5. SUMMARY

Human immunodeficiency virus (HIV) displays a high degree of genetic variability, which is mainly attributed to its high replication rate and the error prone nature of the viral reverse transcriptase. This genetic variability necessitated its classification into two types (HIV-1 and –2), each subdivided into several groups and subtypes. Even within a single infected host, many genetically different viruses exist and this swarm of sequences has been termed viral quasispecies. Furthermore, the genomes of viruses of different subtypes and groups may recombine to yield circulating recombinant forms (CRF), some of which show a high prevalence in certain areas of the world. Quite clearly, the current AIDS pandemic is caused by a large number of genetically different viruses, which are originally derived from few cross-species transmissions, but have evolved and are continuing to evolve at a rapid rate. Currently, there is little known about potential functional differences between the various subtypes and recombinant forms. Most studies have been performed with laboratory strains of the virus and few reagents directly derived from the primary patient-derived virus are currently available. To study specific differences between various strains and recombinant forms, one needs to obtain primary isolates of the virus reflecting the viral quasispecies within a given person. Detailed molecular analysis of the functional properties and biological phenotypes of specific viruses further requires the availability of full length molecular clones of the respective viral genomes. Transfection of such proviral clones leads to production of infectious virus particles, whose properties should be identical to the primary isolate the clone was derived from. It was the aim of this study to obtain a panel of primary HIV isolates from Cameroon and to generate infectious molecular clones derived from relevant viral variants.

Blood samples were collected from HIV-1 infected individuals mostly from the Western Provinces of Cameroon. A total of 19 HIV-1 isolates were derived from these samples by co-cultivation of patient-derived blood cells with either peripheral blood mononuclear cells from HIV negative donors or with PM-1 cells. PM-1 is a permanent T-cell line, which expresses the two main coreceptors of HIV-1 (CXCR4 and CCR5), and therefore allows propagation of most viral isolates, irrespective of their coreceptor usage. Genetic subtyping of the virus isolates was performed by PCR amplification and direct sequencing of discontinuous portions of the three major

structural genes *gag*, *pol* and *env*. Sequences were phylogenetically analysed and classified into the following HIV-1 main (M) group subtypes: $A^{gag}/A^{pol}/A^{env}$ (n=4); $G^{gag}/G^{pol}/G^{env}$ (n=3), $F2^{gag}/F2^{pol}/F2^{env}$ (n=1), $A^{gag}/AG^{pol}/AG^{env}$ (n=2), $AG^{gag}/A^{pol}/AG^{env}$ (n=1), $AG^{gag}/U^{pol}/AG^{env}$ (n=1), $AG^{gag}/AG^{pol}/AG^{env}$ (n=6) and a novel $A^{gag}/J^{pro/rt}/A^{int}/U^{env}$ complex recombinant (n=1). Thus, only few viral isolates displayed a pure subtype and there was a high prevalence of recombinant forms, mostly corresponding to the CRF02.AG type. Two recombinant strains were selected for further analysis: An AJU recombinant because it is the first isolate of this kind to be reported from Cameroon and a CRF02.AG isolate because this is the most prevalent HIV-1 strain currently circulating in West and Central Africa. While the recombinant structure of the AJU virus was different from other subtype J recombinants, the CRF02.AG isolate was very similar to other viruses of this lineage.

The biological phenotype of most isolates correlated with the clinical status of the patient. Five of six isolates from patients with advanced disease induced syncytia (SI) on MT-2 cells while 13 isolates, 12 of which were from patients at earlier stages of the disease, were of the non-syncytium inducing phenotype (NSI). All SI isolates used CXCR4 as their co-receptor, while all NSI isolates used CCR5. Sequencing of the V3 region within the envelope gene revealed additional feature which had been attributed to the respective biological phenotype in previous studies. To determine whether drug resistance mutations were present in these isolates, which had been obtained from drug-naive individuals, the protease and part of the reverse transcriptase coding region was screened for known resistance polymorhisms. However, only variations considered to be compensatory polymorhisms were observed and no primary resistance signal sequence was detected.

In order to obtain reference tools for HIV research, the complete genomes of an HIV-1 group O strain and of a CRF02.AG isolate were amplified by long range PCR and full-length molecular clones were generated. Production of infectious virus was detected after transfection and infectious molecular clones were completely sequenced. Transfection-derived viruses exhibited comparable biological properties and infectious titers as their respective parental isolates. The HIV-1 group O proviral plasmid represents the first infectious molecular clone of this group of HIV-1, and the CRF02.AG clone is the first CXCR4-using infectious clone from this highly prevalent recombinant variant. These reagents will be very important for further studies aimed

at elucidating the biological and functional differences that might exist between HIV subtypes. In addition, they may serve as valuable tools in HIV vaccine studies.