HIV-1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications.

Buonaguro L.*, Tornesello M.L., Buonaguro F.M.


*Corresponding author:
L. Buonaguro, M.D.
Lab. of Viral Oncogenesis and Immunotherapy
& AIDS Ref. Center
Ist. Naz. Tumori "Fond. G. Pascale"
Via Mariano Semmola, 1
80131 NAPLES - ITALY
Tel. +39-081-5903.609
Fax. +39-081-545.1276
E-mail: irccsvir@unina.it
HIV-1 is the causative agent of AIDS\(^{(10,38,105,114)}\). It is characterized by extensive and dynamic genetic diversity, generating variants falling into distinct molecular subtypes as well as recombinant forms; these forms display an uneven global distribution\(^{(55)}\). This diversity has implications for our understanding of viral transmission, pathogenesis and diagnosis, as well as profoundly influencing strategies for vaccine development.

Here we review selected aspects of HIV-1 genetic diversity, with particular emphasis on its pathogenetic and therapeutic implications.

**HIV-1 genetic subtypes.**

The Human Immunodeficiency Virus type 1 (HIV-1) is characterized by extensive genetic heterogeneity, driven by several factors, such as: the lack of proof-reading ability of the reverse transcriptase (RT) \(^{(96,108)}\), the rapid turnover of HIV-1 in vivo \(^{(56)}\), host selective immune pressures \(^{(84)}\), and recombination events during replication \(^{(122)}\). Due to this variability, HIV-1 variants are classified in three major phylogenetic groups: **group M** (main), a **group O** (outlier) as well as a **group N** (non-M/non-O) \(^{(6,52,116)}\). The **group M**, responsible for the majority of infections in the HIV-1 worldwide epidemic, can be further subdivided into 10 recognized phylogenetic subtypes or clades (A – K), which are approximately equidistant from one another (Fig. 1). Within the group M, the average inter-subtype genetic variability is 15%, for the *gag* gene, and 25% for the *env* gene \(^{(58,64,68,70,92,109)}\).

Moreover, within a subtype, it is possible to identify groups of viral isolates forming genetically related sister clades, termed sub-subtypes \(^{(109)}\), which appear to be phylogenetically more closely related to each other than with other subtypes. This is the case with the A and F clades, whose members are currently classified into A1 - A2 and F1 - F2 sub-subtypes, respectively \(^{(42,123)}\). Also B and D clades are more closely related to each other than to other subtypes, with D clade to be considered as the early B-African variant,
but their original designation as subtypes is retained by authors for consistency with earlier published work (40,74).

The original HIV-1 subtype classification was based on sub-genomic regions of individual genes. However, with the increasing number of viral isolates available worldwide and the improvement of sequencing methods, HIV-1 phylogenetic classifications are currently based either on nucleotide sequences derived from multiple subgenomic regions (gag, pol and env) of the same isolates or on full-length genome sequence analysis. This approach has revealed virus isolates in which phylogenetic relations with different subtypes switch along their genomes. These inter-subtype recombinant forms are thought to have originated in individuals multiply infected with viruses of two or more subtypes. When an identical recombinant virus is identified in at least three epidemiologically unlinked people, and is characterized by full-length genome sequencing, it can be designated as circulating recombinant forms (CRFs) (99,110) (Fig. 1).

More than 20 CRFs, whose origin can be tracked in areas where the parental strains are co-circulating, have been reported (Fig. 2). The co-circulation of multiple subtypes and CRFs in the same population is increasing the probability of individuals “superinfected” with different HIV-1 genetic forms which can swap parts of their genetic material. This is resulting in the generation of several recombinants called “unique recombinant forms,” or URFs, which, if spread to other people, will lead to their classification as CRFs (79).

Geographic distribution of HIV-1 subtypes.

