV-1 variants. The *nef* gene sequences did not show any phylogenetic differences. The information will be used as a background for further investigations of the epidemiological and biological characteristics of the HIV1 viruses prevailing in Russia.

A26 Probing the compartmentalization of HIV-1 in the central nervous system through its neutralization properties

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Compartmentalization of HIV-1 has been observed in the cerebrospinal fluid (CSF) of patients at different clinical stages. Compartment specific modifications have been frequently described in the variable loops and the glycosylation sites of the envelope glycoproteins, a known mechanism to escape neutralizing antibodies (NAb). Considering the low permeability of the blood–brain barrier, we wondered if a lower NAb selective pressure in the central nervous system (CNS) could favor the evolution of NAb-sensitive viruses in this compartment. Single-genome amplification (SGA) was used to sequence near full-length HIV-1 envelope variants (453 sequences) from paired CSF and blood plasma samples of nine subjects infected by HIV variants of different clades and suffering from neurologic syndromes. Dynamics of viral evolution were evaluated with a Bayesian coalescent approach for individuals with longitudinal samples ($n = 4$). For six subjects, pseudotyped viruses expressing envelope glycoproteins variants representative of the quasispecies present in each compartment were generated, and their sensitivity to autologous neutralization, broadly neutralizing antibodies (bNAbs) and entry inhibitors was assessed. Significant compartmentalization of HIV populations between blood and CSF were detected in five out of nine subjects by all tests ($P < 0.01$). Bayesian analyses revealed independent evolution of CSF viral populations for extended periods of time (up to eight years for one patient). There was no difference in sensitivity to autologous neutralization between blood- and CSF-variants, even for subjects with compartmentalization. However, we observed major differences of sensitivity to sCD4 or to at least one bNAb targeting either the N160-V1V2 site, the N332-V3 site or the CD4bs, between blood- and CSF-variants in all cases. Our data show that selective pressure by autologous NAb is not the main driver of HIV evolution in the CNS. Given that each of the conserved neutralizing epitopes is associated to a specific property for cell entry, our data suggest that functional properties of the envelope are responsible for compartmentalization. Considering the possible migration from CSF to blood, the CNS could be a reservoir of bNAb-resistant viruses, an observation that should be considered for immunotherapeutic approaches.

A27 Exploring novel mechanisms of HIV-2 mutagenesis

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Over thirty-six million individuals are infected with HIV worldwide. Nearly 95 per cent of these individuals are infected with HIV type 1 (HIV-1), which has a high rate of viral mutation that helps drive immune evasion, disease progression, and rapid emergence of drug resistance. HIV type 2 (HIV-2) accounts for fewer than two million infections overall, remains primarily restricted to West Africa, and exhibits a significantly attenuated disease phenotype compared to HIV-1, characterized by lower rates of transmissibility and a slower progression to AIDS. HIV-2 has recently been found to have a significantly lower rate of mutation compared with HIV-1, which may be related to the differences in viral disease progression and persistence. Although the main driver of HIV mutagenesis is the low fidelity of the virally encoded reverse transcriptase, host factors may contribute to the mutation rate as well. The host protein SAMHD1 has been previously shown to restrict HIV-1 infection in myeloid lineage cells by depletion of dNTP pools through a triphosphohydrolase activity. In addition to inhibiting reverse transcription, this disruption of cellular dNTP levels may contribute to misincorporation of nucleotides and result in mutation of the virus. Here, we propose the use of NGS to explore the role of SAMHD1 on HIV-1 and HIV-2 mutagenesis. Using HIV-1 and HIV-2 Vpx-viruses (which are sensitive to SAMHD1 restriction), we will use

high-throughput sequencing to observe how SAMHD1 expression alters the mutational profile (frequency and spectra) of integrated proviruses. We will explore how mutation rates of HIV-2 can be manipulated through the use of nucleoside analogs and RNRI drugs to explore what effects these compounds have on the HIV-2 mutation profile. Using single-cycle infectivity assays as well as long-term spreading experiments, we will be able to correlate mutagenesis with viral evolution and infectivity data to explore how sensitive these two viruses are to changes in viral mutation. This work will serve to understand how HIV-2 operates at a lower mutation frequency than HIV-1, elucidate the relationship between mutagenesis and infectivity for the two viruses, and provide insights into the contrasting phenotypes observed between the viruses.

