HIV-1 subtype C in Ethiopia: genotypic and phenotypic variation
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CHAPTER IX

General Discussion
Since the first cases of AIDS were diagnosed in the early 1980s, HIV-1 has caused a world-wide pandemic, with the continent of Africa greatly affected. Ethiopia is one of the African countries where the HIV-1 toll has been very high. Although the epidemic started relatively late, in the mid-1980s [1,2], it has spread rapidly and has dramatically affected the productive and active age groups in society, those individuals essential for the sustained economic growth of a developing country. [3-9] The urgent need for the characterization and further investigation of the HIV strains contributing to the epidemic within Ethiopia provided both the input and basis for this project. The major goal was to study the diversification amongst the circulating HIV strains in the different towns and population groups within Ethiopia, as well as to analyze virus evolution over time. For a pathogen like HIV-1 with such an extremely high mutation rate, its effective surveillance and control is a challenge for the developing countries of the world, where the public health resources are limited. The short replication cycle, large numbers of viral progeny, missing proof reading mechanism for the reverse transcriptase enzyme, and both DNA and RNA recombination have together resulted in the emergence of multiple HIV-1 subtypes with the capacity to evolve independently. The African continent is an HIV battlefield where not only are most subtypes widely distributed but also there is the emergence of an increasing number of recombinant viral strains. Three different groups of individuals within Ethiopia believed to be at risk from HIV-1 infection commercial sex workers (CSWs), pregnant women and blood donors were studied. The CSWs were likely to be the first target-group for the influx of the HIV infection into the population, and generally it is this group that has the highest prevalence of HIV-1 infection. It has been shown amongst the CSWs in Addis Ababa that the seropositivity prevalence was 25% in 1989 and 74% in 1998, a dramatic increase over a relatively short period of time. [9, Aklilu M., et al submitted]
Moreover, pregnant women and blood donors are population groups that need active surveillance, with the mother-to-child infection incidence resulting in large numbers of HIV-positive children in Africa and blood transfusions being the main cause of nosocomial HIV infection.\(^{10-15}\)

**HIV-1 subtype(s) circulating in Ethiopia**

Chapter II and chapter III describe an epidemiological study focusing on the molecular characterization of the HIV strains circulating in the capital, Addis Ababa. Three different groups of asymptomatic HIV-1 infected individuals described above (CSWs, pregnant women and blood donors) were studied between 1989 and 1997. It was found that subtype C is the most dominant HIV-1 strain circulating in Addis Ababa. Analysis of the C2-V3 region of the gp120 envelope protein revealed that 159 of the 161 sera samples included in the study contained subtype C viruses. Only two non-C subtype isolates were found: one subtype-A and the other subtype-D. This finding is in agreement with a number of previous reports, but here larger numbers of samples provide more solid data. The presence of subtype C in Addis Ababa had been demonstrated by sequence data on a few AIDS patients\(^{16,17}\) and peptide ELISA and DNA sequencing studies\(^{16-21}\) had revealed subtype C as the major HIV subtype in Ethiopia. Taken together, these results demonstrate that sequencing of DNA isolated from cells or cDNA derived from genomic RNA in serum can be an extremely useful for HIV-1 subtyping as well as the most accurate method. In addition, the first full-length HIV-1 subtype C sequence published was from a 1986 isolate from Addis Ababa.\(^{20}\)

In order to gain insights into the HIV-1 epidemic in Ethiopia, it was important to carry out studies in other urban areas of the country as performed in the capital, Addis Ababa. This aim is addressed in Chapter IV, which describes the study of the genetic diversity and geographic distribution of HIV-1 strains from several Ethiopian towns including the capital city. This study utilized sera
containing HIV-1 antibody and virus that was collected in 1988 and in 1996/7 from two HIV-1 risk-groups, CSWs and blood donors. It was found that of the 104 viruses analysed in the C2-V3 region of the gp120 envelope protein, all but two were subtype C. These two viruses were from the 1996/97 period and, as in the Addis Ababa study described in chapter II and chapter III, one was subtype A and the other subtype D. This finding confirmed the data from the Addis Ababa study showing that subtype C is the predominant cause of the HIV-1 epidemic in Ethiopia as a whole. Also important in this study was our finding that the circulating subtype C viruses could be separated phylogenetically into two sub-groups, which have been designated main group C and subcluster C'. These two groups are significantly different and distinguishable in their nucleic acid as well as in their amino acid sequences.

