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Chapter 15

Simian-Human Immunodeficiency Viruses and Their Impact on Non-Human Primate Models for AIDS

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1. Introduction

Non-human primates (NHP) have been indispensable to the study of simian immunodeficiency (SIV)/human immunodeficiency (HIV) infection, pathogenesis, and the development of prophylactic and therapeutic interventions to prevent transmission and progression to disease. A number of SIV and chimeric simian-human immunodeficiency virus (SHIV) challenge stocks have significantly advanced the NHP model, making it possible to identify and better understand factors that influence virus transmission, acute infection, pathogenesis and the eventual progression to AIDS. The development of SHIV recombinant viruses, in particular, has been especially advantageous in that it provides a more relevant research tool for studying properties of HIV-1 infection in a NHP setting. These include HIV-1 envelope characteristics that affect transmission and pathogenesis. SHIV constructs also allow for the evaluation of the efficacy of anti-HIV microbicide formulations and vaccines that are directed against envelope and other critical virus components such as reverse transcriptase. While beneficial, the vast number of virologically-distinct challenge stocks and the growth of the NHP challenge model repertoire to now include rhesus, pigtail and cynomolgus macaques, have collectively introduced an increased level of complexity with regard to experimental design and data interpretation. Furthermore, some virus stocks have virological properties that limit applications in novel areas of drug discovery, prompting the development of new generation SHIV challenge stocks. The purpose of this chapter is to therefore summarize efforts that have been made to characterize both SIV/SHIV challenge stocks and NHP hosts, to highlight the development of new generation SHIV, and how these novel challenge stocks have advanced the SHIV NHP challenge model and anti-HIV drug and vaccine development.

2. Current NHP models: Overview of NHP species origin, susceptibility to infection and pathogenesis/disease course

2.1. Old World NHP natural hosts

As many as 40 NHP species have been identified to be naturally infected with SIV, with each species exhibiting distinct virus lineages that share a considerable degree of genetic identity. Epidemiological and phylogenetic analyses have established that the origin of the HIV-1 and HIV-2 strains is the result of cross-species transmission of the NHP lentivirus equivalents SIVcpz and SIVsm from Eastern/Central African chimpanzees and the West African sooty mangabey, respectively [1-8]. More recently, there was also evidence demonstrating cross-species transmission of a distinct SIV lineage from gorillas [9]. Due to cost and ethical reasons, the study of SIVcpz infection has been limited to small numbers of chimpanzees tracked in the wild or in captivity, with limited opportunities to monitor natural SIVcpz infections. Laboratory-adapted HIV-1 strains were initially tested in chimpanzees and were found to recapitulate some, but not all, aspects of pathogenesis observed in HIV-1 infected humans [10]. Changes in the environmental protection status of this species have led to a halt in invasive studies thereby limiting their research capacity. Experimental SIV infection of sooty mangabeys has also ceased due to their endangered status, but previously acquired samples and animals with existing SIV infections are permitted for study. Two other animal models of natural SIV infection that are available for experimental AIDS research include the African green monkey (AGM), and more recently, mandrills that are native to Gabon [11, 12]. Although initial studies were performed in AGM of African origin, the import of this NHP species to the Caribbean has facilitated their availability, making AGM of Caribbean origin the source of more recent SIV studies. The breadth of research in mandrills is not yet as extensive as that conducted in the other Old World NHP species, thus the majority of information on non-pathogenic SIV infections have been gained from studies in sooty mangabeys and AGM. Research on these natural NHP SIV hosts has collectively revealed what have come to be known as the hallmarks of SIV infection in Old World NHP natural hosts: attenuated anti-SIV immune responses and a typical lack of progression to an AIDS-like disease.

Natural hosts of SIV generally exhibit elevated acute innate and adaptive immune responses in the early phase of infection, followed by a downregulation of Type I interferon responses during the chronic phase of infection [13, 14]. In addition, while humoral immune responses are mounted during SIV infection, these are relatively minimal as demonstrated by the detection of low neutralizing antibody titers in SIV-infected sooty mangabeys and AGM [15, 16]. This immunologic attenuation collectively contributes to limited T-cell apoptosis and maintenance of peripheral CD4+ T-cells even though viral loads comparable to those in pathogenic SIV infection are observed [17-19]. The precise mechanism that triggers the downregulated immune response is unclear, but is likely to involve a combination of proposed processes that include, (1) enhanced responses of immunosuppressive regulatory T-cells and IL-17 producing Th17 cells (2) a robust early innate immune response that is swiftly constrained, and (3) controlled regulation of cellular factors or receptors associated

with activation, apoptosis, or virus binding [20-32]. Furthermore, in contrast to the massive immune depletion that occurs in the gastrointestinal (GI) tract of non-natural hosts of SIV or HIV infection, natural NHP SIV hosts maintain GI epithelial integrity and exhibit a lack of microbial translocation that may in part account for minimal systemic immune activation [33, 34]. Limiting the pool and/or proliferative capacity of target cells may also play a role in disease resistance, as demonstrated by studies in sooty mangabeys in which SIV replication was shown to be restricted to primarily short-lived activated CD4+ T-cells, which likely contributes to the preservation of central memory CD4+ T-cells [35, 36]. A population of double negative (CD4-CD8-) T-cells capable of producing Th1, Th2 and Th17 cytokines have also been identified in sooty mangabeys, and are thought to compensate for CD4+ T-cell helper functions in SIV-infected animals [37].

Although the vast body of evidence points toward a disease-resistant phenotype, a low level of AIDS-like mortalities have been described among natural NHP hosts of SIV. An increasing number of studies suggest that SIV-infected chimpanzees in particular do not necessarily follow the disease-resistant paradigm and can in fact develop AIDS-like symptoms that include depleted CD4+ T-cell counts, reduced fertility in females, low offspring survival rates and increased risk of death following infection [38]. A case of immunodeficiency was also observed in a sooty mangabey that had been naturally infected with SIV for nearly two decades [39]. Collectively, these reports suggest different incubation periods for the SIVcpz and SIVsm lineages and/or that the asymptomatic period in SIV-infected Old World NHP may be longer than what is typically noted for HIV-infected humans. These NHP models have therefore provided valuable insight into both host and virus factors that have co-evolved to result in this attenuated disease phenotype. The slow progression to AIDS-like symptoms, if at all, in these species share important parallels with HIV-1 infected individuals who are long-term non-progressors and with HIV-2 infected individuals who typically exhibit a less severe clinical course , thereby providing clues about protective immune correlates of HIV infection that will undoubtedly influence vaccine and therapeutic design. It has also become increasingly apparent that immune factors alone may not influence the course of disease, as targeted CD4+ T-cell, CD8+ T-cell or CD20+ B-cell in vivo depletion via cell-specific antibody infusion in sooty mangabeys or AGM had negligible effects on viremia and disease progression [40-42], adding an additional layer of complexity and also highlighting that the virus itself needs to be taken into account. Continued research on the intricate interplay between host and virus factors in natural NHP hosts will continue to shed light on mechanisms that may have applications for health preservation in individuals already living with HIV infection.

2.2 .Old World NHP non-natural hosts

While natural NHP hosts of SIV have afforded a wealth of information about non-pathogenic infections, current environmental and ethical laws alluded to above restrict the availability and/or experimental infection of some of these species thereby limiting studies involving SIV transmission and evaluation of early anti-SIV immune responses. Furthermore, the non-pathogenic status of these natural hosts is not applicable to studies

that seek to develop and test new prophylactic and therapeutic tools aimed at preventing and/or treating HIV infections. Such research has however been greatly facilitated by the utilization of Old World NHP macaque species since virus isolates and derivatives of SIVsm and SIVagm readily infect these animals, resulting in a pathologic process that is strikingly similar to that observed in HIV-1 infected individuals who progress to AIDS. Also of note, baboons (*Papio cynocephalus*) are readily infected with HIV-2, and exhibit a disease course resembling the slow progression that is observed in chronic HIV-1 infection in humans [43]. Baboons have therefore proven to be useful in studies evaluating viral latency and clinical stages of the disease. However, due to a number of factors that include differences in the HIV lineages, animal resource availability, and the time to disease development, studies modeling HIV-1 infection and vaccine development have primarily involved macaque species. The degree of susceptibility to infection and severity of disease course is highly dependent on both the macaque species and the challenge virus. Infection of Rhesus macaques (*Macaca mulatta*) with SIVsm isolates and its derivatives, but not SIVagm, typically leads to simian AIDS [44, 45]. Pigtail macaques (*Macaca nemestrina*) succumb to AIDS-like symptoms after infection with SIVsm and SIVmac, with only certain strains of SIVagm, and with SIVl'hoest and SIVsun which are isolates of l'Hoest (*Cercopithecus lhoesti*) and Sun tailed monkeys (*Cercopithecus solatus*), respectively[45-47]. Cynomolgus macaques (*Macaca fascicularis*) are readily infected with SIVmac251, an isolate from a captive rhesus macaque thought to have been infected with SIV from sooty mangabeys, but this species exhibits diminished pathogenicity and lower viremia when compared to SIV infected rhesus macaques [48].

Thus, while there is a fairly broad repertoire of macaques as animal models for AIDS research, their distinct pathogenic outcomes and innate physiological and biological makeup have to be carefully accounted for prior to selection for experimental studies. There is healthy skepticism regarding the extent to which macaques can accurately reflect HIV pathogenesis and predict efficacy of vaccines or other prophylactic tools in humans, and this is especially highlighted by negative results of the vaccine clinical trials AIDSVAX and STEP [49]. However, NHP macaques continue to be the best available model that researchers can utilize to study in vivo host-virus interactions in a system that is similar to HIV infected individuals. Furthermore, macaque models can be utilized to conduct retrospective studies to recapitulate vaccine clinical trials that have been conducted in humans. The most recent Phase IIb vaccine clinical trial, RV144, demonstrated a modest level of protection (31.2%) with a prime-boost platform involving ALVAC HIV (vCP1521) and AIDSVAX B/E gp120 candidate vaccines, and a working group has been set up to identify correlates of protection conferred by this vaccination in macaques in order to compare and contrast degrees of protection and associated protective immune responses . Drawing parallels between macaque models and vaccine trial participants may inform the design of future clinical trials as well as guide the choice of NHP model for prospective pre-clinical studies. The nature of the challenge virus itself has to also be considered. Indeed, as reviewed below, virus stocks utilized in NHP research have grown past SIV to include chimeric SHIV strains as well as simian-tropic HIV-1 strains, to better reflect properties of HIV-1 specific transmission and

associated immune responses, and to facilitate experimental studies on anti-retroviral treatments and anti-HIV vaccines in NHP models. The selection of a NHP model will also require careful thought of the scientific questions being evaluated, the impact of these studies on the design of clinical trials in humans, as well as the cost and availability associated with each macaque species. These considerations are detailed below and are summarized in Table 1.