A28 Phylogeographic analysis of HIV-2 ANRS CO5 cohort reveals new trends in HIV-2 epidemic patterns in West Africa

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The early spread of HIV-2 in Western Africa is imperfectly described for group B and the recently identified subtype A2. Recent HIV-2 epidemiological data are also scarce outside of Guinea-Bissau. The sequence database of the HIV-2 ANRS CO5-cohort, one of the largest to date, was used to explore the early migration patterns of these strains by phylodynamic's means. All publicly available (forty-nine and eight for A and B, respectively) and ANRS CO5-cohort (125 and 68 for A and B, respectively) pol sequences with available time of sampling and patient's country of birth were included. Bayesian phylogeographic reconstructions and effective population size estimations were performed under the best fitting combination of evolutionary, demographic, and molecular clock models using BEAST 1.8. The tree topology was assessed with maximum likelihood trees using RAxML 8.0.0. The estimated introduction of group A in humans was 1945 [95 per cent HPD: 1935–1953], as previously reported. Subtype A1, present in Senegal, Gambia, Guinea-Bissau, and Guinea, experienced an early diversification around 1946 [1936–1954] with two distinct early epidemics in Guinea-Bissau and Senegal. Subtype A2, present in Ivory Coast and Mali, experienced a latter diversification (1956 [1947–1963]) in Ivory Coast with two introduction events in Mali (1963 [1957–1969] and 1967 [1960–1974]). Group B was originally introduced in Ivory Coast in 1962 [1953–1913]. Changes in effective population size over time revealed initial exponential growth phases occurring sequentially for the three HIV-2 strains and followed by a population decline starting in the 2000s for all HIV-2 strains. The rate of this decline was slower for A2 and B subtypes (Ivory Coast, Mali) than for A1 (Guinea-Bissau, Senegal). This phylogeographic study is the first to reconstruct the early dispersal of A2 and B HIV-2 clades in Western Africa. Our results suggest that subtype A1 was circulating in Guinea-Bissau and Senegal before the independence war of the former, believed to have contributed to the dispersal of HIV-2. Both A2 and B clades emerged in Ivory Coast and experienced latter diversification and population expansion (starting in 1980 and 1990, respectively) than A1. There is indication of slow decreasing incidence rates of HIV-2 in Ivory Coast or Mali where recent data are scarce.

A29 Development of a full-genome sequencing platform to study norovirus diversity

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Norovirus is a major cause of acute gastroenteritis worldwide. Noroviruses are very infectious and highly diverse, with two different genogroups (GI and GII) and almost thirty different genotypes infecting humans. Over the last two decades a single genotype (GII.4) has been shown to be the predominant cause of viral gastroenteritis outbreaks worldwide, therefore, most of the research was focused on this genotype. However, the epidemiological picture has changed during the last three seasons, where two different genotypes GII.17 (2013–2015) and GII.2 (2015–2016) have emerged as the major causes of gastroenteritis in different countries. Thus, a better understanding of the evolution of all different norovirus genotypes is needed for vaccine development. Much of current research on norovirus evolution has been focused on the major capsid protein (VP1), the major target for vaccine development. The VP1 is encoded by the open reading frame (ORF) 2, which constitutes only about one-fourth of the whole genome. However, much is unknown about the evolution, functional, and immunological roles of ORF1, a 5,000-nucleotide segment of the genome that encodes six nonstructural proteins. Because only 0.3 per cent of the norovirus sequences deposited in public databases correspond to full-length genomes, we developed an RT-PCR assay that amplifies the full-length genome of different norovirus genotypes; the resulting amplicons are sequenced using next-generation sequencing platforms. Using this platform, we successfully sequenced and assembled over fifty norovirus genomes from eleven different genotypes. The full-length sequences of two genotypes, GII.9 and GI.7, were obtained for the first time. Using neighbor-joining phylogenetic trees, we determined that the GII.9 presented an ORF1 region very similar to the one associated to GII.6, GII.7, and GII.14 noroviruses. On the contrary, sequences of GII.17 strains circulating pre-2013 clustered in different branches, for both ORF1 and ORF2 regions, than the ones circulating during 2013–2016. In addition, ORF1 sequences from different GII genotypes showed the clustering into at least two different groups. Together, this suggests independent evolution of the two different regions of the genome. We expect this method will encourage full-genome sequencing in the norovirus field, and create an improved database to expand our genomic analyses.

A30 Norovirus epidemiology and diversity in South Africa, 2009–2016

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Norovirus is a major cause of viral gastroenteritis in all age groups. The virus is classified in the Caliciviridae family of small, icosahedral viruses with a ~7.6-kb linear positive-sense RNA genome. The genome encodes three open reading frames