Molecular epidemiological studies show that, with the exception of sub-Saharan Africa, where almost all subtypes, CRFs and several URFs are detected, there is a specific geographic distribution pattern of HIV-1 subtypes (55,97). This seems to be the consequence of either accidental trafficking (viral migration), with a resulting “founder” effect, or a prevalent route of transmission, resulting in a strong advantage and local predominance of the subtype prevalently transmitted in that population (17,81,91,93,98).
On a global scale, according to recent studies, the most prevalent HIV-1 genetic forms are subtypes A, B, C, with subtype C accounting for almost 50% of all HIV-1 infections worldwide (Fig. 3). In particular, subtype A viruses are predominant in areas of central/east Africa (Kenya, Uganda, Tanzania, and Rwanda), and in east European countries formerly constituting the Soviet Union. Subtype B is the main genetic form in western and central Europe, the Americas, and Australia, and is also common in several countries of southeast Asia, northern Africa, the Middle East, and among South African and Russian homosexual men. Subtype C viruses are predominant in Countries accounting for >80% of all global HIV-1 infections, such as southern Africa and India. The relevance of CRFs in the global HIV-1 pandemic is increasingly recognized, accounting for 18% of incident infections (55,97) and representing the local predominant form in Southeast Asia (CRF01-AE) (82,89,104) or in West and West-Central Africa (CRF02-AG) (80,87) (Fig. 4).

**HIV-1 diversity and its origin.**

Strong phylogenetic evidences suggest that HIV-1 has originated by a zoonotic cross-species transmission of the simian lentivirus SIVcpz from the chimpanzee subspecies *Pan troglodytes troglodytes* to humans (39). This could have occurred in West Equatorial Africa from direct exposure to animal blood as consequence of hunting, butchering and consumption of raw meat (53). In fact, this region includes Countries (Gabon, Equatorial Guinea, Cameroon, and the Republic of Congo) where conditions in support of this hypothesis are found: 1) HIV-1 groups M, N, and O co-circulate in human populations; 2) the HIV-1 group M viruses show the greatest diversity (28,59,85,86,88,101,127); and 3) chimpanzees (*Pan troglodytes troglodytes*) have been found to be infected with genetically closely related viruses (26,39,78,102,116). All conditions, therefore, support both a long-lasting circulation of the whole spectrum of the know HIV-1 genetic forms, possibly resulting from multiple founder effects, and the plausible zoonotic transmission from infected animals to humans.
Moreover, the interspersion of HIV-1 group M, N, and O sequences between different SIV<sub>cpz</sub> (*Pan troglodytes troglodytes*) lineages in phylogenetic trees requires that HIV-1 viruses from the three groups originated from no fewer than three separate SIV<sub>cpz</sub> transmission events (Fig 5.). However, while the Group M viruses appear to have efficiently adapted to the new host species, spreading all around the world and generating multiple genetic subtypes, the other two Groups show a worse adaptation to humans. In fact, HIV-1 group O seems to be endemic in Cameroon and neighboring countries in west-central Africa, where it represents only approximately 1–5% of HIV-1-positive samples (102); likewise, group N viruses have only been identified in a limited number of individuals from Cameroon (6,116). Moreover, Group N viruses appear to be the result of a recombination event between an SIV<sub>cpz</sub>-like and an HIV-1-like virus (39), and the high similarity to chimpanzee viruses may indicate a significantly recent zoonotic cross-species transmission. These evidences suggest that cross-species zoonotic transmission to humans of additional primate lentiviruses, and/or recombinations between HIV-1 viruses and primate lentiviruses, may still occur giving rise to new HIV-1 Groups with unpredictable virulence.

Considering that African primates are the natural hosts of several different lentiviruses (100), it is quite reasonable that such cross-species zoonotic lentivirus transmissions to humans have periodically occurred during the centuries in West Equatorial Africa. The exposure to SIV infection in natural settings is a common finding, in individuals exposed to blood and body fluids of naturally SIV-infected non-human primates. However, in contrast to a significant percentage of seropositivity to SIV antigens in such high-risk groups (17.1%), no productive infection has been detected (60). This has been proposed as consequence of either an exposure to non viable or defective SIV, or a non-productive cleared infection, or sequestering of virus in lymphoid tissues. The reason why only during the 20<sup>th</sup> century the HIV-1 has given rise to the AIDS pandemic is not yet determined, but could reasonably be the sum of significant cultural and socio-behavioral changes, plus the use of non-sterile needles for parenteral injections and vaccinations, as well as the
unwitting contamination of biological products for medical treatment (i.e. Oral Polio Vaccine, OPV) \(^{(46,57)}\). In particular, the earliest case of HIV-1 infection, dating from 1959, has been identified in the Democratic Republic of Congo \(^{(134)}\) and the origin of the HIV-1 group M radiation has been estimated around the ’30s, using different methods of molecular clock analysis \(^{(63,113)}\).