Macaque model	**Pros**	**Cons**
Rhesus macaques (*Macaca mulatta*)	-intravenous, intra-rectal, intra-vaginal and penile-exposure models established (single high and repeat low dose challenge). - SIV/TB co-infection models. - Well characterized MHC allelic profiles in Indian origin macaques. -Model of choice for vaccine candidates.	-Supply of Indian rhesus macaques dependent on domestic breeding capacity. -Chinese rhesus macaques: Poorly characterized MHC allelic profiles, exhibit low viral loads and not suitable for vaccine studies. -Primarily seasonal breeders: shortage of female macaques, not suitable for comparative menstrual cycle-related SIV/SHIV studies.
Pigtail macaques (*Macaca nemestrina*)	-Vaginal ecology and physiology similar to women. -Well-characterized repeat low dose model of intravaginal virus challenge. -SIV/SHIV and STI co-infection models. -Shows promise as a macaque model utilizing modified HIV-1.	-SIVmac infections typically aggressive and not reflective of HIV-1 infection. -Limited breeding facilities in the US, expensive. -Less established as a model for testing vaccine candidates.
Cynomolgus macaques (*Macaca fascicularis*)	-Smaller in size, easier to handle. -Widely available. -Mauritian origin macaques exhibit high MHC allele homogeneity.	-Smaller size restricts volume and/or frequency of blood and specimen collections. -Exhibit low viral loads. Limited suitability for vaccine studies. -Repeat low dose model not optimized.

Table 1. Summary of advantages and disadvantages of current NHP models for AIDS.

2.3. Research applications and species-specific advantages of macaque models for AIDS

2.3.1. Rhesus macaques (Macaca mulatta)

Rhesus macaques have been extensively used in AIDS research, which was in part facilitated by their availability. These macaques are now less easily obtained in part due to demands for these animals in non-HIV areas of research. This species is highly susceptible to infection with a wide range of SIVmac and SHIV strains via intravenous, intrarectal and intravaginal routes of infection [50-52], and it is perhaps the best characterized NHP model of low dose penile exposure studies [53, 54]. Co-infection models involving SIV and tuberculosis have also been established in rhesus macaques [55]. SIV replicates to high levels in macaques of Indian origin. This can be advantageous in applications that involve stringent testing of vaccine and/or therapeutic efficacy that utilize viral load readouts as primary endpoints. However, the high levels of viremia and relatively rapid decline in CD4+ T-cells lead to simian AIDS in an average of 2-3 years, which is not reflective of the typical rate of pathogenesis in HIV-1 infected humans who tend to develop AIDS over a longer period of 10 years. The comparatively faster disease course in rhesus macaques may underestimate the efficacy of prophylactic or therapeutic interventions in preclinical studies. Nonetheless, this NHP model still has wide applications in vaccine studies since experimental and disease outcomes can be determined in a shorter time frame. Furthermore, certain HIV-1 infected individuals do exhibit rapid disease progression (2-5 years) [56] and thus SIV-infected rhesus macaques could serve useful in this context as well. In humans rapid progression to AIDS and death in individuals homozygous at one or more loci (A, B, and C) and the association of rapid development of AIDS and the presence of HLA class I alleles B*35 and Cw*04 was demonstrated previously [57]. Genes of MHC class I alleles such as HLA B*5701, HLA C and the specific combination of KIR3DS1 with HLA-B alleles that encode molecules with isoleucine at position 80 (HLA-B Bw480I) were associated with an efficient immune control of the kinetics on AIDS progression [58]. Variant genotypes of the chemokine receptors of HIV CCR2 (CCR2-64I) and CCR5 (CCR5-Δ32) in the homozygous or heterozygous states have been implicated in combating the progression of AIDS [59].

The lower viral load and slower decline in CD4+ T-cells observed in most HIV-1 infected individuals is recapitulated better by SIVmac infection in rhesus macaques of Chinese origin[60]. However, there is limited genotypic information on Chinese rhesus macaques [61, 62], with major histocompatibility (MHC) Class I alleles and relevant SIV epitopes being more extensively characterized in Indian macaques [63-66]. MHC class I-restricted CD8+ T-cell responses are a critical component of adaptive immunity that contributes to HIV-1 and SIV control. MHC-typing can be especially informative when selecting cohorts for studies that may require exclusion of animals expressing protective alleles known to confer disease resistance. Paradoxically, the advances in genotyping rhesus macaques have contributed to high levels of demand for animals with specific protective alleles such as Mamu-A*01, driving up their cost and limiting their availability. Homologues of the MHC class I alleles HLA-A and HLA-B exist in rhesus macaques. The high frequency of MHC class I (Mamu-

A*01) in rhesus macaques of Indian origin resulting in the restriction of epitopes in different regions of SIV has been reported [67]. Mamu-A*01 positive rhesus macaques naturally restrict SIVmac251 replication and significantly contain viremia following intrarectal challenge. Significant preservation of absolute CD4 counts but the absence of viremic control was observed in Mamu-A*01 positive macaques upon intravenous infection with SIVmac251 or SIVsmE660 [68]. The ability of Mamu-A*01 positive macaques to restrict SIVmac251 replication at peak and set-point following intravenous challenge was demonstrated recently [69].

Elite controllers or long-term non-progressors have a high frequency of HLA-B27 and HLA-B57. The presence of homologues in rhesus macaques of the above HLA alleles led to the identification of Mamu-B*08 in a high frequency (38%) in a group of macaques defined as elite controllers (geometric mean of chronic phase of plasma viremia is below 1000 copies/mL). The association of MHC class I alleles Mamu-B*17 and Mamu B*29 and Mamu-A*01 with several fold reduction in chronic-phase plasma viral load was established in a group of 181 rhesus macaques infected with SIVmac239 [70].

Although the genotype of rhesus macaques has been well characterized, this species has limitations in studies involving SIV/SHIV infection and the reproductive cycle. Rhesus macaques are seasonal breeders [71], with female macaques exhibiting irregular menstrual cycles during non-breeding periods. These reproductive patterns put further restrictions on the general availability of female macaques and their applications in SIV/SHIV studies involving the role of the female reproductive tract and/or hormonal cycle on virus infection. Some researchers have circumvented the problem of irregular cycling by using Depo Provera, a progestin-based contraceptive, to thin the vaginal wall of rhesus macaques and to generate a prolonged luteal phase-like state that allows for consistent vaginal infections. An in depth description of this is provided below, in the section detailing Routes and Dose of Virus Inoculation.

2.3.2. Pigtail macaques (Macaca nemestrina)

In recent years, pigtail macaques have increasingly become an alternative NHP model for AIDS research. However, recent closures of several breeding facilities in the US have created logistical challenges for their acquisition and resultant increased cost. The benefit of this macaque model is that this species is readily infected with SIVmac, SIVagm, and SHIV strains [72]. SIVmac-infected pigtail macaques tend to progress rapidly to AIDS and can potentially develop thrombocytopenia [136] which is a common autoimmune disease that can also manifest in untreated HIV-1 infected individuals. Pigtail macaques in some breeding colonies may exhibit certain pre-existing immunologic conditions such as compromised mucosal integrity, increased microbial translocation and lower levels of naïve and central memory CD4+ T-cells,have been described [34, 73]. SIV/SHIV infected pigtail macaques also exhibit considerable variability in set point viral loads which is a trend that is noted in HIV-1 infected humans. This may be partially dependent on host genetics, which in part prompted the study of MHC Class I alleles in this macaque species (Mane). To date, 16

Mane-A and 22 Mane-B MHC Class I alleles have been identified, and further characterization of the frequency and distribution of at least 10 of these alleles was performed in pigtail macaques of Indonesian, North American, and Australian origin [74, 75]. New alleles continue to be identified as the genetic characterization of pigtail macaques progresses, and this is likely to increase their application in vaccine research.

Although pigtail macaques have not served as the primary NHP model for HIV vaccine studies, other similarities that are shared by this species and humans have encouraged their use in other areas of AIDS research. One of the main advantages of pigtail macaques is that the females exhibit continuous lunar menstrual cycles as do women [76], making this species particularly valuable for studies examining the impact of the menstrual cycle and accompanying changes in the vaginal environment on SIV/SHIV susceptibility. Research on the role of the female reproductive system in HIV acquisition and transmission is especially critical given recent findings that demonstrated a higher risk for women receiving the Depo Provera synthetic progesterone injection for the purpose of birth control [77]. Indeed, studies in pigtail macaques have demonstrated a similar increase in susceptibility to infection during the late luteal phase of the menstrual cycle when progesterone levels are high, which is when thinning of the vaginal epithelium, reduced local immunity and other factors conducive to virus infection occur [78]. Furthermore, pigtail macaques are a well-characterized model for repeated low dose SHIV challenge studies involving the intravaginal route [79] which are more reflective of infectious HIV doses that are mucosally transmitted in humans. In addition, a co-infection model has been developed using this species, allowing for the study of sexually transmitted infections in the context of SHIV infection [80].

An intriguing feature of pigtail macaques is that they are partly permissive to HIV-1 and HIV-2 infection although virus replication and persistence are transient in vivo [72]. Nonetheless, the implication that this macaque species could potentially serve as a primate model that utilizes HIV strains as the challenge virus is incredibly appealing given that current models depend on SIV or SHIV viruses that exhibit enough divergence from HIV-1 to impede their applications in certain preclinical studies. The discovery that pigtail macaques carry a variant form of the host restriction factor TRIM5alpha [81-83] that fails to inactivate incoming HIV particles has led to the design of new generation recombinant SHIVs (described below) that exploit this feature, opening doors for the application of this macaque species as a challenge model in studies evaluating a number of pre-exposure prophylaxis (PrEP) approaches targeting HIV-1.

2.3.3. Cynomolgus macaques (Macaca fascicularis)

Like pigtail macaques and humans, cynomolgus macaques also have monthly menstrual cycles. While high dose virus challenge is widely used in these macaques, they are less well characterized for the repeat low dose challenge model. Cynomolgus macaques are small and easier to handle than rhesus or pigtail macaques, but this can restrict peripheral blood sampling volumes and the frequency of other specimen collections. Perhaps one of the

biggest advantages of this NHP species is that they are more widely available, with the Indian Ocean island, Mauritius, being its largest exporter. A caveat of cynomolgus macaques in AIDS research is that in order to establish a pathogenic SIV infection, a higher inoculation dose is required, at least when challenged mucosally, and the resulting viral loads are typically lower than levels observed in rhesus macaques, and are more similar to those noted in HIV-infected humans [84]. This can pose a problem for vaccine studies that depend on virus load reductions as end points. However, due to the natural geographical isolation of the Mauritian species, cynomolgus macaques exhibit a rather homogeneous genetic profile, with the majority of animals possessing the allele combination Mafa-B*430101, Mafa-B*440101 and Mafa-B*460101 [85, 86]. This degree of MHC identity can be immensely beneficial to vaccine studies that require evaluation of CD8+ T-cell immune responses to defined viral epitopes. Furthermore, the well characterized and limited diversity of MHC alleles in this macaque species allows for their application in studies to evaluate non-MHC correlates of protection. As with rhesus macaques, an SIV/tuberculosis model has also been established in cynomolgus macaques, with this species being particularly informative with regard to latent/reactivated tuberculosis [87].

