CHAPTER 3

HIV/AIDS Infection in the World with a Special Focus on Africa

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3.1 INTRODUCTION

HIV/AIDS represents the prototype of an emerging disease with its worldwide dramatic consequences. The Acquired Immune Deficiency Syndrome (AIDS) is caused by two lentiviruses: the human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) [3,6]. AIDS was first recognized in the 1980s and is presently the leading cause of death in developing countries. It is believed that 40 million individuals have been infected with HIV of which about two-third live in Sub-Saharan Africa [57].

It is increasingly evident however that the virus was present in humans many decades previously when the conditions required for its epidemic dissemination were not present [9]. Recent phylogenetic analysis of different strains of HIV-1 suggests that the pandemic originates from Central Africa, two different methods pointing to the 1930s [23,48]. The oldest known HIV-1-antibody positive serum dates from 1959 and comes from the Democratic Republic of Congo [69]). HIV-2 was initially identified in two patients from West Africa, and the oldest antibody positive sera also comes from a survey done in West Africa in 1965–1969 [51].

It is likely that the human infection is due to zoonotic transmission from chimpanzees (*Pantroglodytes troglodytes*) in the case of HIV-1 and sooty mangabeys (*Cercocebus atys*) in the case of HIV-2 [15]. The passage of these viruses is readily explained by the close contact between monkeys and humans in this part of the world, and especially by hunting and butchering primates for consumption of their meat. It is very probable that sporadic isolated cases occurred on several occasions, over several decades, without provoking an epidemic. The epidemic was probably the consequence of profound social upheavals in the 1970s, combining massive urban migration, poverty, civil wars, and, as a corollary, sexual promiscuity [17,40,50].

The patterns of spread to HIV-1 and HIV-2 are highly dissimilar. In particular, the HIV-2 epidemic seems to have stabilized and may even be declining. In addition, the HIV-1 epidemic itself is geographically heterogeneous. Knowledge of the multiple factors that explain this heterogeneous spread is important for the prevention of the epidemic.

Another characteristic of HIV is its very high genetic diversity. In this chapter, we will examine how complex and evolutive the variability of HIV-1 is and analyze its consequences. Finally, we will make the point on the access of treatment in the developing world in 2005.

As Africa is the continent far most severely affected by the HIV pandemic, this chapter will be focused on this continent.

3.2 CURRENT STATE OF THE EPIDEMIC

3.2.1 Prevalences and Incidences in the World

At the end 2005, it was estimated that 40.3 (36.7–45.3) million people are living with HIV, according to the UNAIDS report [57] (Fig. 3.1). Africa represents 70% of all infections worldwide. More than 25 million Africans are now living with HIV, of whom 55% are women. The second area with highest

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Fig. 3.1. Adults and children estimated to be living with HIV in 2005.

prevalences is Southeast Asia with more than 7 million infected persons.

In Africa, 24 million infected persons are aged between 15 and 49 years, and 1 million are children. The overall prevalence among adults in this part of the world is estimated at 8.8%, but regional variations are such that this figure has little practical value. In general, it is in southern Africa that the highest level (≥15%) is observed among the general adult population, especially in Botswana, Lesotho, Malawi, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe. In Botswana, the country most directly affected, levels are particularly alarming: 40% of pregnant women living in the capital are infected with the virus, and so are 60% of patients with other sexually transmissible infections.

High prevalence rates (10–15%) have also been reported in countries such as Cote d'Ivoire, Ethiopia, Djibouti, Kenya, Central African Republic, Burundi, Rwanda, and Mozambique.

In countries such as Ethiopia, Ghana, Cote d'Ivoire, Togo, and Zimbabwe, more than two-thirds of prostitutes living in large urban centers are seropositive.

It is remarkable that although the first known HIV infections occurred in Central (HIV-1) and West (HIV-2) Africa, and in contrast with the dynamic of the epidemic in the Great Lakes area, the epidemic is recent and explosive in southern Africa where prevalences, low in 1980s except in Zambia and Zimbabwe, increased rapidly in the 1990s, making now southern Africa the leading affected part of the world.

In addition to the UNAIDS surveillance system, data from epidemiological surveys are helping to determine the epidemiological profile of HIV infection in providing complementary figures. Thus, these surveys highlight large differences in prevalences between regions, between rural and urban areas, and between population subgroups.

Recently demographic surveys using the cluster sampling method have been proposed. They provided new informations suggesting that the UNAIDS sentinelle surveillance system had overestimated the HIV prevalences.

HIV-2 has a far more restricted geographical distribution. Its epicenter is in West Africa, but more or less sporadic cases are also reported in Lusophone countries such as Angola and Mozambique [51]. The highest levels were found in Guinea Bissau, with 6.8% in a survey of semiurban areas in 1996 and 4.6% among pregnant women from Bissau, the capital, in 1997 [25]. Levels are far lower in other West African countries, ranging from 0.5% to 1.6% among pregnant women [51]. Contrary to HIV-1, the highest HIV-2 prevalence rates are in elderly people [25,51].

Incidence rates, which are used to study the dynamics of the HIV epidemic, show general upward trend, with the noteworthy exception of Uganda, where the incidence fell from 0.8 to 0.5 per 100 person per year in rural areas between 1990 and 1996 [22]. In South Africa, in contrast, the incidence in pregnant women residing in rural areas rose from 4 to 10 per 100 person per year between 1992 and 1997 [65].

Contrary to HIV-1, the incidence rate of HIV-2 infection tends to be stable, and is even falling in Guinea Bissau for example [25,51].