**HIV-1 subtypes and transmission.**

Diverse risk behaviors sustain the HIV-1 transmission in different regions of the world and, within the same region, multiple transmission routes can be involved in spreading the epidemic. For example, considering the 2005 as reference year, in Eastern Europe and Central Asia, 67% of the prevalent HIV infections were due to needle sharing among intravenous drug users (IDUs). In South and South-East Asia, not including India, 49% of the prevalent HIV infections were reported in commercial sex workers (CSW) and their clients, while 22% were reported in IDUs. In Latin America, instead, 26% of the HIV infections were in men-who-have-sex-with-men (MSM) and 19% in IDUs \((http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp)\). In Western Europe unprotected intercourse among heterosexuals accounted for 45% and among MSM for 28% of the HIV infections \((http://www.eurohiv.org/reports/report_73/pdf/report_eurohiv_73.pdf)\).

As reported in the previous paragraph, the HIV-1 epidemic in these regions is sustained by different subtypes and, within each region, a segregation of subtypes in different risk groups has been reported. In particular, the co-circulation of B subtype among IDUs and CRF01_AE (originally defined as E subtype) among heterosexuals has been originally described in Thailand \(^{(41)}\); the segregation of B subtype in homosexuals and C subtype in heterosexuals has been described in South Africa \(^{(126)}\); more recently, two concurrent epidemics in Argentina have been reported, one among MSM, sustained by the B subtype, and the other one among heterosexuals and IDUs, sustained by BF recombinants \(^{(5)}\). In Europe, where the B subtype has sustained the HIV-1 epidemics among the “historical“
IDUs and Homosexual risk groups, non-B subtypes and CRFs are progressively introduced in association to the increased HIV-1 heterosexual transmission between migrants/immigrants from endemic regions for HIV-1 and their European partners (\(^{19,20,121}\)).

All these observations, reported in different phases of the HIV-1 epidemics around the world, may suggest different biological properties for the subtypes, resulting in their segregation among individuals with different risk behaviors for HIV-1 infection. Nevertheless, a consistent demonstration of this association remains still to be proven. On the contrary, it is clear the absence of a predetermined linkage between a specific subtype and a unique mode of transmission. In fact, the A subtype is transmitted among heterosexuals in Sub-Sahara Africa and IDUs in Eastern Europe; similarly the B subtype is transmitted among all the historical risk groups in Western Countries.

Therefore, the apparent segregation of HIV-1 subtypes by risk behavior, rather than being a result of virologic factors (cell tropism, co-receptor specificity), could derive from genetic, demographic, economic, and social factors that separate the different risk groups for HIV-1 infection. Moreover, the overwhelming predominance of the C subtypes in areas where the unprotected heterosexual intercourse is the main transmission route, could result from a "founder effect" with a fast colonization outcome.

**Impact of HIV-1 genetic diversity on specificity and sensitivity of diagnostic tests.**

The genetic variability of HIV-1 may pose significant problems in the specificity and/or sensitivity of serological and molecular diagnostic tests which may represent a serious risk factor for the spreading of unidentified infections.

Fourth-generation of HIV immunoassays are designed to detect both HIV p24 antigen and antibody in single test, in order to reduce the seroconversion window (\(^{131}\)). The sensitivity of these assays for the p24 antigen and seroconversion, however, can vary significantly (\(^{76,130}\)). Nevertheless, recent comparative studies have shown that some of
these immunoassays have a specificity and sensitivity comparable to second and third-generation assays designed to detect either antigens or antibodies, identifying most of the known Group M subtypes, the most diffused CRFs (CRF01_AE and CRF02_AG) and Group O viruses \(^{(69)}\).

In parallel, a similar comparative study has been reported on molecular diagnostic tests, which might be even more affected by the high variability of the HIV-1 target nucleotide sequences. The analysis has shown that widely used real time RT-PCR for viral load measurements is able to identify all the tested Group M and Group O isolates within a target concentration ranging from approximately 316'230 copies/ml (5.5 \(\log_{10}\)) to below 50 copies/ml (1.6 \(\log_{10}\)), with a coefficient of correlation between 0.991 and 0.999 \(^{(120)}\).