3. SIV/SHIV challenge stocks

3.1. SIV strains utilized in AIDS research: Origins, phylogeny, characteristics and applications

To date, at least seven distinct lineages of the primate lentivirus SIV have been identified [5, 88-94], and these share up to 50% identity in Gag and Pol proteins, which are the most conserved and encode structural and enzymatic viral proteins, respectively. The genomic organization for SIV lineages is generally LTR-gag-pol-vif-vpr-tat-rev-env-nef-LTR, but some differences exist, with the vpu gene being unique to SIVcpz and HIV-1 and a number of strains from Cercopithecus monkeys . However, SIVsm, HIV-2 and SIVmac strains harbor a vpx gene upstream of vpr. Genes expressing Vpr or Vpu are absent in the all other SIV lineages that include SIVagm.

Sooty mangabey SIV is the origin of most virus challenge stocks for studies involving NHP non-natural hosts, although SIVagm.sab from the AGM species sabaeus has also been used to infect rhesus macaques in a number of studies. Commonly utilized SIV strains are listed in Table 2. The ‘parent’ SIVmac strains, SIVmac251 and SIVmac239, have been derived from rhesus macaques that are thought to have to been infected by SIV+ sooty mangabeys [95-98]. SIVmac251 is a swarm, containing different quasispecies, that was isolated from a lymphoma of an infected rhesus macaque. Further passage of this isolate through additional macaques yielded a clonal stock , SIVmac239. The SIVmac316 clone was generated in a similar manner following passage of SIVmac239 [99]. Several other isolates, either swarms or clones, were derived from the plasma or PBMC of sooty mangabeys that were passaged through rhesus macaques, or were expanded in cell lines in vitro. It is also important to note that several attenuated SIV strains, primarily from the SIVmac239 and SIVmac251 lineages, have been designed for the purposes of vaccine research. Live attenuated strains, that

include SIV-Δvpr, SIVmac239Δnef, and SIVmac251Δnef, have been utilized to study protective effects against intravenous or mucosal challenge with heterologous or homologous virus stocks in rhesus macaques (reviewed in [100]). While the risk associated with a live attenuated HIV vaccine precludes use in humans, vaccine studies in macaques serve to provide an understanding of the basis of protection that is conferred by attenuated strains, by shedding light on immune memory mechanisms and virus targets that could be applied in HIV vaccine design.

Strain	**Source**	**Stock composition**	**References**
SIVsmE660	Passaged in rhesus macaques originally infected with SIVsmE038	Swarm	[236]
SIVsmE543 SIVsmE543-3	Passaged in rhesus macaques originally infected with SIVsmE038	Swarm -Clone	[112]
SIVmac251	Rhesus macaque isolate	Swarm	[95, 97, 98]
SIVmac239	Passaged in rhesus macaques infected with SIVmac251	Clone	[95, 96]
SIVmac316	Passaged in rhesus macaque infected with SIVmac239	Clone	[99]
SIVagm.sab92018	African green monkey plasma and PBMC isolate	Swarm	[12, 18]
SIVPBj14 SIVsmPBj6.6 SIVsmPBj6.9	Rhesus macaque isolate originally infected with SIVsm9	Swarm -Clone -Clone	[237, 238]
SIVmne SIVmneCl8 SIVmne170 SIVmne027	Pigtail macaque lymph node isolate	Swarm -Clone -Clone -Clone	[230, 231, 239-243]

Table 2. Commonly utilized SIV strains.

Although the SIVmac and SIVsm strains share common ancestry, differences in the source, number of animal passages and/or laboratory in vitro propagation techniques can confer distinct virological properties, such as replicative capacity and pathogenicity, to each

challenge stock. The selection of a challenge stock for any study therefore requires careful consideration of these virus-specific characteristics. SIVmac239, being a molecular clone, allows for better experimental reproducibility, and the nature of escape mutations from CD8+ T-cell responses are well defined for this stock. However, a clonal virus stock is not representative of human exposures where a number of quasispecies exist per exposure. This issue can be circumvented by utilizing swarm virus stocks such as SIVmac251 and SIVsm strains. These viruses are typically more aggressive but can serve as stringent challenges in vaccine studies. However, as mentioned earlier, their high pathogenicity could also underestimate the efficacy of vaccines and other prophylactic interventions. Furthermore, lab-specific propagation techniques can affect swarm challenge stocks, such as SIVmac251, leading to variations in the composition of quasispecies within what should in theory be the same stock. It is unclear if these differences can significantly affect the infectivity and course of pathogenesis in macaques, but nonetheless highlights the importance of addressing the phylogeny of challenge stocks used in NHP experiments.

Phylogenetic analysis of challenge stocks has greatly advanced since the advent of single genome amplification (SGA) which accurately determines the number and nature of viral quasispecies during the stages of transmission, acute, and chronic infection . This technique has demonstrated that the majority of mucosal HIV-1 infections (60-90%) originate from a single virus variant [101, 102]. This phenomenon, termed the genetic bottleneck, does not necessarily apply to high-risk individuals, who are typically infected with a more heterogeneous population that also correlates with more rapid disease progression [103-105]. An in-depth understanding of lentiviral phylogeny can therefore contribute immensely to the design of preventative strategies aimed at viral variants that are transmitted and go on to establish infection. Importantly, the features of HIV-1 transmission and early diversification are mirrored in SIV-infected macaques. SGA analysis of swarm challenge stocks SIVmac251 or SIVsmE660, and isolated virus soon after intra-rectal or intravaginal inoculation of macaques have demonstrated the presence of low diversity env sequence lineages that share a high level of genetic identity to the env spectrum in the challenge stock [53, 106, 107]. Indeed, it was found that a limited number of transmitted variants (1-10 species) establish infection, thus offering strong support for and confirmation of the observed patterns of HIV-1 transmission. Studies have also been performed on SIV evolution in the male genital tract of rhesus macaques, with results demonstrating similar virus sequence distribution in the blood and semen at peak viral load, while a compartmentalization of quasispecies begins to develop after set point [108]. Thus, SIV phylogenetic studies of challenge stocks and transmitted strains combined with knowledge of the dose and timing of transmission allows for more defined evaluation of virus evolution. In addition to shedding light on virus factors influencing transmission, analysis of SIV by SGA, or other next generation sequencing methods, can help identify variant-specific cellular or humoral responses generated by the host at early and late stages of infection.

Some SIV strains, such as SIVmac251, SIVmac239, and SIVsmE543-3, can be resistant to neutralizing antibodies, restricting their application in vaccine studies designed to elicit

humoral immune responses [84, 109-114]. In contrast, SIVmac316 is sensitive to antibody neutralization while others, that include SIVsmE660, demonstrate variable sensitivity [109, 110]. Furthermore, while neutralizing antibodies may be produced in response to some of these viruses following infection, successful control of viral replication is still not achieved. Given these variations, evaluation of neutralizing antibody responses typically involve a tiered approach, and patterns of sensitivity are defined for viruses based on whether there is very high (Tier 1A), above average (Tier 1B), moderate (Tier 2), or low (Tier 3) sensitivity to antibody-mediated neutralization [115, 116]. Although levels of elicited humoral immune responses are inconsistent among the various challenge stocks, a close evaluation of these differences can help delineate molecular determinants of neutralization.

A unique characteristic of SIV strains is that CCR5 is the primary co-receptor of choice, with few utilizing CXCR4, and this CCR5-specificity is reflective of the majority of HIV-1 strains. Furthermore, a number of alternate co-receptors have also been identified for SIV, including GPR15, STRL-33, GPR-1, ChemR23 and CCR8 [117, 118]. The affinity for one or more of these co-receptors varies for each challenge stock. In addition to co-receptor usage patterns, several of the commonly utilized SIV strains, such as SIVmac251 and SIVmac316, are M-tropic, and SIV-infected macaques switch from M-tropism (macrophages, memory or activated CD4+ T-cells) to dual or T-tropism (naïve/resting and memory T-cells) during infection, as do many HIV-infected individuals [119].

Given that the cellular tropism of SIV strains affects cell populations other than CD4+ T-lymphocytes, such as macrophages and dendritic cells, NHP macaques are also utilized to address other aspects of SIV pathogenesis. HIV-1 infection in humans has been shown to cause complications that include encephalopathy, neurological diseases, interstitial pneumonia, and nephropathies [120]. Some of these pathologies have been successfully modeled in SIV-infected rhesus and pigtail macaques, and studies have focused on elucidating viral determinants of macrophage tropism, since the infection of this cell population was observed in perivascular, meningeal and microglial cells, and in alveolar macrophages, which contribute to neurological diseases and interstitial pneumonia, respectively [121]. However, macrophage tropism alone is not sufficient to cause these diseases, and these pathologies only manifest in a fraction of SIV-infected macaques or HIV-infected humans. Efforts to determine host and virus factors that influence the development of these diseases are ongoing.

While SIV strains have a wide range of applications in NHP macaque models of AIDS, the genetic, structural and antigenic differences between SIV and HIV-1, particularly in the virus envelope (Env), pose limitations in areas addressing cellular tropism or co-receptor affinity, antibody neutralization, and immune-driven evolution and adaptation of Env. These differences in Env and other viral components can restrict the utility of SIV challenge models when evaluating Env-based vaccine strategies, or when testing methods of PrEP that employ entry inhibitors and/or post-entry inhibitors. To circumvent this issue, chimeric simian/human immunodeficiency viruses have been developed to create challenge stocks that better mimic the infectivity and pathogenic properties of HIV-1 in a macaque model setting.