In Southeast Asia, the epidemic is also very heterogeneous as illustrated in Figure 3.2. The dynamic of the epidemic is also complex. In Thailand, for instance, the incidence rate is now declining, whereas in more recent infected countries such as Myanmar, the incidence rate are increasing.

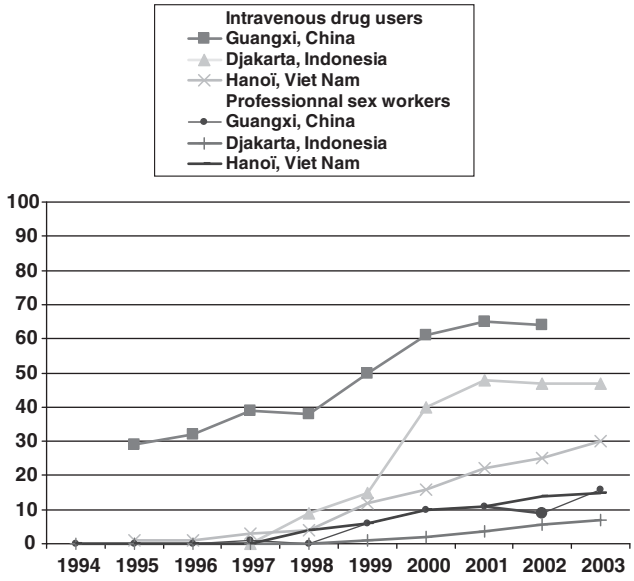

Fig. 3.2. HIV-1 prevalence rates among intravenous drug users and professional sex workers in Asia (1994–2003).

In China, the epidemic is recent but explosive. It is one of the countries where the HIV incidence is highest in the world. For instance, in Guangxi, the HIV prevalence among intravenous drug users (IVDU) is reaching 65%.

In industrialized countries, the high incidence initially observed among men having sex with men (MSM) and IVDU is now globally controlled, but the recent increase of sexually transmitted infections (STI's) among MSM is a concern. In Western Europe, the incidence rate is increasing in the heterosexual population, which is partly explained by a high proportion of migrants from Africa.

In Eastern Europe, especially in Russia or Ukraine, the epidemic is beginning to explode. For instance, the HIV prevalence rate is reaching 100% and 10% among IVDU in St Petersburg and Moscow, respectively. Predictions vary widely, but some estimate that by 2020, 14.5 million persons would be infected if denial remains the only strategy in Russia! [10].

3.2.2 Mode of Transmission

The HIV virus can be transmitted by sex, blood products, or from an infected pregnant woman to her infant.

In Sub-Saharan Africa, the predominant mode of transmission is heterosexual intercourse. Despite the same major mode of transmission, there are extraordinary differences in the spread of HIV infection among African countries, for reasons that are complex, multiple, and poorly documented.

In general, the risk of transmission depends on the infectivity of the index person, the type of sexual intercourse, and the susceptibility of the person thus exposed.

Many longitudinal epidemiological studies and direct studies of factors favoring genital HIV carriage have identified parameters influencing infectivity. One major factor is viral load in peripheral blood, as recently demonstrated in Uganda [41].

Factors leading to genital inflammation and/or infection are also important. STIs, whether or not they cause ulceration, clearly favor HIV transmission [11]. In particular, the role of herpesvirus type 2 has clearly been shown [7]. Imbalances in the vaginal flora also influence the risk of transmission [54]. A wide variety of intravaginal practices such as "dry sex" which are likely to cause irritation and disruptions of the genital mucosal epithelium have been described in Sub-Saharan Africa, but there is conflicting evidence for an association between these practices and HIV infection [49]. Concerning sexual practices, multiple partnership is clearly a risk factor, but a study comparing populations living in areas with low and high incidence rates showed no significant behavioral differences [5]. In contrast, this study, and a meta-analysis, showed that male circumcision was clearly a protective factor [63], and this was recently confirmed through a prospective interventional study comparing the incidence rate between circumcised and uncircumcised men [2]. No genetic factor specific to African populations has yet been demonstrated, either in co-receptors or the HLA system.

Among the other factors involved in the risk of transmission, the role of viral factors has been suggested [7]. Indeed, subtype C (see paragraph on HIV diversity) is associated with an explosive epidemic in eastern and southern Africa. This subtype appears to have biological particularities that could favor transmission. Several studies have shown that the phenotype of this subtype is preferentially non- syncytium inducing (NSI), implying that the asymptomatic phase, and thus the contagious period, could be longer. In addition, NSI strains use the CCR5 co-receptor present on macrophages in the genital mucosae (contrary to lymphotropic SI strains), and this could potentially favor sexual transmission of the former.

The control of mother-to-child transmission is a major challenge in Africa as in the rest of the developing world [31]. Indeed, without preventive measures, the HIV-1 transmission rate is 25–40% according to various studies versus less than 2% with adequate prophylactic measures [67]. By comparison, the mother-to-child transmission rate of HIV-2 is below 5% [51].

Two-thirds of these transmissions take place at the end of pregnancy, during delivery or very early after birth, and the viral load in the mother's peripheral blood is the main risk factor. Hence, the importance of prevention with antiretroviral drugs. Various trials have assessed the most feasible and cost-effective strategies based on the use of ZDV alone, ZDV combined with 3TC, or nevirapine monotherapy [8, 14, 29, 46, 64]. Some of these studies were based on antiretroviral treatment of the mother only, but some also included treatment of the child for a few days or weeks. Preventive efficacy is 30–50%, compared with 68% using the lengthy, complex, and costly regimens prescribed in industrialized countries. A single oral dose of nevirapine for mother and child was the strategy with the best cost-effectiveness, but the emergence of resistance is a real problem [20] and so this regimen alone is not still recommended. Recent studies in Thailand have shown the benefit of combined ARV drugs for the PMTC infection [24]. Maternal breast-feeding is a supplementary factor, doubling the risk of transmission. Nearly 75% of cases of postnatal transmission occur within 6 months after birth, as demonstrated by a clinical study in Kenya [34]. Importantly, the promotion of formula feeding in Africa must take into account the nutritional and infectious context, the poor hygiene, and the risk of stigmatization.

