Adaptation of a Novel Simian-tropic HIV-1 Clade C Progeny Virus

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The human immunodeficiency virus (HIV) is the causative agent of one of the most challenging epidemics humanity ever had to face. Overall, 0.8% of the human population carries HIV-1. Approximately 30% do not know their status. Over 19 million carriers, which are 44% of all HIV-1-infected individuals, live in Southern or East Africa. Only 2.1 million, which sums up to not more than 5.7%, live in Central and Western Europe and North America. Since the outbreak in the 1980s, more than 76 million people have become infected and more than 35 million people have died from AIDS-related illnesses [1]. To the date, prevention by a vaccine is still not in sight. Although highly active antiretroviral therapy (HAART) is potent and prevents disease progression to AIDS in HIV-infected people, the epidemic is still ongoing. Therefore, the development of a vaccine gains more and more importance.

However, one of the biggest challenges in HIV-1 research is the lack of an animal model that fits all the requirements to represent HIV-1 infection in humans. Unfortunately, attempts to establish HIV-1 replication in mice, rats, or rabbits were unsuccessful. Although cats are hosts for the feline immunodeficiency virus (FIV) [2], which is a relative to HIV-1, cats did not support HIV-1 replication. In addition, FIV infection of cats significantly differs from HIV-1 infection of humans. Therefore, efforts were made to establish a model among non-human primates (NHPs). Taxonomically, HIV-1 is a direct progeny of SIVcpz, endemically infecting mainly Central African chimpanzees (*Pan troglodytes*). Historically, cross-species transfer of SIVcpz from chimpanzees to humans opened the door to the HIV-1 pandemic. Later, a jump of SIVsmm from sooty mangabeys (*Cercocebus atys*) to humans in West Africa resulted in the outbreak of HIV-2 [3]. The SIVmac strain is pathogenic in Asian rhesus macaques. Although there are more than 40 different SIV strains that are endemically infecting African primate species, efforts to develop AIDS disease models in African NHPs remained unsuccessful for a long time. This can be explained by the coexistence of virus and host over centuries, making African monkeys and apes resistant to SIV/HIV-1 disease progression [4]. This theory can be vindicated by the fact that SIVmac causes severe AIDS, including CD4+ T-cell depletion, high viral loads and opportunistic infections in rhesus macaques (*Macaca mulatta*, RMs) of Indian origin [5] – and to a lesser degree – in Chinese-origin RMs [6]. Asian RMs are not natural hosts for SIV. Pig-tailed macaques (*Macaca nemestrina*, PMs) on the other hand are susceptible to SIVmac. Interestingly, they progress significantly faster to AIDS than RMs. In addition, PMs have a very important genetic difference to RMs. This genetic difference is crucial for the development of a macaque model for HIV-1 infection [7,8]. It should be noted that the envelopes of SIV strains, which cause AIDS-like disease in Asian macaques, and HIV differ so much that antibodies developed in response to such SIV strains do not recognize HIV and vice versa.

To counteract this dilemma, chimeric strains have been developed, designated simian-human immunodeficiency viruses (SHIVs). These strains contain the HIV *env* (envelope) gene within a SIV backbone. The first strains SHIV-1 to SHIV-4 [9] facilitated vaccine studies in macaques targeting envelope glycoproteins of HIV-1. However, immune prevention studies that target other HIV gene products than *env* are impossible. Furthermore, these initial SHIV strains were not capable of mucosal transmission, which was unfortunate as 70% of all infections are transmitted over a mucosal route [7]. Although nearly 50% of all HIV-1-infected people carry a clade C virus, research on this genetic subtype is not as advanced as for clade B. The latter is mainly responsible for infections in the western world, such as Europe, the Americas and Australia. By that time only two proviral DNA templates of HIV-1 clade C strains have been developed, although clade C is one of the most predominant subtypes all over the world.