3.2. SHIV challenge stocks

3.2.1. Strains, transmissibility, and in vivo virological characteristics

The similarity in the genetic organization and composition of HIV-1 and SIV make it possible to construct replication-competent recombinant viruses that exhibit properties of both lineages. The genetic backbone of the majority of SHIV strains is SIVmac239. These viruses have been engineered to contain not only HIV-1 env, but also genes encoding Tat, Rev, Vpu, Vpr, Nef, integrase and/or reverse transcriptase. Initial SHIV constructs were found to have attenuated pathogenesis in macaques compared to parental SIVmac strains. However, the isolation of variants obtained from serial passage and in vivo adaptation in macaques yielded challenge stocks that variably increased their pathogenicity. The dual-tropic chimera SHIV89.6 that was originally developed by Reimann et al, contains the env gene from cytopathic primary patient isolate HIV-1 89.6, and although CD4+ T-cell loss and some degree of persistent infection was observed following intravenous inoculation in rhesus macaques, no disease developed [84]. In contrast, serial transfusion of peripheral blood from a rhesus macaque infected with SHIV89.6 yielded more pathogenic variants, SHIV89.6P (isolated from PBMC, LN and spleen) and SHIV89.6PD (plasma-derived), that had primarily CXCR4-tropism and resulted in higher viral loads and CD4+ T-cell decline, as well as simian AIDS [84, 122]. This SHIV construct and its derivatives have since been utilized to decipher host and virus factors influencing transmission and early T-cell and antibody responses following intravenous and intravaginal inoculation in macaques, and have also been applied in a number of pre-clinical vaccine trials. However, the suitability of HIV89.6P as a challenge virus in rhesus macaque models, particularly when evaluating vaccine candidates, has been called into question given their CXCR4-tropism. The affinity for the CXCR4 co-receptor allows for infection of naïve CD4+ T-cells which has a major impact on the kinetics of CD4+ T-cell depletion, resulting in rates of lymphopenia that are not reflective of that caused by HIV-1 and most SIV strains that are CCR5-tropic.

Another CXCR4-specific construct, SHIVSF33, which encodes Env from the patient PBMC isolate HIV-1SF33, exhibited similar properties in that the original molecular clone was minimally virulent, with in vivo adaptation in rhesus macaques cells yielding a more pathogenic isolate termed SHIVSF33A [123, 124]. This virus stock resulted in productive infections in rhesus macaques via both intravenous and intravaginal routes of challenge. The increased virulence of these SHIV derivatives were mapped to distinct amino acid changes throughout Env. Indeed, certain CXCR4-tropic constructs, such as SHIVku-1 which is a pathogenic variant derived by sequential passage of SHIV-4 (Table 2) in pigtail macaques leads to CD4+ T-cell depletion within 4 weeks of infection and simian AIDS in as early as 8 months [125]. However, this particular construct and its rhesus macaque-passaged counterpart SHIVku-2, lead to productive infection in the central nervous system and glomerulosclerosis of the kidney [126, 127], and so have applications in modeling neuropathogenesis and renal diseases that manifest in some HIV-1 infected individuals.

Thus, despite the drawbacks of utilizing CXCR4-tropic SHIV in vaccine studies, these chimeric constructs are still highly relevant in other areas of AIDS research. For instance, the

infection of cynomolgus macaques and rhesus macaques of Chinese origin with SHIV89.6 results in a robust acute immune response, lower viremia, slower decline in CD4+ T-cells, and general maintenance of virus-specific immune responses and prolonged survival [84], similar to the scenario in HIV-1 infected humans. The early robust cell-mediated responses to SHIV89.6P infection and subsequent reduction in viral replication offer clues about immune correlates of protection and help determine prophylactic or therapeutic interventions aimed at inducing strong immune responses during the acute infection period. Furthermore, the transmission of SHIV89.6P, SHIVSF33 and SHIVku-1 constructs via the vaginal route in macaques demonstrate that CXCR4-utilizing virus strains can successfully cross the cervicovaginal mucosa to result in persistent viremia, CD4+ T-cell loss and simian AIDS [128-130]. Thus, although there is a higher prevalence of CCR5-tropic transmitted HIV-1 variants, these SHIV constructs can be utilized to address early T-tropic pathogenesis, as well as prophylactic and/or therapeutic strategies aimed at CXCR4 HIV-1 variants.

SHIV CCR5-tropic chimeric viruses have been developed in recent years. One of the best characterized of these is SHIV162 [124]. Recombinant virus was generated by replacing the tat, rev and env genes of SIVmac239 with those of HIV-1SF162. Intravenous challenge of rhesus macaques with SHIV162 yielded lower viral loads than the parental SIVmac239 strain, and although viremia persisted for over a year, immunodeficiency did not develop in any of the animals under study. However, three sequential blood-bone marrow transfusions in naïve rhesus macaques resulted in pathogenic variants termed SHIV162P3 and SHIV162P4 [131], the former being isolated from lymph node mononuclear cells. Infection of macaques with these in vivo adapted strains leads to peak viral loads of 10^6-10^8 viral RNA copies/ml plasma, viral set points of 10^3-10^6 viral RNA copies/ml, a gradual CD4+ T-cell decline, severe weight loss, and opportunistic infections, which are reflective of HIV-1 infection in humans. Importantly, productive infection via intrarectal, intravaginal or intravenous routes can be consistently obtained in rhesus, pigtail and cynomolgus macaques, therefore making this virus chimera a useful tool in a wide range of studies that involve host-specific immune responses and/or prophylactic or treatment regimens targeting HIV-1 envelope protein.

Another clade B chimera that productively infects rhesus, pigtail and cynomolgus macaques is SHIV Ba-L, which expresses tat, rev, vpu and env genes from R5-tropic HIV-1 Ba-L [132]. However, HIV-1 Ba-L is a laboratory adapted strain which may not truly reflect virologic properties of primary isolates. Furthermore, virus persistence of SHIV Ba-L is comparatively shorter (42 weeks) than has been observed for SHIV162P3/P4. A dual R5- and X4-tropic Clade B chimera, SHIVDH12 replicates to high titers and causes immunodeficiency in pigtail macaques, while its derivative SHIVDH12R induces CD4+ T-cell loss in rhesus macaques [133]. However, mucosal transmission of this construct has not been described. Nonetheless, the utilization of these recombinant viruses, which encode HIV-1 subtype B Env, has collectively contributed greatly to our understanding of host and viral determinants influencing the transmission of an HIV-1 clade that is highly prevalent in North America and Europe. However, diverse HIV-1 genotypes exist due to the high genetic variability of this virus, leading to classification of distinct classes (Group M, N and O) and

subtypes based on env or gag nucleotide sequence comparisons. Group M is the major class and consists of 9 subtypes (A-D, F-H, J and K) that collectively constitute greater than 90% of HIV infection cases across the globe. Clades A, C, and D are predominant subtypes in sub-Saharan Africa, with Clade C being prevalent also in India and China. CRF01_AE is widespread in Thailand and neighboring regions of Southeast Asia. Recombinants of HIV-1 have also been identified in areas with populations infected with two or more subtypes. Thus, SHIV constructs have been tailored to encode clade-specific Env proteins in order to develop virus and macaque models that better reflect HIV transmission that is local to these regions.

Strain & derivatives	**SIVmac components**	**HIV-1 components**	**Source HIV-1 Clade**	**Tropism**	**Reference**
$SHIV_{89.6}$ $SHIV_{89.6P}$ $SHIV_{89.6PD}$	*gag, pol, vif, vpx, vpr, nef*	*env, tat, vpu, rev* from 89.6/HXBc2	B	X4/R5X4	[122]
$SHIV_{SF33}$ $SHIV_{SF33A}$	*gag, pol, vif, vpx, vpr, nef*	*env, tat, rev, vpu* from SF33	B	X4	[124]
SHIV-4 $SHIV_{KU-1}$ $SHIV_{KU-2}$	*gag, pol, vif, vpx, vpr, nef*	*env, tat, rev, vpu* from HXBc2	B	X4	[229]
$SHIV_{162}$ $SHIV_{162P3}$ $SHIV_{162P4}$	*gag, pol, vif, vpx, vpr, nef*	*env, tat, rev, vpu* from SF162	B	R5	[124]
SHIV Ba-L	*gag, pol, vif, vpx, vpr, nef*	*env, tat, rev, vpu* from Ba-L	B	R5	[132]
$SHIV_{DH12}$ $SHIV_{DH12R}$	*gag, pol, vif, vpx*, 20% *vpr*	80% *vpr* from NL43 and DH12; *tat, rev, env, vpu, nef* from DH12	B	R5X4	[133, 232]
$SHIV_{1157i}$ $SHIV_{1157ip}$ $SHIV_{1157ipd3n4}$ $SHIV_{ipEL}$	*gag, pol, vif, vpx, vpr, nef*	*tat, rev, vpu* from HXBc2; *env* from 1157i isolate from Zambian infant	C	R5	[134-136]
$SHIV_{CHN19}$	*gag, pol, vif, vpx, vpr, nef*	*env, tat, vpr, vpu, rev* from CHN19	C	R5	[137]
SHIV-E-CAR SHIV-E-P4	*gag, pol, vif, vpx, vpr, nef*	*rev, tat* and *env* ectodomain from CAR-402; *rev, tat* and *env* TM domain from SF33	E	X4	[228]

Table 3. List of common SHIV recombinant viruses and their genetic composition.

The chimera SHIV-1157i is an infectious molecular clone that encodes Env from an R5 clade C HIV-1 strain that was isolated from a 6 month old Zambian infant born to an HIV+ mother. As can be expected, the in vivo passage of this recombinant virus in rhesus macaques yielded a pathogenic isolate, SHIV-1157ip, which induces simian AIDS but at a relatively slow rate [134]. A clonal derivative, SHIV-1157ipd3N4, was designed to contain an additional NF-KB site to accelerate viral replication and also contains the 3' half of provirus isolated from the PBMC of a SHIV-1157ip infected rhesus macaque that progressed to simian AIDS [135]. This construct was found to exhibit a Tier 2 neutralization phenotype. A Tier 1 SHIV derivative, SHIV-1157ipEL, was generated to encompass both the neutralizing-sensitive Env from SHIV-1157ip and the increased replicative capacity of SHIV-1157ipd3N4 [136]. The passaged virus, SHIV-1157ipEL, retains its R5 tropism, is mucosally transmissible and induces a pathogenic profile that is consistent with HIV infection. SHIV strains with different sensitivities to neutralizing antibodies can also provide a tiered testing platform for humoral-based vaccine candidates. Another Clade C recombinant virus, SHIVCHN19, encodes Env from an HIV-1 isolate local to China [137]. The passaged virus exhibits viral loads of up to 10^9 vRNA/ml of plasma in pigtail macaques and persists for 28-31 weeks, but its pathogenicity in rhesus macaques requires further characterization. This and other SHIV constructs are summarized in Table 3.

The sizeable repertoire of studies involving SHIV recombinant viruses has revealed a number of characteristics about these chimeras. First, they are highly versatile and can be engineered to reflect viral envelope properties of a wide range of HIV-1 subtypes. Second, these viruses have varying levels of transmissibility, pathogenicity and host specificity, thereby providing investigators with options that can be tailored to fit their research interests and available resources. These SHIV constructs also successfully establish infections when applied in repeat low dose models. Finally, despite pathogenic effects in some macaques, SHIV viremia in many of these animals is typically controlled or is ultimately cleared due to either intrinsic replicative properties of the recombinant virus and/or early robust host cell-mediated responses or neutralizing antibodies. While this may limit their application in some studies, SHIV constructs are still tremendously useful for evaluating prophylactic strategies aimed at preventing mucosal virus transmission, and for examining viral evolution in the context of prophylactic or therapeutic regimens during the acute infection period. The inclusion of env from different HIV-1 subtypes in these recombinant viruses has shed light on envelope-related factors such as cellular tropism that influence transmission, as well as host immune defense mechanisms that are required to inhibit envelope-mediated entry and infection. However, the constantly evolving nature of HIV-1 env poses a challenge for certain existing prophylactic methods, prompting a focus on other virus components as potential drug targets. This has yielded a new generation of SHIV chimeras that allow for preclinical evaluation of a wide range and/or combination of antiviral drugs.