The importance of parenteral transmission is difficult to evaluate in developing countries. According to WHO estimates, it accounts for approximately 5% of all new cases. The main causes are blood transfusion, as transfusion safety leaves much to be desired, especially in rural areas. This mode of transmission also involves inadequate (or absent) sterilization of reusable injection materials. Some specialists consider that this has played a major role in the initial spread of the virus in Africa.

3.2.3 Impact of HIV Infection on Other Endemic Diseases

The spectrum of endemic diseases in developing countries is extensive, so we will focus on the other two main diseases, tuberculosis and malaria.

Tuberculosis is the leading cause of morbidity and mortality of HIV-infected people in both urban and rural areas.

HIV-infected patients with tuberculosis have a shorter survival and a higher tendency to acquire new opportunistic infection. For instance, active tuberculosis was present at autopsy in half HIV-1-positive cadavers in Nairobi and Kenya [42]. The risk of developing active tuberculosis among persons co-infected with HIV-1 is 5%–15% per year [43]. Thus, the AIDS epidemic is also a powerful factor facilitating the spread of tuberculosis. Several randomized controlled trials have now demonstrated that preventive therapy against tuberculosis is effective in preventing tuberculosis in HIV-infected individuals. However, feasibility studies have showed that this intervention is complex and rather inefficient [68]. Malaria is also one of the most common infections in Sub-Saharan Africa. In most ancient studies, no interaction between these two infections has been documented. However, in Malawi, it has been reported that postnatal mortality in HIV-infected infants was greatly increased in case of placental malaria infection [4]. Furthermore, in a recent cohort study performed in rural Uganda, it has been demonstrated that HIV-1 infection is associated with an increased frequency of clinical malaria and parasitaemia [66]. Taking into consideration the frequency of HIV and malaria, theses interactions could have important public health implications.

3.2.4 Demographic, Social, and Economic Consequences

AIDS is now the leading cause of death in Africa, with 3.1 million deaths in 2004 (twice the number of deaths due to malaria!) and a total of more than 17 million since the beginning of the epidemic [57].

Thus, in eastern and southern Africa, mortality rates, which had been declining in the last decades, have doubled or tripled in the last 15 years [56]. It is estimated that in 2025, the population in the 20 hardest hit African countries will be 30–120 million lower than it would have been in the absence of the AIDS epidemic [53].

Contrasting with the higher HIV prevalence rate in women, AIDS-related mortality rate is higher in men.

Infantile mortality is also affected in highly endemic countries, where all the gains made before the 1980s have been lost [53]. In southeast African countries, AIDS is responsible for up to 74% increase in deaths among children under 5 years of age, with a mortality rate of up to 30 per 1000. In a study in Uganda, the median survival time of an infected child was 21 months. As a result of the epidemic, the population of several African countries (Botswana, South Africa, and Zimbabwe) has started to decline [45]. The impact on life expectancy has also been assessed. It is estimated that each 1% rise in the prevalence rate in the general population cuts the overall life expectancy by a year.

In the next two decades, the standard age pyramid will be deeply modified by the AIDS epidemic in the countries most severely affected, with an abrupt broadening at around 20 years and a rapid decrease in the 20–40 category.

The social consequences of the epidemic are evident. In particular, the cumulative number of orphans due to AIDS in Sub-Saharan Africa increased to 12.1 million, representing 90% of all orphans in this part of the world. In Zimbabwe, 7% of all children are orphans because of AIDS.

The epidemic also has major consequences for education and general development. In Zambia, for example, more than 1300 teachers died in 1998, representing two-thirds of all teachers trained annually. The epidemic affects every socio-professional strata. As most adults fall ill during their most productive years, the economic consequences for households, enterprises, and states are considerable. Excess health expenditures are also a major burden in these countries: 40% of beds at Kenyatta hospital in Nairobi are occupied by people with AIDS, and this figure reaches 70% at Prince Regent hospital in Bujumbura [1].

3.3. MOLECULAR EPIDEMIOLOGY

3.3.1 Classification of HIV

3.3.1.1 HIV-1 One of the major characteristics of HIV is its extremely high genetic variability, which is the result of the high error rate, the recombinogenic properties of the reverse transcriptase enzyme [19], and the fast turnover of virions in HIV-infected individuals [8].

The greatest genetic diversity of HIV-1 has been found in Africa. Phylogenetic analysis of numerous strains of HIV-1, isolated from diverse geographic origins, has revealed the distinct clades of viruses, which have been named groups M (Main), N (New or non-M, non-O), and O (Outlier). Each of the three HIV-1 groups is thought to represent independent cross-species transmissions with a closely related virus. Thus, based on inferences from phylogenetic tree topologies, HIV-1 originated from simian immunodeficiency virus (SIVcpz) from *Pan troglodytes troglodytes* chimpanzees in West-Central Africa [15,47].

The vast majority of strains found worldwide belong to the group M. Within group M, there is further phylogenetic structure, which has allowed the classification of strains into subtypes.