Therefore, these reports indicate that the currently used serological and molecular HIV-1 diagnostic tests are still sufficiently specific and sensitive to detect the worldwide most prevalent HIV-1 genetic forms. Nevertheless, anecdotal unidentified HIV-1 isolates have been reported in primary HIV-1 infection cohorts in mono-clade HIV epidemics \(^{(21)}\). Therefore, the continuous genetic variation and inter-subtype recombination events require frequent evaluation analyses of the diagnostic tests and, possibly, national registries to report undiagnosed cases. This would greatly help in keeping updated the diagnostic capacity of the tests.

**Correlation between HIV-1 genetic diversity and drug resistance.**

The genetic variability characterizing the different HIV-1 subtypes and CRFs affects also the protease (PR) and reverse transcriptase (RT) genes coding for the viral enzymes mainly targeted by antiretroviral (ARV) drugs. These polymorphisms, if conferring drug resistance, can be selected by the drug-selective pressure and dramatically influence the therapeutic outcome. Alternatively, the polymorphisms do not confer drug resistance but may change the “genetic barrier”, which is defined as the number of viral mutations required to develop escape mutations able to overcome the drug-selective pressure. In general, several
mutations are generally required for the virus to become resistant to protease inhibitors (high genetic barrier), whereas a single amino acid substitution can induce resistance to non-nucleoside reverse transcription inhibitors (NNRTIs) (low genetic barrier)\(^{(12)}\).

The frequency and pattern among HIV-1 subtypes of polymorphisms inducing resistance \emph{per se} or leading to a faster emergence of drug resistance, under pharmacological pressure, has been evaluated in different recent studies. In particular, considering the lack of extensive information on non-B subtypes, which predominate in Countries where the availability of ART is very limited, great emphasis is given to B versus non-B subtypes comparative studies. In a large European study, performed on approximately 2000 protease and RT gene sequences (>600 non-B) from anti-retroviral-naïve patients, a similar genetic barrier was found for B and non-B subtypes at almost all positions \(^{(125)}\).

In addition, specific data are available for individual ART drug classes. In regards to the few positions of the protease sequence where differences were found, such as the 82A and 82T polymorphism, the genetic barrier was either lower for subtype C or higher for subtype G, suggesting that non-B subtypes may even require more mutations than B subtypes to develop drug resistance. Moreover, specific polymorphisms in protease positions may confer greater susceptibility to protease inhibitors (PI) in other non-B subtypes. In particular, subtype C and CRF02_AG recombinant viruses from drug naive patients show an \emph{in vitro} hyper susceptibility to PIs \(^{(1,48)}\), which needs to be confirmed by \emph{in vivo} clinical studies.

On the contrary, extensive differences between the subtypes were found for the minor protease substitutions, which do not impair drug susceptibility, but may affect the genetic pathway of resistance once the virus generates a relevant major substitution \(^{(47,83,106)}\). This implies that a faster emergence of drug resistance to a particular protease inhibitor could be expected in some non-B subtypes.
In regards to NRTI compounds, non-B subtypes do not show a lower genetic barrier in any resistance-associated substitutions, indicating an evolution to drug-resistance phenotype comparable to B subtype isolates. For NNRTI resistance-related substitutions, the most relevant finding is the reduced genetic barrier for the V106M substitution in subtype C, observed in different studies, which confers high-level resistance to all NNRTIs (\(^{16,124,125}\)). In particular, the V106M mutation in RT is facilitated in subtype C by a single transition (GTG to ATG), compared to two transitions needed in viruses of other subtypes (GTA to ATG). Moreover, natural resistance to NNRTIs has been observed in patients carrying group O HIV-1 viruses\(^{29,107}\) and this is believed to have a genetic basis concerning the natural RT Y181C polymorphism, similar to the Y181I divergence seen in naturally NNRTIs-resistant HIV-2 \(^{117}\).