3.2.2. New generation SHIV recombinants

While the SHIV constructs designed thus far have contributed significantly to the field of NHP preclinical AIDS research, the genetic distance between these recombinant viruses and

HIV-1 is still substantial (up to 70%), which makes it difficult to definitively predict the efficacy of proof-of-concept studies in NHP models in downstream clinical trials. Furthermore, emerging drug resistance and/or drug toxicities underscore the need for alternate targets and novel anti-retroviral (ART) drugs. To facilitate these studies in NHP models, SHIV recombinant viruses encoding one or more HIV-1 genes in addition to env have therefore been developed and will be highlighted in this section.

Current HIV-1 treatment regimens involve nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) either alone or in combination with other ARTs. The reverse transcriptase (RT) protein of HIV-1 shares approximately 60% identity with that of SIV. For this reason, NNRTIs, which exhibit high specificity for HIV-1 RT, do not effectively inhibit SIV or SHIV chimeras that contain SIV RT. To overcome this limitation, the construct RT-SHIVHXB2 in which the entire RT of SIVmac239 was replaced with RT from the HXB2 clone of HIV-1 IIIB, was designed [138, 139]. The initial construct exhibited a severe impairment in replicative capacity, but this was significantly improved by introducing a T to C substitution at position 8 of the SIV tRNA primer binding site. The resulting chimera has been shown to replicate to high levels (10^5-10^7 vRNA copies/ml) following intravenous or intra-rectal challenge in rhesus macaques, or intra-vaginal challenge in pigtail macaques both with and without Depo Provera treatment. In addition, RT-SHIVHXB2 also exhibited sensitivity to a number of NNRTIs (efavirenz, nevirapine and UC781) and NRTIs (tenofovir and emtricitabine) both in vitro and in vivo. Similar studies have been performed in pigtail macaques utilizing RT-SHIVmne, which contains HIV-1 RT in the genetic background of SIVmne, a pathogenic isolate from the lymph node of an infected pigtail macaque [140]. Importantly, plasma virus isolates from macaques infected with either RT-SHIVHXB2 or RT-SHIVmne, and treated with NRTIs/NNRTIs, contained genetic mutations known to confer drug resistance such as K65R (tenofovir), V108I (efavirenz,) and K103N and M184I (emtricitabine), making these chimeras useful tools for the preclinical evaluation of in vivo drug resistance [140-143].

Newer prevention and treatment strategies typically employ ART combinations that target two or more components of HIV-1. The utilization of RT and entry inhibitors, in particular, has gained momentum since this approach targets the virus at both early and post-entry stages of its life cycle, potentially increasing efficacy. This combined method has been difficult to model in macaques since the majority of SHIV constructs, containing either Env or RT from HIV-1, have limitations in evaluating certain combination drug strategies for prevention or treatment. To facilitate this line of study in NHP models an RT Env SHIV construct which used RT-SHIVHXB2 and SHIV162P3 as templates to generate a chimera containing both RT and Env from HIV-1was developed [144]. In vivo passaging in rhesus macaques yielded a virus stock that infects intravenously, intra-rectally or intra-vaginally (without Depo Provera treatment). Viral loads of 10^6-10^7 vRNA copies/ml are observed, with viremia being detected up to 20 weeks post-challenge [145]. Furthermore, this virus retains sensitivity to both RT and entry inhibitors that include the NNRTI dapivirine, the NRTI tenofovir, and the CCR5 antagonist maraviroc. In vitro testing of dual drug combinations indicated additive inhibitory effects on RT Env SHIV replication. Collectively, these studies

open the door for further in vivo applications of this chimera in macaques and allow for the evaluation of dual drug combinations in prophylactic and treatment strategies.

In order to stay one step ahead of drug resistance mutations that develop, ARTs targeting components other than RT and Env have also been developed. These include a broad range of HIV protease inhibitors (PIs), fusion inhibitors and integrase inhibitors. The recombinant virus SHIV-pr, which contains the pol segment encoding protease from HIV-1 NL432, replicates to levels of 10^6-10^7 vRNA copies/ml following intravenous challenge in rhesus macaques, reaching a set point of approximately 10^5 copies/ml with infection persisting for up to 12 weeks [146]. Importantly, in vivo viral load was lowered to or below the detection limit when macaques were treated with a combination of the PIs lopinavir and ritonavir. However, further characterization of this virus stock in mucosal transmission applications and in other NHP models is required.

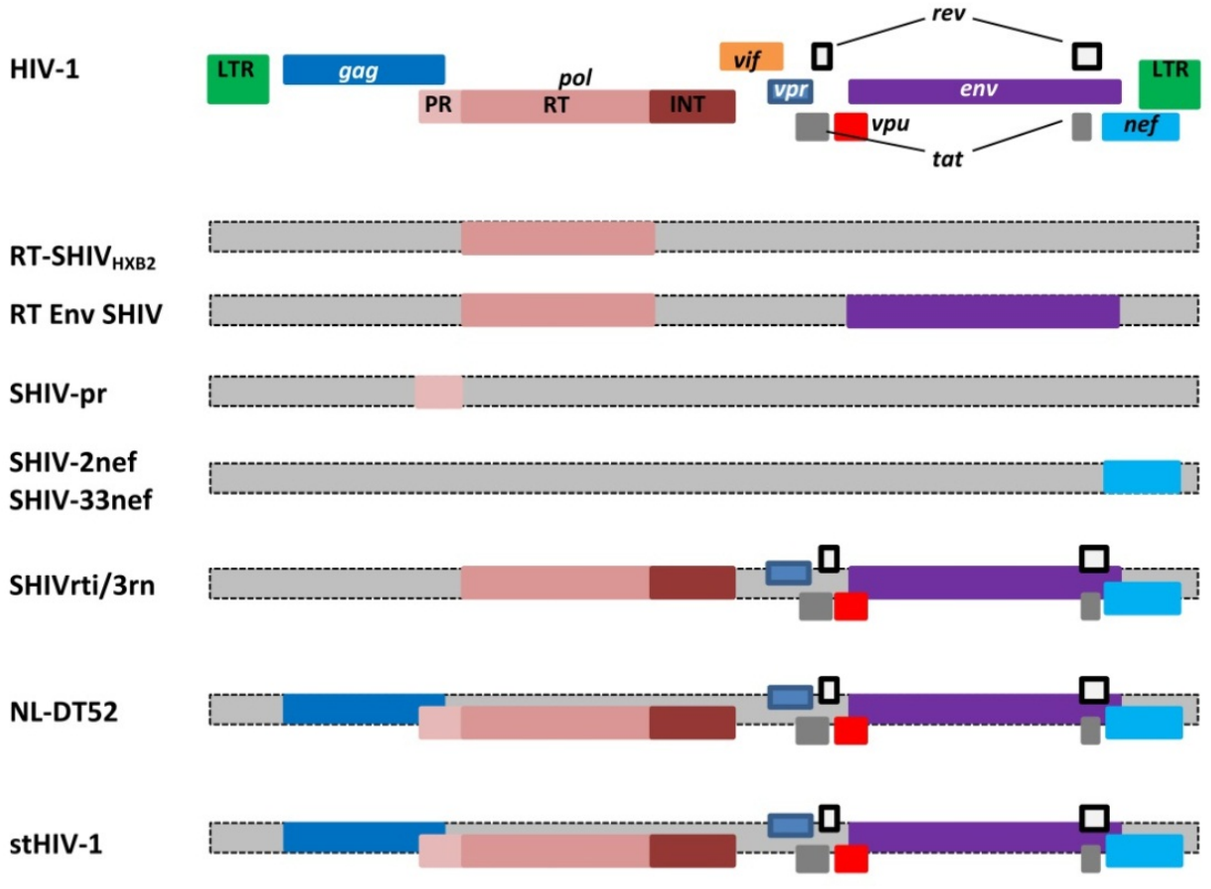

Figure 1. New generation SHIV constructs. The schematic illustrates HIV-1 genetic components in an SIV background (pale grey) for the various SHIV recombinants.

In recent years, scientists have worked toward generating recombinant SHIV constructs that are minimally divergent from HIV-1, such that they share greater than 90% genomic identity. This would undoubtedly expand the utility of these chimeric viruses and would also reduce the complexity of data interpretation when comparing prophylactic and

treatment studies in NHP macaque models and human clinical trials. However, the creation of such chimeric viruses has proven to be difficult with the major reason being innate restriction factors of macaque cells that inhibit HIV-1 replication. In particular, TRIM5alpha and APOBEC3 proteins in rhesus macaque cells prevent HIV-1 infection, while the capsid and Vif protein sequences of HIV-1 enable it to overcome the human forms of TRIM5alpha and APOBEC3. Interestingly, pigtail macaques express TRIMCyp, which is a fusion protein of CypA and TRIM5, and does not inhibit HIV-1 transmission and replication [81-83]. However, HIV-1 replication is not sustained in vivo in these macaques. Some degree of persistent viremia was noted for two modified HIV-1 constructs, SHIVrti/3rn and NL-DT5R, in pigtail macaques. SHIVrti/3rn contains the reverse transcriptase and integrase-encoding regions of HIV-1 in addition to the 3' half genomic region of HIV-1, while NL-DT5R is an HIV-1 derivative that contains sequences encoding a 7 amino acid segment of capsid protein and the entire vif gene from SIV. Persistent replication of both these strains in pigtail macaques was attributed to replacement of HIV-1 vif with that from the HIV-2/SIVsm/SIVmac lineage. However, replication levels were low and plasma viremia was cleared by 5-12 weeks post-infection. In vivo treatment with a CD8+ T-cell-depleting antibody was also necessary to establish infection in some of the macaques. A promising study by Hatziioannou et al described the generation of simian-tropic (st) HIV-1 strains that not only persisted for greater than 20 weeks after intravenous inoculation, but also replicated at levels comparable to that in HIV-infected humans (10^5-10^6 vRNA copies/ml plasma) [147, 148]. Furthermore, the stHIV-1 strains demonstrated sensitivity to a wide range of RT and protease inhibitors in vitro, and the PrEP application of a tenofovir/emtricitabine/efavirenz triple drug combination in two naïve pigtail macaques demonstrated protection against a high dose intravenous challenge by stHIV-1. In depth characterization of the stHIV-1 model is still necessary, but results thus far show promise for its application in candidate vaccine studies and alternative PrEP research involving drug combinations. A limitation of the modified stHIV-1 constructs described above is that they encode a Clade B env that is primarily CXCR4-tropic and so do not model the dominant CCR5-mediated mode of transmission. More recently, a study by Humes and Overbaugh described the generation of HIVAQ23/SIVvif, which is a CCR5-tropic subtype A HIV-1 molecular clone encoding the vif gene from SIVmac239 [149]. Two adaptive mutations in Env were found to confer increased infectivity and replication in pigtail macaque cells in vitro. Thus, while further in vivo characterization of this modified HIV-1 construct is necessary, it is clear from the studies described above that the SHIV molecular virology field is making great strides in generating strains that not only maximally mimic transmitted HIV-1 strains but are also viable tools that can be implemented in vivo in NHP macaque models. Figure 1 summarizes the genomic content of the new generation SHIV/modified HIV-1 constructs.