The subtypes are approximately equidistantly related with difference of 25–35% amino acid sequence in their ENV proteins. To be considered as a subtype, isolates should resemble each other across the entire genome. In this light, there are only nine subtypes of HIV-1 group M (A, B, C, D, F, G, H, J, K) because the viruses of subtypes E and I have been found to be recombinants. Within some subtypes, further distinct sequence clusters exist, leading to the classification into sub-subtypes. For instance, subtypes A and F are subdivided into two, A1 and A2, and F1 and F2. It is clear that subtypes B and D would be better considered as sub-subtypes of a single subtype, but for historical reasons, it is difficult to change these designations (Fig. 3.3).

As our knowledge of HIV sequences improved over time, it became clear that some isolates clustered with different subtypes in different regions of their genome in phylogenetic

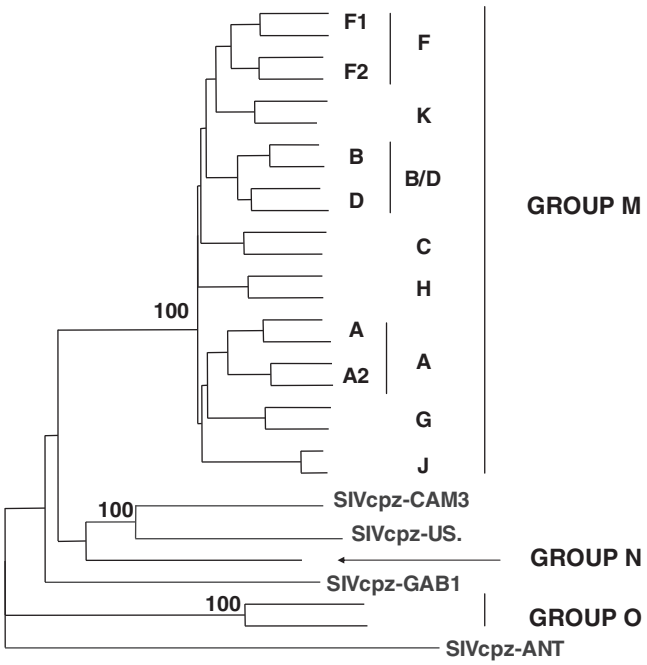

Fig. 3.3. Neighbor-joining phylogenetic tree of near full-length genome sequences of representative HIV-1 isolates from group M subtypes and sub-subtypes, group O and group N. Branch lengths are drawn to scale (the bar indicates 10% divergence). The numbers at the nodes indicate the percent bootstrap values supporting the cluster to the right (only values >80% are shown).

tree analyses [44]. Presently, some of these mosaic HIV-1 genomes play a major role in the global AIDS epidemic and are now designated as "Circulating Recombinant Forms," or CRFs. By definition, CRFs should resemble each other over the entire genome, with similar breakpoints reflecting common ancestry from the same recombination event(s): 16 CRFs of HIV-1 exits, each is designated by an identifying number, with letters indicating the subtypes involved, the letters are replaced by "cpx," denoting "complex" if more than two subtypes are involved (Fig. 3.4).

3.3.1.2 HIV-2 strains Compared to HIV-1, only a limited number of HIV-2 strains have been genetically characterized. Close phylogenetic relationship and similarities in the organization of the viral genome indicate that HIV-2 is also a result of a zoonotic transmission from SIVsm from sooty mangabeys to humans in West Africa [15]. The natural habitat of sooty mangabeys coincides with the geographical region where HIV-2 is prevalent in West Africa, and sooty mangabeys are regularly hunted for food or kept as pets, thus allowing direct contact between mangabeys and humans. More detailed phylogenetic analysis showed even that cross-species transmissions from SIVsm to humans occurred on several occasions [15]. Several of the HIV-2 subtypes have only been found in countries where sooty mangabeys are present in large numbers. Seven subtypes (A–G) of HIV-2 have been described so far. Only subtypes A and B are largely represented in the HIV-2 epidemic, with subtype A in the western part of West Africa (Senegal, Guinea-Bissau) and subtype B being predominant in Ivory Coast [22, 25, 30, 65]. The other subtypes have been documented in one or few individuals only. The different clades of HIV-2 must be the result of multiple independent cross-species transmissions of SIVsm into the human population [17]. Based on genetic distances, HIV-2 subtypes correspond to what is considered as groups for HIV-1.

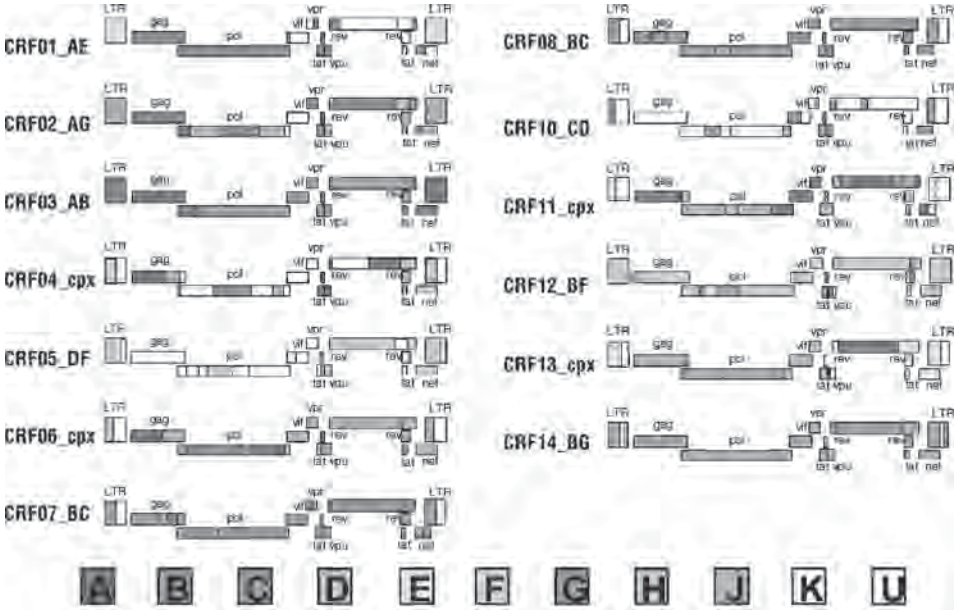

Fig. 3.4. Mosaic genome of different Circulating Recombinant Forms (CRFs). See color plates.