Unfortunately, HIV-1 cannot replicate in PMs due to the antiviral factor *APOBEC3G*. This immune factor can be inhibited by the v*if* gene from SIV, which explains why SIV is able to replicate in PMs and HIV is not [10]. For this purpose, a new strain has been developed by the Ruprecht lab, containing up to 90% HIV-1 clade C and 10% SIV information in its genome. The novel virus was designated clade C simian-tropic HIV (stHIV-C). This virus contains only three genes from SIVmac239 – *vif*, *vpr* and *vpx*. The core of stHIV-C shares the essential genes of SIV, which are crucial for immune resistance in PMs, while the rest is composed of HIV-1 clade C. The initial stHIV-C clone was designated stHIV-C8457 and capable of

replicating in PMs, which are not natural hosts for HIV. This novel construct would serve as a model for HIV-1 infection in animal models and improve animal studies of vaccines and antivirals significantly. However, stHIV-C needs further adaptation for improved viremia. In order to improve the virus to its new host species, stHIV-C8457 was passaged in PMs by rapid animal-to-animal blood transfer by the Ruprecht lab. By now, 13 animals have been infected with the virus.

In this thesis, we investigated the serology of all infected PMs for anti-HIV-1 antibodies via western blot over time. We showed that all animals produced antibodies against HIV-1 – even years after the initial virus inoculation and even though these animals' immune systems suppressed viremia after the acute infection; some of these PMs had been aviremic for many months or years. The persistent anti-HIV Env antibody responses imply that the PMs' immune systems were unable to eliminate virus-infected cells that continued to provide low-level antigen stimulation to sustain the antiviral antibody responses. We observed only one case of possible seroreversion – gradual disappearance of anti-stHIV-C antibodies. Our studies showed that stHIV-C8457 is capable to persistently infect PMs. However, further adaptations are necessary to improve the replication fitness of stHIV-C in PMs in order to better serve as model of HIV-1 infection in humans and are discussed in this thesis.

References:

1. UNAIDS. (2017). *Unaids: Fact Sheet*. Retrieved January 19, 2018, from UNAIDS: http://www.unaids.org/en/resources/fact-sheet
2. Hartmann K (2012). Clinical aspects of feline retroviruses: A review. *Viruses*, 4(11), 2684-2710. doi: 10.3390/v4112684
3. Sharp PM & Hahn BH (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med*,1(1), a006841, doi: 10.1101/cshperspect.a006841
4. Klatt NR, Silvestri G & Hirsch V (2012). Nonpathogenic simian immunodeficiency virus infections. *Cold Spring Harb Perspect Med,* 2(1). doi: 10.1101/cshperspect.a007153
5. Marthas ML, Lu D, Penedo MCT, Hendrickx AG & Miller CJ (2003). Indian- and Chinese-origin rhesus macaques for AIDS-related research: Comparison of vaginal transmission efficiency of simian immunodeficiency virus (SIV), viral loads, and virus-specific antibody responses. *International Perspectives: The Future of Nonhuman* (128-131). National Research Council.
6. Zhou Y, Bao R, Haigwood NL, Persidsky Y & Ho WZ (2013). SIV infection of rhesus macaques of Chinese origin: a suitable model for HIV infection in humans. *Retrovirology,* 10(89). doi: 10.1186/1742-4690-10-89
7. Hatziioannou T & Evans D (2012). Animal models for HIV/AIDS research. *Nat Rev Microbiol,* 10(12), 852-67. doi: 10.1038/nrmicro2911.
8. Evans DT & Silvestri G (2013). Non-human primate models in AIDS research. *Curr Opin HIV AIDS,* 8(4), 255–261. doi: 10.1097/COH.0b013e328361cee8
9. Shibata R, Kawamura M, Sakai H, Hayami M, Ishimoto A & Adachi A (1991). Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J Virol*, 65(7), 3514-3520 .
10. Hatziioannoua T, Ambroseb Z, Chungb NP, Piatak M, Yuanc F, Trubey CM, Coalter V, Kiser R, Schneider D, Smedley J, Pung R, Gathuka M, Estes JD, Veazey RS, KewalRamani VN, Lifson JD, Bieniasz PD (2009). A macaque model of HIV-1 infection. *Proc Natl Acad Sci USA,* 106(11), 4425–4429. doi: 10.1073/pnas.0812587106