3.2.3. Routes and dose of virus inoculation

Researchers have struggled for decades to mimic human HIV transmission and pathogenesis in animal models. A wide variety of combinations of NHP species, route and

dose of infection, and recombinant viruses have been established over the last few decades. The specific combination to be used by an individual researcher is determined primarily by the research question being addressed. The varying degrees of resistance to different mucosal routes of infection with SIV or SHIV make the process even more complex. This is quite pronounced in vaginal transmission models. Unlike the rectum, the vaginal cavity has naturally evolved to resist and fight pathogens like bacteria and viruses and foreign material introduced via intercourse. The existence of multiple layers of the squamous epithelium in the vagina, innate immune factors, vaginal microflora, and mucus are some of the factors that occur in vivo to protect the female reproductive tract against sexually transmitted infections.

Earlier NHP models used supraphysiological doses that are far greater than the viral inoculum seen in human semen to demonstrate the efficacy of HIV therapeutics . Traditional methods of SIV/SHIV infection of macaques involve the administration of a single high dose of virus sufficient to infect all of the naïve controls. However, much lower doses of HIV exist in mucosal fluids of humans during sexual transmission. Thus these high dose inoculums may underestimate the degree of efficacy when evaluating HIV vaccines or antiretroviral drugs that are effective in preventing HIV infections at a physiologically relevant dose. Infection of macaques with lower intermediate doses of virus was therefore adopted. The intermediate dose in rhesus macaques generally includes the exogenous administration of Depo-Provera (a progestin-based contraceptive) to thin the vaginal epithelium and therefore increase the susceptibility to infection with SIV/SHIV [51, 150]. Although this model is reliable in obtaining consistent infection rates, it does not model HIV transmission in humans that are not on hormonal contraceptives.

The repeat-low dose model developed in the early 2000s closely resembles to the mucosal exposures of humans to HIV in the following ways. A physiologically relevant viral dose to what is seen in humans during exposure to seminal fluid is used, and repeated exposures mimic multiple sexual transmission events. Unlike the models which use a single high viral inoculum exposure, the repeat low-dose model [79, 151-153] allows the investigator to assess the efficacy of anti-HIV regimens in a repeated fashion that is closer to human use patterns.

Intravenous transmission - The intravenous transmission route in the SIV/SHIV non-human primate model has contributed to our understanding of HIV/AIDS associated disease pathogenesis and in the development of effective vaccines and anti-HIV therapies. Rhesus macaques infected intravenously with SIVmac251 or SHIV89.6P were analyzed for virological outcomes at peak, set-point and viral decline. A positive correlation was found to exist between peak and set point viral load among animals infected with both viruses. However, rhesus macaques infected with SIVmac251 had a greater variability for set point viral load and viral decline than among those that were positive with SHIV-89.6P [84]. The association between plasma viral kinetics and the development of AIDS and death in humans has been well characterized. A similar correlation can be found among rhesus macaques infected with SIV [154]. Rhesus macaques inoculated with chimeric SHIV showed variability in their disease progression. Intravenous inoculation of rhesus macaques with

SHIV-HxBc2 or SHIV-89.6 caused persistent viremia but no decline in their CD4 numbers. On the other hand, inoculation with SHIV-89.6 P, a biological isolate derived from in vivo passages of SHIV-89.6, and SHIV-KB9, a molecular clone of SHIV-89.6P, caused high viremia and rapid and profound loss of CD-4 T cells and immunodeficiency [155]. Intravenous infection of juvenile rhesus macaques with SIVmac251 led to development of AIDS like symptoms and the rapid progression to death as is seen in some patients with HIV. However it was also reported that 8 monkeys were persistently infected for prolonged periods of time. There was an effective correlation between the presence of a strong antibody response to SIV and the clinical outcome in these long-time survivors [156]. Pig-tailed macaques are also susceptible to intravenous inoculation with SIVmac251. Persistently high levels of viremia associated with a gradual decline of CD4+ T cells mimic closely the outcomes of HIV-1 infection in humans [72]. These examples illustrate the importance of choosing the most relevant virus/host combination when defining efficacy of anti-HIV strategies.

Although the intravenous route is used rarely in the NHP model today, the early proof of concept experiments using the intravenous route of infection in macaques helped to formulate the parameters of pathogenesis, disease progression and correlates of protection that are now being evaluated with mucosal routes of exposure. The NHP model has progressed more towards mucosal routes of transmission in both vaccine evaluation and PrEP studies in an effort to keep pace with the clinical strategies being developed.

Intrarectal transmission - The estimates of relative risk of HIV-1 acquisition for the different routes of sexual transmission has been determined to be the highest for rectal, followed by vaginal, and finally the urogenital route. The incidence of acquisition of HIV with unprotected anal intercourse was estimated to be 0.65%- 1.7% per act risk of transmission [157, 158]. Rectal intercourse is prevalent among men who have sex with men as well as within the heterosexual exposure group. It has been reported that among the heterosexual population surveyed for rectal intercourse, additional high risk behavior for HIV/STD such as the lack of condom use and multiple sex partners exist [159]. The per-act risk of HIV transmission associated with receptive anal intercourse is five times greater than that for receptive vaginal intercourse [160]. The presence of a single cell-layer of columnar epithelium along with increased expression of the CCR5 and CXCR4 receptors required for HIV entry augments the vulnerability of the rectal mucosa to infection with HIV [161]. An understanding of the rectal environment and mucosal mechanisms involved in rectal transmission of HIV through animal models will greatly facilitate the development of effective microbicides and vaccines.

A more appropriate model to mimic rectal transmission of HIV is achieved with a repeated low-dose exposure to virus. Consistent rectal infection can be achieved in rhesus macaques within the first four exposures with an inoculum of 10^5 RNA copies of SHIV162p3 which is within the range of HIV-1 RNA levels in semen (10^3–10^6 copies/ml). It was demonstrated that low-dose SIV infection of rhesus macaques, unlike high dose, gave rise to a longer eclipse phase (the time period between infection and first appearance of systemic viremia), and lowered activation of innate immunity [106]. No association of adaptive immune T-cell

responses upon repeated rectal exposures and inherent resistance or delayed susceptibility to infection with SHIV162P3 among some rhesus macaques validates the use of this model as an effective approach to test various HIV prevention strategies .

Alternative models such as cynomolgus macaques have also been utilized for vaccine and PrEP studies with rectal SIV and SHIV exposures. Cynomolgus macaques of Philippine origin were infected intrarectally with multiple-low dose exposures to SIVmac239. However, there was a need for an escalating dose regimen to infect some of the macaques [162].Cynomolgus macaques are susceptible to infection by the rectal route with SIVsm, but have lower steady state plasma RNA concentrations than rhesus macaques [163]. Successful intrarectal inoculation of cynomolgus macaques with a single high dose of SIVmac239 and the ability to generate escape variants to CD8 T-cell responses has been demonstrated as well [164, 165]. Rectal transmission in cynomolgus macaques of hybrid SHIV viruses such as the pathogenic variant SHIV89.6P was achieved with a high dose of 1000 TCID50. These animals exhibited CD4+ cell depletion and a significant decline of their CD4+/CD8+ ratios [166].

Pig-tailed macaques, though highly utilized for intravaginal transmission studies, have been used sporadically for rectal transmission [167]. Intrarectal SIVmac251 infection of pig-tailed macaques led to persistently high levels of plasma viremia and continuous gradual decline of the CD4 cell counts [72]. The utilization of a pathogenic CCR5 variant clade C SHIV-1157ipd3N4 intrarectally in pig-tailed macaques results in an immunopathogenesis similar to SIV infection in rhesus macaques [168].

Intravaginal transmission - The lower transmission probability of vaginal infections with SIV/SHIV requires the administration of a higher inoculum of the virus vaginally compared to the rectal dose, or the use of Depo-Provera to thin the vaginal epithelium to increase the susceptibility of the macaques to vaginal SHIV/SIV transmission . The inherent resistance of the vaginal cavity to mucosal transmission and the need for the administration of high doses of SIV for successful transmission was established in the late 1980s [169]. Transient viremia and no seroconversion to SIVmac251 were achieved upon a single intravaginal inoculation [170]. Rhesus macaques of Chinese origin were equally susceptible as those of Indian origin to infection with high physiological doses of SIVmac251. Lower plasma viral loads than rhesus macaques of Indian origin were seen 6 weeks post infection among the Chinese rhesus macaques [171]. Intravaginal infection of pig-tailed macaques, the species that is widely sought after owing to its menstrual cycling similarity to humans, with 6x 10^3 TCID50 of the CCR5-tropic SHIVSF162P3 over 2 days infected all macaques with a moderate depletion of the CD4+ T cells. Although the mean peak viral load was similar to those infected intrarectally with SIVmac251, three of the eight macaques controlled their viremia to very low levels owing to their robust SHIV-specific cellular and humoral immune responses [72]. Rhesus macaques were also successfully infected intravaginally with chimeric SHIV89.6 in which the envelope glycoproteins were derived from HIV-1 89.6, a primary isolate that is CXCR4/CCR5, lymphotropic and monocytotropic, and not with SHIV (HxBC2) where the env fragment was derived from the CXCR4 T-tropic HIV-1 IIIB/LAI. Thus the ability of chimeric SHIVs to establish an infection mucosally is influenced by the properties provided by the cloned HIV-1 env fragments [172]. Unlike natural HIV infections

that are primarily initiated by CCR5 tropic viruses, the chimeric SHIVs such as SHIV89.6 that produced successful vaginal infection in rhesus macaques are dual tropic (CCR5 and CXCR4). The above mentioned studies in rhesus macaques require the administration of high doses of virus to achieve mucosal infection in naïve control animals. A more relevant model with a repeated weekly exposure to a physiologically relevant dose of the SHIV162P3 in pig-tailed macaques is effective and more appropriate for preclinical evaluation of therapeutics targeted to early transmission events .