All these in vitro genetic studies, showing both the absence of naive drug-resistance mutations in non-B subtypes and a substantial similarity between B and non-B subtypes in the possibility to develop drug resistance, are confirmed by a generally conserved drug susceptibility in vivo. In fact, HIV-1 subtypes do not appear to affect the first-line therapy efficacy and currently available protease and RT inhibitors are equally active on all HIV-1 subtypes \(^{3,13,37,103}\). In particular, a global collaborative study based on non-B subtype sequences from 3,686 persons, showed that 1) most of the protease and RT positions associated with drug resistance in subtype B viruses are selected by antiretroviral therapy in one or more non-B subtypes as well; and 2) no evidence is available that non-B viruses develop resistance by mutations at positions that are not associated with resistance in subtype B viruses \(^{61}\). However, few exceptions to this general rule have been reported in ART-treated patients. Subtype C viruses show a faster emergence of drug resistance to NNRTIs, for the appearance of the V106M mutation\(^{73}\). A Ugandan study to prevent mother-to-child HIV-1 transmission showed that a single-dose nevirapine (NVP) induced a more frequent selection of genotypic mutations associated with resistance to NVP in women infected with subtype D than in women infected with subtype-A viruses \(^{34}\).
The overall observed genetic similarities among the HIV-1 subtypes in the drug resistance-related codons, in contrast with the overall extensive genetic variability along the HIV-1 genome, can be explained by the biological relevance of such sites. In fact, sequences containing major drug resistance-associated substitutions show a reduced fitness which results in a less efficient replication and spread of these viral populations (75,128).

**HIV-1 genetic diversity and vaccine development.**

The HIV transmission in the general population can be possibly blocked by an effective, safe and affordable anti-HIV-1 vaccine, which should induce a strong humoral as well as cellular immune response against the whole array of HIV-1 genetic forms.

The significant intra- and inter-subtype variability of the HIV-1 envelope protein(58,64,68,70,92,109), the main target of a preventive and possibly sterilizing vaccine approach, together with the undeniable “fiasco” of the first phase III vaccine clinical trial based on a monomeric gp120 (35,50), has drastically driven in the last years the HIV-1 vaccine field toward the development of CTL-inducing vaccine approaches. This is based also on early observations showing the strong correlation between anti-HIV-1 CTL response and disease progression (67,90,95). Moreover, CTL responses can be raised against gag and pol proteins, which are less variable proteins, possibly circumventing the viral variability as well as the necessity of using HIV vaccine candidates corresponding to the HIV-1 strains prevalent in the target population.

In support of this strategy, promising broad cross-clade cellular immunity against specific HIV-1 epitopes has been described (27,45,51,133); however, only few epitopes are conserved across different subtypes and single amino-acid substitutions are selected by the CTL immune pressure, allowing the virus to escape with a dramatic deterioration of clinical conditions (8,9). Moreover, sequence variability within, as well as proximal to characterized optimal epitopes have been identified, which can either modulate binding to the HLA
molecule, or reduce the binding affinity to the cognate T cell receptor or interfere with efficient antigen processing, resulting in the escape from CTL surveillance (4,14,31,71).

As consequence, it is now currently believed that an anti-HIV-1 vaccine should elicit efficient cellular as well as humoral immune responses and, in particular, broadly anti-envelope neutralizing antibodies able to target the largest number of HIV-1 genetic forms. Most of the envelope immunogens developed, however, have failed to achieve this objective (11,23,77).

Broadly-neutralizing monoclonal antibodies, targeting different epitopes in gp120 and gp41, have been obtained from natural infected individuals (for a comprehensive review, see Srivastava et al.) (119), suggesting that envelope immunogens able to raise such broadly-neutralizing response can be generated. However, a polyspecific reactivity with auto-antigens of some of these monoclonal antibodies has been recently reported, raising serious doubts on the real possibility of eliciting such broad anti-HIV humoral response in vaccinees (2,54). Nevertheless, along this concept, few labs are currently focused on designing an HIV vaccine following a "working backwards" strategy, consisting of isolation and characterization of human/animal antibodies that can neutralize a broad range of HIV strains, and then using these antibodies to identify the HIV target regions to be incorporated into vaccines. The development of such vaccine candidates and their immunoprotective effectiveness will be shown in the near future.

In the mean time, several strategies have been developed to engineer envelope immunogens presenting epitopes able to induce neutralizing antibodies with different broad efficacy against T-cell adapted as well as primary HIV-1 isolates (7,18,22,32,36,118,132).

Furthermore, the HIV-1 genetic diversity and the distribution of different subtypes in each population is currently addressed using vaccine approaches based on either multivalent formulations, or consensus and ancestral sequences. The first approach is based on using envelope molecules from different HIV-1 subtypes, in order to induce antibodies with broader breadth of neutralizing activity (15,24,25,49,111,112,115). The second
approach, instead, is based on computer-derived sequences ("consensus sequences"), generated aligning circulating primary isolates and selecting the most common nucleotide at each position. The resulting inferred genes are in an intermediate position within an evolutionary tree, being equidistant from the currently circulating isolates (30,33,44,62,94). However, these presumed consensus sequences are dependent on sampling bias and may include polymorphisms, post-translational modifications (i.e. glycosilation) not reflecting the natural spreading viruses, which may severely influence their antigenicity and immunogenicity. In particular, out of five generated envelope consensus and ancestral sequences (30,43,65,66,72,129), only one envelope consensus of the Group M sequences has been shown to elicit high titer of neutralizing antibodies effective against primary isolates from three different group M clades (72).