3.3.2 Distribution of HIV-1 in Africa

The classification of HIV strains has helped in tracking the course of the HIV pandemic [52]. Extensive efforts have been made to collect and characterize HIV isolates from around the world and Africa, and a broad picture of the distribution of HIV strains has emerged. As mentioned above, HIV-2 is restricted to West Africa, and the prevalences remain low and are even decreasing in some areas [51]. HIV-1 group O seems to be endemic in Cameroon and neighboring countries in West-Central Africa, and represents only about 1%– 5% of HIV-1 -positive samples in this region [37]. Elsewhere in the world, group O viruses have been identified mainly from persons with epidemiological links to Central Africa, mainly Cameroon and some neighboring countries. Interestingly, group N viruses have only been identified in a limited number of persons from Cameroon only [52].

The global pandemic is due to HIV1 strains belonging to group M. The distribution of the different HIV-1 group M variants in the world is summarized in Figure 3.5.

In Africa, subtypes A, C, and CRF02-AG are most frequent, but the distribution of the different HIV strains is very heterogeneous [38,39].

All groups and subtypes are found consistent with this continent being the source of the epidemic. As expected, given the presence of numerous co-circulating subtypes, a high frequency and a wide variety of recombinants have also been reported in Africa. In South and East Africa, subtype C predominates. In West and Central Africa, as judged by *env* sequences, subtype A-like viruses are most common. Full-length genome sequences of env subtype A viruses from West Africa, Senegal, Cote d'Ivoire, and Cameroon, showed that these viruses have the same recombinant structure involving subtype A and G as CRF02-AG viruses, and it seems likely that the majority of viruses with subtype A *gag* and/or *env* sequences in West Africa and West Central Africa belong to this CRF [55]. In contrast, in East Africa, the subtype A viruses are predominantly nonrecombinant. Subtype D is present at frequencies of 5–40% in Central and East Africa, whereas subtype G has been documented in many West and Central African countries. Subtypes F, H, J, and K as well as CRF01-AE, are mainly seen in Central Africa.

In occidental countries, the epidemic is mainly due to the subtype B, but there is an increasing number of non-B-subtypes in Western Europe; for instance, in France, it is estimated that about 25 % of the new infections are due to non-B-strains. In Eastern Europe, the initial infections were due to subtype B (among intravenous drug users) and subtype A (heterosexual contamination), and it is now a CRF A/B which is predominant. In South east Asia, the CRF01-AE is predominant, whereas in China, subtypes B and C and now a CRF0- BC are circulating.

In addition to CRFs, which play a major role in the global epidemic, many unique recombinant viruses have also been documented. Because only few systematic studies have been conducted, the exact prevalence of recombinant strains is unknown. Based on preliminary data, the proportion of discordant subtypes between *gag* and *env* vary from <10% to>40% according to the countries or regions studied. Peeters et al. [39] illustrate the estimated prevalences of unique recombinant HIV-1 viruses based on discordant subtype/CRF designations in different regions of the genome. The subtypes involved in these discordant samples depend on the subtypes that co-circulate in the region. For instance, in Nigeria, only subtypes A and G co-circulate, and these are the only subtypes involved in the 37% discordant samples. As expected, because subtypes circulate concurrently, a wide

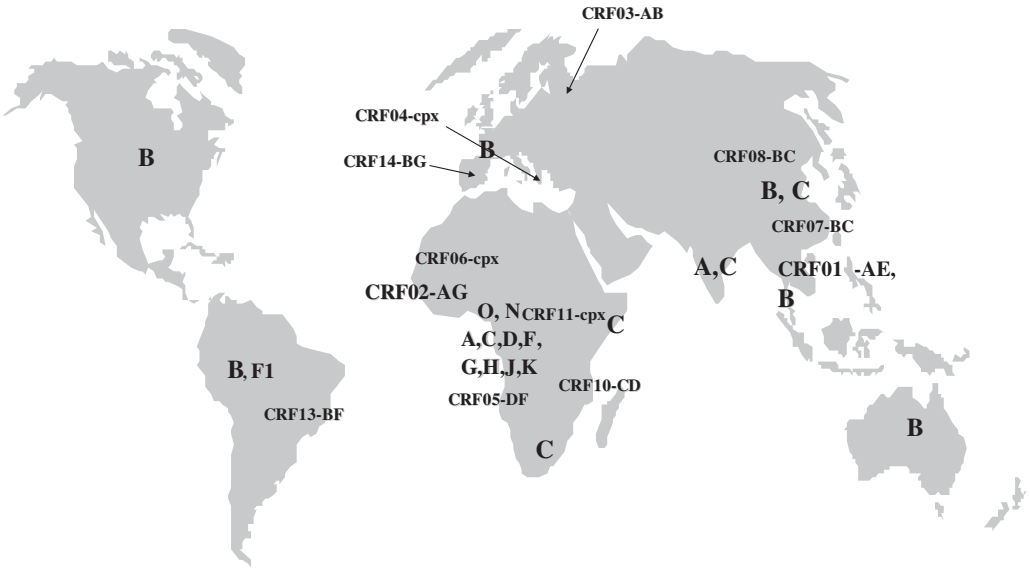

Fig. 3.5. Geographical distribution of HIV-1 subtypes and CRFs.