Pigtail macaques have a menstrual cycle very similar to women and have become a very important model for pre-exposure prophylaxis (PrEP) studies where inhibition of vaginal transmission is the primary endpoint. A low-dose titration in pig-tailed macaques showed that systemic infection can be achieved with 3 once-weekly intravaginal exposures to 10TCID50 of SHIV162P3 [152]. It was reported recently that the susceptibility to infection in normally cycling female pigtail macaques is substantially greater in the luteal phase when the challenge regimen used is the repeat low dose model [78]. This study has had great implications on study design for vaginal topical PrEP preclinical studies, and in the interpretation of clinical trial results. Currently, PrEP trials for vaginal transmission require that the enrolled participants must be on contraceptives . Given that we now know that contraceptive use can lead to a luteal-like state as observed with the Depo-Provera treated rhesus macaques, and susceptibility is increased in this state, the interpretation of protection in clinical trials is changing. It will be very important to model these different scenarios in the pigtail macaque for topical as well as systemic PrEP regimens. It is easy to treat all of the animals with Depo-Provera to mimic what is happening in clinical trials, but we must keep in mind that in a real world situation not all women will be taking hormonal contraceptives. Indeed, many women prefer non-hormonal forms of birth control, and it is imperative that we design our NHP studies to answer questions for this group as well [77].

Penile transmission - Men are infected through penile exposure to HIV in heterosexual and men who have sex with men (MSM) relationships . Limited preclinical research is available regarding the penile mode of transmission and an emphasis has been placed on vaginal and rectal transmission studies. The presence of foreskin has been associated with an approximately 50% increase in risk of acquisition of HIV [173-175]. The human penis, foreskin has potential HIV target cells such as CD4+ T cells, Langerhans cells, macrophages, and submucosal lymphoidal aggregates that are rich in CD3+ and CD4+ cells [176, 177]. Early penile transmission studies in rhesus macaques were limited to urethral exposures to SIVmac251 [169]. Successful infection of two adult and four juvenile rhesus macaques with urethral exposures to SIVmac251 was obtained in the above study. Recent epidemiologic evidence has prompted an increase in interest in establishing a penile transmission model. Infection of rhesus macaques with SIVmac251 through penile exposure has been recently reported [53, 178]. However, repeated exposure of macaques to a dose of 10 TCID50 (n=5) or 10^3 TCID50 (n=2) over 14 inoculations was insufficient to infect the animals Exposures to a high dose of SIVmac251 (10^5 TCID50) twice within the same day was needed to infect 3 of 5 animals. One of two macaques exposed twice within the same day to 10^5 TCID50 of SIVmac251 for a total of three-times over an 8 week period became systemically infected as

well. The above rhesus macaque penile transmission study was used in an attempt to recapitulate the findings of the Merck Step trial which revealed enhanced HIV-1 infection in Ad5 seropositive individuals [179].

4. Vaccine research in NHP models

The high degree of variability among HIV-1 strains and the lack of defined correlates of immune protection in HIV-1 infected individuals or SIV/SHIV NHP models have collectively posed a considerable challenge for the development of a vaccine that confers sterilizing immunity. Vaccine design thus far has focused heavily on the induction of T-cell immunity, since HIV-1 neutralizing antibodies have been difficult to induce and do not play a dominant role in the control of viral load. Early vaccines employing recombinant HIV-1 envelope glycoprotein were efficient in neutralizing lab-adapted HIV-1 strains but not primary isolates . Furthermore, several in vivo studies in macaques have demonstrated that while certain humoral-based vaccine candidates conferred partial protection to animals challenged with SHIV strains, no protection was observed against challenges with more pathogenic SIVmac strains . However, this does not entirely rule out a role for humoral immunity, since a prime boost vaccination approach in a study involving SIVmne gp160 was shown to be effective in protecting cynomolgus macaques against intrarectal challenges with uncloned SIVmne and protection was associated with the development of SIV-specific neutralizing antibodies [180]. Similar approaches involving a recombinant vaccinia virus or baculovirus expressing SIVmac239 gp160 did not protect rhesus macaques against intravenous challenges with homologous SIVmac239 or heterologous SIVmac251 strains [181].

Although it is evident that a skewed focus on eliciting broad neutralizing antibodies will not suffice, it has also become clear that while HIV-1 gag vaccines can be strongly immunogenic, a potent T-cell response does not necessarily translate to protection. This was especially highlighted by the Merck STEP clinical trial which showed that following administration of a replication defective recombinant adenovirus 5 (rAd5) expressing HIV-1 subtype B Gag/Pol/Nef, those vaccine recipients who were already seropositive for Ad5 had a higher incidence of HIV-1 infection [182]. This is in contrast to preceding preclinical studies in which rhesus macaques exhibited a high level of both the magnitude and duration of virus-specific immune responses following a DNA prime- rAd5 boost regimen, and were protected against challenges with SHIV89.6 [183]. Subsequent studies demonstrated that the Ad5 vaccine did not protect against challenge with SIVmac239, and reduced viral loads only in animals with the protective MHC class I allele Mamu A*01 [184]. Furthermore, male rhesus macaques that were chronically infected with a host-range mutant Ad5 prior to immunization with an Ad5 vector expressing SIVmac239 Gag/Pol/Nef had a higher rate of infection following challenge with an escalating dose of SIVmac251 via penile exposure, recapitulating the outcome of the human clinical trial [179]. However unlike the Merck trial, the Ad5 immunized macaques showed a lower acute-phase viremia than the unimmunized animals.

The STEP Trial outcome led to a significant overhaul in the design and execution of vaccine studies. It was suggested that NHP models could not always be relied upon as a

"gatekeeper" for determining go/no-go criteria. However NHP are the only animal models that best reflect many facets of HIV infection in humans, and therefore continue to a play a pivotal role in comparative and retrospective studies which can simultaneously inform vaccine strategies of both ongoing and future clinical trials.

Since the Merck STEP Trial, several NHP studies employing various types and combinations of HIV-1 antigen prime-boost vaccines have been conducted, with varying degrees of success. Rhesus macaques receiving a plasmid DNA prime and rAd5 vector expressing SIVmac239 env/gag/pol boost vaccine regimen, and challenged intrarectally for 12 weeks with either SIVmac251 or the heterogeneous SIVsmE660, exhibited 50% protection from infection with the latter virus strain [185]. In addition, among the SIVsmE660-infected animals, those expressing the Mamu-A*01 MHC class I allele were found to have a log lower plasma peak viremia. The vaccinated Mamu-A*01 negative animals in the SIVsmE660 group that were protected were also shown to express low levels of neutralizing antibodies and an envelope-specific CD4+ T cell response, highlighting roles for both humoral and cellular arms of the immune system. The presence of homozygous restrictive, allelic forms of the TRIM5alpha was shown to be associated with protection from infection [185]. The most recent, and perhaps most successful, vaccine study was the RV144 trial conducted in Thailand [186]. The vaccine candidates included a canarypox viral vector vaccine encoding clade B gag/pro and Clade E env as the prime (ALVAC-HIV vCP1521), and a boost with AIDSVAX gp120 B/E which is genetically engineered HIV-1 gp120 from both Clade B and E. Spanning over a six year period, this Phase IIb trial had an approximately 31% protection rate against HIV acquisition. While modest, this level of protection nonetheless re-energized the vaccine field, and several studies are underway in NHP models to recapitulate the results from the clinical trial, with the hope of identifying the specific immune response(s) that is responsible for protection.

The outcomes of HIV/SIV/SHIV vaccine trials thus far have made it apparent that the rate and level of virus acquisition and/or replication are at present, the only reliable factors when deciding the efficacy of a vaccine candidate, since the immune responses required for vaccine efficacy remain undefined. However, it is clear that NHP studies need to carefully account for the challenge virus as well as the genetic background of the macaque species, and perhaps standardize or implement more rigorous vaccine protocols to afford better predictive power and/or help in the identification and exclusion of confounding factors.

5. The role of the NHP model for Pre-exposure Prophylaxis

Microbicides are inhibitory compounds that when applied vaginally or rectally will prevent or reduce the likelihood of HIV transmission . PrEP is defined as the use of antiretroviral (ARV) drugs among HIV- negative individuals to prevent the acquisition of HIV. The successful use of ARVs in the treatment of HIV-infected individuals as post-exposure prophylaxis (PEP) and the associated knowledge gained on their safety have led the way for their use as PrEP agents. Various formulations of microbicides have been developed for rectal and vaginal application such as gels, films, suppositories (tablets) and intravaginal

rings. It is necessary that a product that is destined for topical use is safe and widely acceptable, thereby promoting and enhancing adherence, cost effective and be able to deliver the drug at a high enough concentration locally to prevent the acquisition of HIV. The primary focus of microbicides has been placed on a coitally-dependent gel strategy, the method of choice for microbicide formulations, and intravaginal rings (IVR) that provide a sustained release of drugs over prolonged periods of time in a coitally-independent fashion.

Though Sub-Saharan Africa is home to only 10% of the world's population it contains every two of three people living with HIV. More than 60% of the people living with HIV in Sub-Saharan Africa are women, and of these 75% are between the ages of 15 and 24 [187]. The increasing risk associated with women and their inability to negotiate consistent condom use or monogamy emphasizes the need for the development of female-controlled methods of prevention of HIV acquisition. It was predicted that a vaginal microbicide that is 50% efficacious may prevent 33% of HIV infections in a period of 25 years upon 75% usage [188].

The effectiveness of PrEP in preventing mucosal infections with HIV will be influenced by the delivery of ARVs to a protective level at the mucosal site of transmission. The NHP model not only provides an experimentally controlled platform for the safety and pharmacokinetic evaluation of microbicides, but can also be used in the evaluation of efficacy in preventing mucosal transmission of HIV [189-192]. It is of utmost important for microbicides that are targeted for topical use in preventing sexually transmitted infections, such as HIV, to be tested in animal models prior to human trials. The sexual transmission of HIV involves a biologically complex milieu comprising initial infection among target cells at the port of entry (vaginal or rectal), the establishment of a small founder population, and local expansion to establish systemic infection . This poorly understood process cannot be properly evaluated in vitro. The recent failure and enhanced transmission observed in the first microbicide efficacy trials with nonoxynol-9 and Savvy [193, 194] warrant the need for controlled and careful investigation of topical products in animal models. A phase II/III trial with nonoxynol-9, an over the counter spermicide, in a vaginal gel formulation increased the risk of acquisition of HIV among users of the gel [195]. The detrimental effects associated with the multiple vaginal application of nonoxynol-9 such as epithelial disruption and inflammatory infiltration was also demonstrated in pig-tailed macaques [196].