Both virus groups C and C' showed significant amino acid differences when compared to all database sequences of subtype C although, the main C group was genetically closer than C' to the data base C consensus sequence. The C and C' groups were equally distributed regardless of geographic location, time of sample collection, or risk group(s) in Ethiopia. It is an interesting observation that in Ethiopia the prevalence of non-C HIV-1 subtypes is extremely low, whilst in the neighboring countries more than one subtype can often be found in relatively high frequencies. Moreover, for the first time, two distinct subtype C strains were identified to spread throughout the same country.

**Year of HIV-1 introduction into Ethiopia**

Our determining of the years in which the HIV-1 subgroups C and C' viral isolates were initially introduced into Ethiopia is described in chapter V and VI. The calculations were carried out separately for the main C group and the subcluster C' group. We compiled the C2-V3 sequences from all our studies, 1988 to 1999, and included in this study the oldest sequences that belong to the C group from 1984 and 1985.
The synonymous sequence-distance of the C2-V3 envelope region of the Ethiopian C strains to the reconstructed common ancestor was shown to increase in the course of the epidemic, as was observed with the subtype B epidemic.\textsuperscript{[26,27]} Based on this observation, evolutionary distance analysis of samples collected in 1984 and 1985 revealed that they were close to the reconstructed common ancestor. Additionally, as in subtype B epidemics\textsuperscript{[28]} a highly significant correlation was observed between the synonymous distances of the C2-V3 sequences to the common ancestor and the sequence sampling years. With extrapolation of the regression analysis back to the date when no synonymous heterogeneity was present in Ethiopian HIV-1 subtype C populations, we were able to estimate 1983 as the introduction year for the main group C and 1982 for the subcluster C'. This finding strongly agrees with serological data showing that the first HIV-1 seropositive sera identified in Ethiopia were from 1984, with more than 1500 sera being analysed between 1982 and 1984 for that study.\textsuperscript{[1]} The synonymous evolution rate of the C2-V3 region in the C' viruses is shown to be two times lower than that observed with the C viruses. A possible explanation could be that there are differences in the replication rates between the C and C' viruses. However, if this were the case, the C virus would be expected to predominate, which was not found to be so. A more detailed comparison of the biological function of the two virus envelopes might provide an answer.

\textbf{Comparison of the two subtype C strains by phylogenetic analysis}

All the above studies were based upon the cDNA sequence analysis of the C2-V3 gp120 envelope region. To confirm our results on the C2-V3 env region a study described in chapter VII compared different regions of the viral genome (gag, pol and env) of viruses randomly selected among the isolates included in the national survey described in chapter IV. Phylogenetic analysis confirmed the existence of two distinct virus populations for all regions analyzed.
Furthermore, a number of viruses, whilst clustering with the main group for the gag region, switched to the C’ subcluster for the V1-V2 and V3 regions, indicating intra-subtype recombination. Based on the phylogenetic results of 30 gag sequences, 16 viruses (including those suspected to be recombinants and representative samples from the main C and subcluster C’ groups) were selected for further analysis. A 2600bp segment of the HIV-1 genome comprising of the gag and part of the pol (protease and half of the RT) genes were sequenced and analysed together with the respective V1V2/C2V3 regions of the envelope. Six isolates were found to be C/C’ intra-subtype recombinants, and whilst they all have different recombination patterns, often the recombination crossover points were the same as the ones described for the inter-subtype recombinants.\(^{[29-33]}\)

Furthermore, we identified one crossover point in the p24/p17 region of gag, one in the 5’end of the Pol gene.