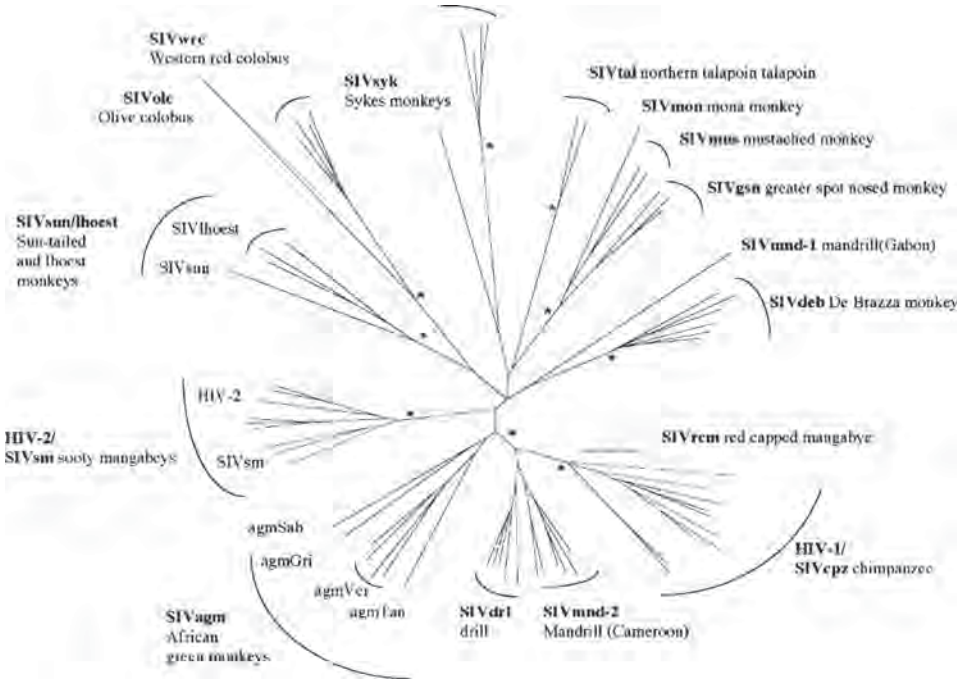

Fig. 3.6. Evolutionary relationship of a large number of SIVs characterized in *pol* region.

variety of recombinants have been reported in the Democratic Republic of Congo with all subtypes involved in the recombination events [61].

3.3.3 Implications of Recombination

As more HIV-1 variants inevitably intermix in different parts of the world, the likelihood of generating new recombinant viruses will increase. Therefore, the global distribution of different forms of HIV-1 will continue to be a dynamic process. Mosaic genomes will become even more complex, as recombination involving viruses that are already recombinant will occur. Mosaics involving CFR02-AG have already been observed in various African countries. Recombination between two CRFs (CRF02/06) has also been described in a study in Niger [28]. Even distantly related viruses have been shown to recombine. For instance, intergroup recombinants between group O and M HIV-1 strains have been documented in Cameroon (Fig. 3.6) [36]. Recombination between strains from distant lineages may contribute substantially to new HIV-1 strains and could have important consequences. Presently, group O viruses represent a minority of the strains responsible for the HIV-1 pandemic. However, if these recombinant intergroup viruses have a better fitness than the parental group O viruses, their prevalence may increase rapidly with consequences on their serological and molecular diagnosis and treatment because differences among susceptibilities to certain antiretroviral drugs have been observed *in vitro*.

Both HIV-1 and HIV-2 are of zoonotic origin, and the current HIV-1 group M pandemic provides compelling evidence for the rapidity and the extraordinary impact that can result from even a single primate lentiviral zoonotic transmission event. We recently showed that humans come in frequent contact with primates in many parts of Sub-Saharan Africa [35]; thus, raising the possibility of additional zoonotic transmission. Figure 3.6 illustrates the diversity of the primate lentiviruses actually described and to which humans are exposed through hunting and handling of bushmeat in Africa. The fact that distant HIVs can recombine begs the question whether distantly related SIVs and HIV can potentially recombine, particularly in individuals who are HIV positive and exposed to SIV by cross-species transmission. Distantly related SIVs can so spread more efficiently into the human population.

Finally, recombination also has important implications for vaccine strategies based on live-attenuated viruses, because these could recombine with infecting strains, even though the two may be quite divergent.

3.4 IMPLICATION OF HIV VARIABILITY ON PATHOGENESIS, TREATMENT, DIAGNOSIS, AND VACCINE DEVELOPMENT IN AFRICA

The majority of our knowledge on HIV is based on the specific HIV-1 subtype B predominant in industrialized countries, whereas the worldwide epidemic is due to non-B-subtype. Importantly, diagnostic tests, antiretroviral drugs, and HIV-1 vaccines have so far mainly been developed for subtype B viruses. Thus, the biological implications of HIV variability are numerous, and some of them are not fully understood.