The pharmacokinetic and pharmacodynamics evaluation of microbicides in animal models allows for the determination of not only the accumulation of ARVs in mucosal tissues , but also of the minimal effective dosing and the optimal timing with regards to the periods of virus exposure. The nonhuman primate model has been pivotal in producing preclinical data that can inform clinical trial design is this new and exciting field of prevention. There are several ARV PrEP candidates that have great potential for topical application and here we describe a few select ARVs and delivery methods that have progressed through initial preclinical evaluation in the NHP model. The implementation of ARVs for pre-exposure prophylaxis (PrEP) of HIV both as oral and topical applications is currently being investigated as outlined among the different trials in Table 4 [197-200].

Trial	Study population	Country	Route of Administration	Drug	Effect Size (95% CI)
Partners PrEP [197]	HIV serodiscordant couples	Kenya, Uganda	Oral	i.TDF/ Emtricitabine ii. TDF	73% (49-85) 62% (34-78)
CDC sponsored TDF2 [233]	Heterosexual men and women	Botswana	Oral	TDF/ emtricitabine	63% (22-83)
iPrEX [227]	MSM	South America, USA, South Africa, Thailand	Oral	TDF/ emtricitabine	44% (15-63)
CAPRISA-004 [198]	Sexually active HIV uninfected women	South Africa	Vaginal microbicide gel	1%TFV	39% (6-60)
VOICE [234, 235]	HIV-negative women	Uganda, South Africa, Zimbabwe	i. Oral ii. Vaginal microbicide gel	i. TFV ii. 1%TFV	Ineffective[a]
FEM-PrEP [200]	HIV uninfected women	Kenya, Tanzania, South Africa	Oral	TDF/ emtricitabine	0% (-69 -41)

TFV- Tenofovir, TDF-Tenofovir disproxil fumarate, [a] Both arms were halted for futility. Final data from VOICE trial has not been announced.

Table 4. ARV based oral and topical PrEP trials among different populations: Adapted from [197].

5.1. Antiretroviral inhibitors implemented in PrEP microbicide products

Several ARV based microbicides are currently under preclinical development in non-human primates to inhibit HIV replication at various stages of its lifecycle. The microbicides are classified based on the step that it inhibits in replication cycle of HIV such as entry, reverse transcription of its RNA genome, integration into the host chromosome, translation of new viral proteins, release and maturation of the progeny virions.

Entry inhibitors - Binding of HIV gp120 to CD4 on T helper cells and macrophages triggers conformational changes in gp120 that allows binding to the CCR5 or CXCR4 co-receptor. Next, the gp41 ectodomain forms a six- helix bundle that allows close proximity of the viral and cell membranes leading to fusion . Small molecule inhibitors that bind gp120 and prevent attachment to CD4 such as BMS-378806 have been shown to be effective as a vaginal microbicide in rhesus macaques upon a high dose challenge. BMS-378806 in combination with C52L, a bacterially expressed gp41-mediated fusion inhibitor peptide, protected

macaques against vaginal challenge [201]. T1249, another fusion inhibitor, was also effective as a vaginal gel formulation against a variety of SHIV in macaques [202]. Cyanovirin, a cyanobacterial protein that binds non-competitively to gp120, was also effective in protecting pig-tailed macaques against vaginal infection [203]. CCR5 antagonists CMPD167 and maraviroc, and modified chemokines such as PSC-Rantes have shown protection in rhesus macaques against RLD vaginal challenge with SHIV [201, 204, 205].

Reverse transcriptase inhibitors - Nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTI) block reverse transcriptase activity. The NRTIs such as zidovudine, lamivudine, and Tenofovir (TFV) require phosphorylation by host cell enzymes to their pharmacologically active triphosphate (TP) anabolite [206-208]. The TPs are analogs of endogenous 2'-deoxynucleotides (dNTPs) and compete for incorporation into the growing HIV DNA chain by HIV reverse transcriptase leading to chain termination .

The potential of TFV for preventing acquisition of HIV derives from its capacity to prevent SIV infection in rhesus macaques. After four weeks subcutaneous administration of once daily TFV beginning 48 hours before and up to 24 hours after intravenous inoculation of SIV, macaques were protected against systemic infection. The macaques showed no evidence of virus in the plasma or PBMC for 56 weeks. Lymphoid tissues and major organs obtained from healthy euthanized animals 40 weeks post inoculation were also free of SIV. The efficacy of TFV and Truvada (TDF and emtricitabine) as PrEP agents was proven with repeated exposures to physiological equivalents of SHIV162P3 in rhesus macaques [153, 209, 210]. Intermittent dosing with an oral pre-exposure dose 1, 3 or 7 days before virus exposure followed by a dose of TDF/emtricitabine 2 hours after exposure was associated with a 16.7, 15.3, and 9.4 factor reduction respectively in comparison to the controls against rectal SHIV162P3 protection. No protection against rectal SHIV162P3 exposure was observed if the first dose was delayed up to 24 hours after exposure emphasizing the need for interdicting the initial replication events [211].

TFV alone (1%) or in combination with emtricitabine (5%) in a vaginal gel formulation was also effective in protecting pig-tailed macaques against a repeat low-dose exposure to SHIV162P3 [212]. The correlation of intracellular TFV-DP levels in vaginal tissue lymphocytes at the time of vaginal exposure and reduced efficacy in protecting pigtail macaques was demonstrated recently with intermittent application of a 1%TFV gel once per week and virus exposures occurring twice weekly. It was estimated that the median TFV-DP concentrations were 1810 fmol/10^6 cells at 4 hours and above 1000 fmol/10^6 cells in the vaginal lymphocytes that were obtained from animals necropsied at 1 and 2 days after gel application. However, the median TFV-DP concentrations dropped to 252 fmol/10^6 cells 3 days after gel application which correlated to 74% efficacy [213]. This study was therefore able to find a direct correlate between intracellular TFV-DP levels and efficacy in the nonhuman primate model.

NNRTIs differ from NRTIs in binding to the reverse transcriptase outside of the active site and have been shown to be efficacious in the vaginal SHIV challenge models in macaques.

Vaginal combination gels containing zinc acetate dehydrate and the NNRTI MIV-150 provided complete protection in rhesus macaques against RT-SHIV up to 24 hours following 2 weeks of daily gel application. Partial protection was seen with formulations containing zinc acetate or MIV alone [214]. MC 1220 in a gel formulation also provided partial protection against RT-SHIV in rhesus macaques [215].

Integrase inhibitors - HIV integrase is essential for incorporation of the viral genome into the host DNA and is an essential event for viral replication. Inhibitors that block this process are actively being developed for therapeutic applications and are just beginning to be investigated for PrEP. Because the integration step occurs later in the replication cycle than entry and reverse transcription, administration of integrase inhibitors may be effective when used as post-exposure prophylaxis (PEP). To address this question in the NHP model, topically applied L-870812 was evaluated for efficacy in preventing vaginal transmission of SHIV162P3 in a repeat low-dose macaque model. Pigtail macaques received 3 mL of a 0.2% L-870812 gel 30 minutes after intravaginal virus exposure with SHIV162P3 and partial efficacy was observed [216]. Further investigation of integrase inhibitors as sole PrEP agents and in combination with other PrEP agents is warranted given these encouraging results. A combination of ARVs that act at different stages of viral replication will theoretically provide broader protection.

5.2. Drug delivery vehicles

There are many different delivery platforms available for PrEP and these are being tested in NHP models. Many of the studies described above employ conventional gel formulations and are associated with problems such as leakage and the need to administer the gel shortly before every act of intercourse to prevent HIV acquisition. In addition there is also the lack of covertness with gel application which makes it difficult for women who need to use the microbicide without the knowledge of their partner. However, topical applications like gels, tablets, and films are administered directly to the site of transmission and very high local tissue levels can be achieved [217, 218]. Oral and injectable dosing is preferable in some settings, but the drug is delivered systemically, not locally, and therefore higher and more frequent dosing may be required for protection.

Alternative delivery platforms are being investigated in the NHP model to overcome some of the problems encountered with conventional dosing methods. For instance, intravaginal rings (IVR), such as those commercially available for contraception, can help overcome some of the barriers associated with conventional gel formulations and delivery. IVRs are torus shaped flexible drug delivery devices that are self –inserted and when placed is located close to the cervix in the upper two-thirds of the vagina and provide sustained release of one or more drugs for mucosal and possibly systemic effects. The advantageous properties of IVRs such as the capacity to provide sustained and controlled release of drugs over extended periods of time, non-coital dependency, and the need for a single application in women of only once a month or every few months, are beginning to be exploited in the field of microbicides [219-223].

The initial safety and size guidelines to develop ring devices that are suitable for use in pig-tailed and rhesus macaques came from the administration of different sized rings and the close monitoring of the safety of these devices. Non-medicated silicone elastomer vaginal rings of 3 different sizes were administered to pig-tailed and Chinese rhesus macaques for a 28 day period [224]. No signs of inflammation or irritation were observed on colposcopic examinations and the animals showed no behavioral changes or other problems following insertion of the rings. Mucosal proinflammatory cytokines were unchanged in the presence of the rings (for 4 weeks) or upon removal (4 weeks post removal). Safety analyses of macaque-sized elastomeric silicone and polyurethane intravaginal rings (IVRs) loaded with candidate ARV drugs were tested in pig-tailed macaques in four studies ranging in duration from 49 to 73 days with retention of the IVR being 28 days in each study. The presence of IVRs not only made of silicone but other polymers such as, polyurethane in pig-tailed macaques does not cause an alteration longitudinally in the levels of the proinflammatory cytokines locally or systemically and in the vaginal microbiological patterns [225, 226]. Efficacy studies in the NHP model with IVRs are just beginning, but preliminary pharmacokinetic studies are very promising [227-235].

6. Summary and outlook

As we move forward in our endeavors to prevent HIV infections, it is clear that having viable animal models are a vital component of a comprehensive approach to develop and test biomedical preventions. The field of HIV treatment and prevention has broadened to include not only vaccine discovery and treatment of infected individuals to PEP, PrEP, combination therapies, and discussions of eradication and cure. The pharmaceutical discoveries of recent years have increased our options for PEP and PrEP, and vaccine designs are becoming much more sophisticated. As the prevention field moves forward we are constantly modifying the macaque model to accommodate new combinations of interventions. The new SHIVs will have to incorporate the elements necessary to evaluate vaccines and other prevention modalities both singly and in combination. The likelihood that future clinical vaccine trials will be conducted in concert with PrEP trials is very high, and the recombinant viruses we use in the NHP models have to keep pace in evaluating promising candidates in the most rigorous way possible. The nonhuman primate model has adapted to aid researchers in answering ever more complex questions surrounding the interaction of the virus, host, and antiretroviral drugs. The coming years will be very interesting and fruitful as we move towards our common goal; to make HIV and AIDS a disease of our past, not of our future.

Disclaimer

The findings and conclusions are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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