Possibly one of the most interesting observations is that all C/C’ recombinant viruses carry the variable V1/V2 and V3 regions of the C’ group, indicating that the C’ envelope maybe advantageous for the survival of the recombined progeny. This finding suggests that upon recombination, the genes or gene fragments that provide competitive advantage are selected. A similar phenomenon has been reported for A/C and D/C recombinants, which all carry the subtype C envelope.\(^{[34]}\) In addition to the two independently evolving C and C’ groups, viral recombination can occur and might increasingly occur between these two groups to create new viruses.

The features of the Ethiopian HIV-1 epidemic are unique, not only because it is composed predominantly of subtype C viruses but also because the few non-C isolates turn out to be subtype-C recombinants (data not shown). The findings from our study are in complete agreement with a previous study reporting on A/C, and A/D/C recombinant viruses from Ethiopia.\(^{[35,36]}\)
Biological phenotypes of the HIV-1 viruses circulating in Ethiopia

Chapter VIII describes a study on the biological phenotypes of the HIV-1 viruses circulating in Ethiopia, compared to other subtypes. The frequency of syncytium-inducing (SI) versus non-syncytium-inducing (NSI) viral isolates and the coreceptor use of these different viral isolates from 48 Ethiopian AIDS patients were investigated. All patients had abnormally low CD4+ lymphocyte numbers and were shown to be infected with HIV-1 subtype C, but only three of the 48 patients were found to harbour SI viruses. From these three individuals, biologically cloned SI and NSI viruses were generated. The NSI clones were shown to use CCR5 as their coreceptor, whereas the SI clones used CXCR4, CXCR4/CCR5 or CXCR4/CCR3. Our finding of the low emergence of SI variants and the predominant usage of CCR5 as the HIV-1 coreceptor in HIV-1 subtype C infections in Ethiopia is in agreement with previous reports.[37,38]

Further studies will be required to identify the cause of this unique feature of subtype C viruses.

Investigation of the virologic aspects of the HIV-1 epidemic in Ethiopia has revealed that subtype C viruses dominate the epidemic and that two different subgroups termed C and C’, co-circulate throughout the country. The increasing genetic heterogeneity of these two virus populations together with their expansion around a stationary consensus sequence has enabled us to estimate 1982/3 as the beginning of the HIV-1 epidemic in Ethiopia. Since then both strains have rapidly spread, saturating the high-risk group network and preventing the introduction of other subtypes. The discovery of intra-subtype recombination and the fact that all recombinants carry the C’ subgroup V1-V2 and C2-V3 region of the envelope points to a competitive advantage for viruses with a C’ envelope, possibly related to more efficient transmission in the population. Other subtypes entering Ethiopia in the early 1990s have recombined with subtype C, perhaps because this was the only way to survive in an environment dominated by C viruses.
The frequency of HIV-1 subtype C SI (CXCR4-using) variants in Ethiopian patients in late stage of HIV infection is found to be very low but it remains to be determined whether this phenotypic difference from the other subtypes correlates with any of the in vivo biological properties of the subtype C virus. In general the HIV-1 epidemic in Ethiopia is a serious public health problem for the country, especially considering the rapid spread of the predominant viral strains. What is needed in Ethiopia, as in many other countries around the world, is an increase in public awareness, along with surveillance of the circulating HIV-1 strains in the population and the early detection of new and possibly more virulent viral strains, in order to curtail or combat this devastating epidemic.

Now that we know more about the viruses circulating in Ethiopia, the next stage will be the development of a vaccine that is effective particularly against HIV-1 subtype C. Diagnostic tools to monitor subtype C infection and the response of these viruses to anti-retroviral therapy will be a crucial step on the way.
REFERENCES