3.4.1 Impact of HIV Variability on Diagnosis

Previous studies have shown that some persons infected with certain highly diverse strains of HIV, such as group O, fail to be diagnosed accurately by some serologic tests with for consequence tha adition of HIV-1 group O antigen in anew generation oof tests. Although enzyme linked immunosorbent assays (ELISAs) and rapid tests are sensitive and specific for diagnosing persons with chronically established infections with HIV-1 group M-non-B subtypes, the challenge still remains in diagnosing persons with recent infections primarily because antigens used for the assays were based on HIV-1 subtype B strains. Presently, in developing countries, much emphasis is being placed on implementing intervention programs resulting from new research findings such as the use of ARV drugs to reduce mother–child transmission of HIV, access to ARV in general, therapy in HIV-infected tuberculosis patients, and voluntary testing and counseling. These programs require that patients be diagnosed accurately and results delivered in a timely fashion; thus, there is considerable use of serologic assays in different countries. HIV diversity being a dynamic process, there is a need to pursue the development and evaluation of HIV diagnostics tests.

Plasma viral load measurement has increased considerably in clinical settings for monitoring patients on ARV therapy. Although, much progress has been made to improve the sensitivity of nucleic-acid-based assays to quantify viral load, some RNA viral load assays still produce erroneous results with highly divergent strains. No commercial available assays exist for HIV-2 quantification.

3.4.2 Impact of HIV Variability and Antiretroviral Therapy

The HIV diversity raises two questions. First, is there a natural resistance to some ARV drugs developed for the subtype B; and secondly, the acquired resistance under drug pressure is the same as for subtypes B and non-B. Concerning the first question, HIV-1 group O and HIV-2 strains are naturally resistant to non-nucleoside reverse transcriptase inhibitors. Limited *in vitro* studies have suggested that some non-B-subtypes may be less susceptible to certain classes of ARV drugs. For instance, subtype G strains are less susceptible *in vitro* to protease inhibitors. Genetic characterization of the protease gene from non-B-strains revealed a high genetic polymorphism with minor mutations [58]. Accessory (or minor) mutations may not result in a significant decrease in susceptibility, but may be associated with an increase in viral fitness (replication capacity) and/or increase in resistance level associated with major mutations, and thus, long-term failure of therapy. However, the implication of the presence of only accessory mutations to susceptibility of ARV drugs still has to be investigated *in vivo*. Results of ARV drug initiatives in Africa (Senegal, Cote d'Ivoire, and Uganda) and studies on African patients in Europe have showed that the presence of accessory mutations in the protease gene at baseline do not influence the clinical outcome of HAART [12]. Indeed, virologic responses (decrease in plasma viral loads and increase in CD4 count) comparable to responses reported among patients infected with subtype B in Western countries have been observed in Senegal and Uganda [26,62]. Other studies have also not found subtype-dependent responses to ARV therapy for subtypes A, C, and D. However, more data and a longer follow-up are needed to identify whether pre-existing accessory mutations could influence the rate of occurrence of resistant viruses during treatment and to what extent they could compensate for the reduced fitness of resistant mutants.

As ARV drugs are beginning to be widely used in Africa, studies are needed to understand the development of ARV drug resistance in patients infected with different subtypes. Development of drug resistance may be influenced by levels of viral loads among the patients, greater quasi-species distribution, and genetic diversity. Subtype G viruses have the V82I naturally occurring polymorphism at a position where major drug-resistant mutations occur. But primary or major mutations related to ART resistance have not yet been documented as natural variants in non-B-group M stains. It is also not known whether primary mutations that confer resistance in subtype B viruses also play a role for non-B-subtypes. However, of the few published studies on ART drug resistance in Africa, a strong correlation has been documented between genotypic and phenotypic resistances, and mutations observed so far correspond to similar mutations seen in subtype B infections under similar treatment regimens, but other studies suggest that mutations associated with drug resistance might differ. An important point is that the algorithm used to interpret the observed mutations is based on B subtypes and that this algorithm is sometimes not relevant for non-B subtypes [62]. Moreover, the few studies reported in Africa have shown that the rate of occurrence of drug resistance depends largely on the appropriate use of the drugs than on the HIV-1 subtypes. For instance, more than 50% resistance has been reported in Cote d'Ivoire and Gabon among patients receiving ARV drug therapy without appropriate clinical and laboratory follow-up. In contrast, in Senegal, after a 24 months follow-up period, drug resistance mutations were seen in only 16% of the patients receiving ART with careful clinical and biological monitoring [60], which is comparable to what has been described for patients infected with subtype B viruses in Western countries. However, one study has shown *in vivo* differences related to subtypes. The study from the HIV Network for Prevention Trials (HIVNET 012 study) in Uganda showed that resistance to nevirapine occurred more frequently in women infected with subtype D than in women infected with subtypes A and C [16]. Overall, in order to avoid the rapid emergence of resistant viruses on a large scale in developing countries, it is important that infrastructure necessary to monitor responses to ART be put in place in these countries and that clinicians are trained in the appropriate use of ART drugs and continuous surveillance of ART drug-resistant viruses has to be organized to guide ARV treatment strategies and policies.

3.4.3 Impact of HIV Variability on Transmissibility and Pathogenesis

Compared with HIV-1, HIV-2 infection is characterized by a much longer asymptomatic stage, lower plasma viral loads, slower decline in CD4+ T cell count, and a lower mortality rate. The existence of many other factors that influence transmissibility and pathogenicity makes it difficult to establish the impact of HIV-1 viral subtypes. Limited studies on subtypes and transmissibility have yielded discordant conclusions. A recent study in Tanzania suggested that subtypes A and C, and recombinants are more likely to be perinatally transmitted than subtype D. On the contrary, a study in Uganda suggests similar rates of perinatal transmission for subtypes A and D, but a study in Kenya has shown that women infected with subtype D were more likely to transmit virus to their infants than those infected with subtype A.