CONCLUDING REMARKS

The genetic complexity of worldwide HIV-1 epidemic is still growing with continuous molecular evolution of existing subtypes and identification of new Circulating Recombinant Forms as well as Unique Recombinant Forms. The B subtype is still predominant in Western Countries, with a progressive introduction of non-B subtypes from high-epidemic Countries, while the C subtype represents the global most prevalent form. A “founder effect” with a fast colonization outcome, along with genetic, demographic, economic, and social factors, appears to explain this epidemic distribution of different subtypes. The available serological and molecular diagnostic tools show a specificity and sensitivity appropriate for identifying the broad spectrum of circulating genetic variants. The anti-retroviral drugs currently used are equally active on all HIV-1 subtypes (with the exception of Group O viruses, which are resistant to NNRTIs), although subtype-related differences in the rate of drug resistance-emergence strongly suggest the need for a HIV-1 pre-subtyping to guide the selection of the appropriate therapeutic strategy. The development of an effective anti-HIV-1 preventive vaccine, and with a broad efficacy against the vast array of HIV-1 genetic forms,
is still a “work in progress” field. However, it is extremely relevant that vaccines targeted to subtypes other than B are now pursued, a model which did not have many fans up to few years ago. This modified attitude, together with the number of achieved information and technologies, should accelerate the process of developing vaccines with the desired broad immunoprotection efficacy. This unprecedented challenge, which is perceived as a difficult-to-achieve goal, may gain extremely useful information from the understanding of exceptional SIV cross-species transmission to humans.

In conclusion, HIV-1 molecular phylogenetic studies are continuously required to trace the viral genetic evolution and keep updated all the diagnostic as well as preventive (vaccine) and therapeutic tools necessary to control the spread of the global HIV-1 epidemic.

ACKNOWLEDGEMENTS

This study was supported by grants from the Ministero Italiano della Sanità (Ricerca Corrente and Progetto Finalizzato AIDS 2006) and the ICSC-World Lab, Lausanne, Switzerland (Project MCD-2/7). We are grateful to Marv Reitz (Inst. Human Virol., Baltimore - MD) for his critical reading of the manuscript.
LEGENDS TO FIGURES

**Fig. 1** Evolutionary relationships among non-recombinant HIV-1 strains. The phylogenetic tree shows the M (main), O (outlier) and N (Non-M/Non-O) HIV-1 groups and, within the M group, subtypes and CRFs. The phylogenetic analysis has been performed on near-full length sequences and is based on neighbor joining method. The reliability of the internal branches defining a subtype has been estimated from 1’000 bootstrap replicates.

**Fig. 2** Mosaic structure of HIV-1 circulating recombinant forms (CRFs). The CRFs described until now are shown (modified from http://www.hiv.lanl.gov/content/hiv-db/CRFs/CRFs.html). Letters and graphic patterns represent the different subtypes of HIV-1 involved in the recombination events; U stands for “Unknown”.

**Fig. 3** Global prevalence of HIV-1 genetic forms. The global prevalence, expressed as percentage of the total number of worldwide identified HIV-1 isolates, is shown. The group O and N HIV-1 isolates are not included in this analysis.

**Fig. 4** Global geographical distribution of HIV-1 genetic forms. Genetic forms predominant in the different World Regions are shown. The pie charts show the prevalence of individual genetic forms in each Region.

**Fig. 5** Phylogenetic tree of primate lentivirus sequences. Phylogenetic tree analysis was performed using the neighbour-joining method, based a 550 bp fragment in **pol** from SIV and HIV isolates. One isolate per each HIV-1 Group M subtype has been used. Branch lengths are drawn to scale (the bar indicates 10% divergence). The reliability of branches has been estimated from 1’000 bootstrap replicates and only bootstrap values >80% are shown.


vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature 415:335-339.


envelope immunogens elicit broad cellular and humoral immunity in rhesus monkeys. J. Virol. 79:2956-2963.