HIV-1 subtype-specific difference in disease progression appear conflicting. For instance, no difference in disease progression was found between patients infected with subtypes B and C in Israel, or among patients infected with subtypes A, B, C, and D in Sweden. In a 4-year prospective multicenter study of 335 patients from Senegal and Cameroon with unknown dates of seroconversion, multivariate analyses showed no difference in survival, clinical disease progression, or CD4 cell decline between patients infected by CRF02-AG strains and those infected with other strains. However, two studies based on incident cases have found HIV-1 subtype-specific disease progression patterns. In a study in Uganda of more than 1000 patients, subtype D was associated with faster progression to death and with a lower CD4 cell count during follow-up, compared with subtype A, after adjusting for CD4 cell counts at enrollment.

3.4.4 Impact of HIV Variability on Vaccine Development

An effective HIV vaccine is the long-term solution to control the HIV/AIDS epidemic in Africa and should protect against infection to all genetically diverse strains of HIV-1. From a vaccine standpoint, the implication of the multitude of HIV-1 subtypes circulating in Africa is unknown, as correlates of protective immunity against HIV-1 are poorly understood. Few and contradictory data exist on the link between genetic subtypes and HIV-specific immune responses [33]. Several studies have not shown a correlation between HIV-1 subtypes and neutralizing serotypes. However, one study has reported subtype-specific neutralization for subtype B and CRF01-AE in Thailand, and one study also suggested geographically clustered neutralization sensitivities within subtype C. Because CTL are important components of the antiviral responses in HIV-infected people, current efforts on HIV vaccines are targeting induction of T-cell responses, particularly against gag and pol proteins, which appear to be more conserved in HIV. Studies have demonstrated CTL cross-reactivity between different HIV-1 subtypes to varying degrees of conservation in the genes. This may suggest that matching HIV vaccine candidates to the prevalent HIV-1 strains might be less important for vaccines targeted at induction of T-cell responses to conserved proteins (for instance, gag and pol). However, intra-subtype CTL responses are usually stronger and more frequently detected than inter-subtype reactivities, and subtype-specific CTL epitopes have also been identified [13, 20,55].

Because of the distribution pattern of HIV-1 subtypes in the world, current vaccine development efforts have been subtype specific.

3.5 ACCESS TO TREATMENT

HAART has dramatically reduced HIV/AIDS-related mortality, morbidity, and hospitalization in industrialized countries, and thus HIV/AIDS can be considered much more a chronic disease rather than a lethal disease, as we can control the replication of the virus, but its eradication in humans is not yet possible. The major public health problem is now to make effective such care for people living in developing countries, that is, for the majority of the persons who need treatment! WHO estimated that in 2005 only 8% of persons living in Africa and who need ARV have access to such treatment.

At the end of 1998, the necessary and legitimate access to antiretroviral drugs was not considered as an evidence. At that time, the cost of drugs and reagents, the need for relatively sophisticated laboratory facilities for treatment monitoring, and the infrastructure required to provide an uninterrupted supply of drugs were considered as important limitations on wide spread use of HAART in poor countries. Other hindrances include the supposed complexity of antiretroviral drug administration, drug interactions, rapid emergence of viral resistance, the frequency of adverse effects, poor adherence, and inadequate knowledge of biological and clinical responses in patient infected by non-subtype-B HIV-1 strains. Furthermore, most patients have advanced HIV disease by the time treatment is initiated, and this could lead to higher toxicity, lower efficacy, and severe immune restoration syndromes. These factors were sometimes used as a pretext for focusing public health intervention exclusively on prevention rather than prevention and treatment. The first governmental African national initiative was set up in Senegal by Dr Ibrahima Ndoye who can be considered as a visionnaire. The result of this program, followed now by numerous others, has demonstrated that the efficiency of such treatment is the same as in occidental cohort. At the same time, the drastic price reduction (90%) of brand-name drugs, the availability of generic drugs and their proven efficacy [69], and the simplification of treatment (a once a day treatment is possible) have changed the landscape for ARV in resource-poor settings. Furthermore, the United Nations global fund the World Bank and numerous other governmental initiatives have generated funds for ARV treatment as never. Despite this, the treatment remains beyond the reach of all. The reasons are multiple. It is clear that the international agencies have generated an extraordinary "bureaucracy" with

nonoperational procedure for the management of such program. In some countries, different international supports are not under the responsibility of the different structures generating conflicts. Furthermore, at the request of WHO and UNAIDS, multisectorial program including different ministries have been created. The result is that in some countries there is competition between AIDS program from the ministry of Health and the multisectorial program. This reform has generated still more "bureaucracy." Beyond these structural problems, factors implementing ARV programs are difficult not only because it is a life-long treatment that requires a good follow-up in order to avoid virological failure and acquired drugs resistance but also because it is a global health program with, for instance, the need to promote voluntary testing and counseling. In order to reach the objective of treating 3 million persons in 2005, WHO has promoted a simplified approach using mainly clinical criteria for the follow-up of the patients. Clearly, this strategy needs to be evaluated through operational research, as we have to be sure that this strategy is not dangerous for the patients in the short time (tolerance, virological failure) and for the community in the mid-term (emergence of resistance).

3.6 CONCLUSION

In the developing world, especially in Sub-Saharan Africa, the HIV epidemic is no longer only a public health problem, it is also affecting the development. A vaccine is eagerly awaited. Several candidate vaccines are now being studied, but it will take several years to develop a safe and routinely effective vaccine covering all the circulating strains. The good news is that we have an effective treatment, but in the meantime, access to antiretroviral regimens is a major concern.

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