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Nonhuman Primate Models for Evaluation of HIV-1 Preventative Vaccine Strategies: Model Parameter Considerations and Consequences

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Abstract

Purpose of review—Nonhuman primate (NHP) models of AIDS are powerful systems for evaluating HIV vaccine approaches *in vivo*. Authentic features of HIV-1 transmission, dissemination, target cell tropism, and pathogenesis, and aspects of anti-HIV-1 immune responses, can be recapitulated in NHPs provided the appropriate specific model parameters are considered. Here, we discuss key model parameter options and their implications for HIV-1 vaccine evaluation.

Recent findings—With the availability of several different NHP host species/subspecies, different challenge viruses and challenge stock production methods, and various challenge routes and schema, multiple NHP models of AIDS exist for HIV vaccine evaluation. The recent development of multiple new challenge viruses, including chimeric simian-human immunodeficiency viruses (SHIVs) and simian immunodeficiency virus (SIV) clones, improved characterization of challenge stocks and production methods, and increased insight into specific challenge parameters have resulted in an increase in the number of available models and a better understanding of the implications of specific study design choices.

Summary—Recent progress and technical developments promise new insights into basic disease mechanisms and improved models for better preclinical evaluation of interventions to prevent HIV transmission.

Keywords

macaque; vaccine challenge; SIV; SHIV

Introduction

Since the earliest evaluations of classical vaccination approaches, nonhuman primate (NHP) models of AIDS have played a key role in HIV vaccine design and development, providing safety and proof-of-concept data for proposed HIV vaccine strategies. Although NHP/AIDS

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Conflicts of Interest.

None.

models have been utilized to evaluate both prophylactic vaccine modalities and therapeutic vaccination approaches, this review will focus primarily on NHP challenge models for use in studies of viral transmission and the evaluation of acquisition prevention approaches.

When designing an *in vivo* NHP experiment, it is critical to select from among the many available NHP/AIDS models that which is best suited to address the specific research questions of interest. The choice of NHP host species/subspecies, challenge virus, challenge route, virus dose, and challenge schema (Fig. 1) can all impact key biological features and outcomes and should be carefully considered. This review will cover our current perspectives on these parameters and considerations.

NHP Host Species

Vaccine studies conducted in NHPs typically involve experimental AIDS virus challenge of Asian macaque species, including rhesus macaques (RMs; *Macaca mulatta*), cynomolgus macaques (CMs; *Macaca fascicularis*), and pig-tailed macaques (PTMs; *Macaca nemestrina*) (Fig. 1), that are not naturally infected with simian immunodeficiency virus (SIV). RMs, specifically those of Indian-origin (IO), are the most widely-used macaques for AIDS research and HIV vaccine studies [6], with an associated large body of historical data describing SIV infection and vaccine immunogenicity within these hosts, and with a greater availability of immunological reagents and genomic information [7,8] than is available for other species. When infected with benchmark pathogenic viruses, such as the SIVmac lineage viruses, IO RMs undergo a generally consistent disease course with characteristically high acute viral loads [9] that provide a robust and reproducible readout for prevention studies. Chinese-origin (CO) RMs can also be used for SIV challenge studies [10–12], but acute viral loads may be 1 to 2 logs lower and chronic phase viral loads may be several orders of magnitude lower in this subspecies when infected with the most widely used viruses, which have been optimized for use in their IO relatives [13–18]. It is unclear if the underlying biological cause of these lower viral loads might also impact viral transmission. Although a less stringent model involving superior control of viral replication even in the absence of vaccination may have advantages for some studies, this apparent limitation for using CO RMs may be surmountable through the use of alternative viruses specifically adapted for robust infection of CO rhesus [18].

Cynomolgus macaques, which based on practical and logistical considerations have been used predominantly in European laboratories, have been used less extensively for AIDS research than RMs. Following infection with SIV strains that are pathogenic in IO RMs, CMs have an infection course that is less consistently pathogenic, with viral load profiles that may be several logs lower and more variable [19], though, like CO RMs, it is possible that this limitation may be overcome by using viruses adapted for this species [20–22]. Despite the more limited historical use of CMs for AIDS research, CMs from the island of Mauritius, which are descended from a small number of founder animals subject to insular geographic isolation resulting in limited genetic MHC class I and class II diversity [23,24], have emerged as a valuable research tool for studies seeking to more completely control for animal MHC haplotypes than is readily feasible with other, more outbred macaque hosts and populations [25,26*,27].

In recent years there has been increased interest in the use of PTMs for intravaginal challenge studies because female PTMs have a normal lunar menstrual cycle with associated hormonal changes that are more akin to human menstrual cycles than other available macaque species [28]. The relevance of PTM vaginal physiology has allowed researchers to assess in this species the impact of factors such as coinfections and contraceptive use on susceptibility to vaginal infection [29*,30], and to evaluate modalities to prevent vaginal virus transmission, including microbicides and other pharmacologic interventions [31–34], all while accounting for menstrual cycle phases [30,35,36*–38]. However, within-species comparisons of intravaginal and intrarectal challenges using PTMs may be confounded by the higher levels of baseline immune activation associated with elevated intestinal permeability and microbial translocation in the absence of infection in PTMs when compared with RMs [39]. This characteristic of PTM intestinal biology may impact fundamental aspects of mucosal viral transmission and early viral replication, although environmental factors may contribute to the described phenotype. Intriguingly, PTMs express a variant form of the TRIM5 viral restriction factor that, unlike rhesus TRIM5α, does not restrict HIV replication [40–43]. This finding has led to ongoing efforts to derive minimally chimeric HIV-1 strains capable of high level replication and pathogenesis in PTMs [44,45], which with further development may become highly useful for HIV-1 vaccine evaluation in NHPs.

Challenge viruses

Factors relevant to the characterization and choice of challenge viruses for a given experiment include identity, clonal or swarm composition, extent of genetic diversity, neutralization sensitivity, pathogenicity, restriction factor susceptibility, and mode of production. To date, the majority of NHP/AIDS research, including vaccine studies, has utilized experimental challenge with SIV isolates and clones derived from chronically infected RMs, though very recent efforts have resulted in the generation of several new pathogenic transmitted/founder SIV clones that may represent more relevant vaccine targets [5] (Fig. 1).

Homologous vs. Heterologous Challenge

Initial NHP vaccine studies relied upon homologous challenges in which animals were challenged with either uncloned SIVmac251 swarm virus or the SIVmac239 infectious molecular clone following vaccination with immunogens derived from the same SIVmac251/239 lineage viruses [46,47]. Although such studies generated important immunogenicity and proof-of-concept data, given the extensive diversity of circulating HIV-1, demonstrating protection against a virus challenge heterologous to the vaccine immunogen has long been of both interest and importance. To evaluate heterologous protection in NHPs, heterologous immunization/challenge designs have been implemented, with SIVmac-based immunogens and challenge(s) with the SIVsmE660 isolate or SIVsmE543 clone, pathogenic viruses which genetically diverge from the SIVmac lineage to an extent approximating the degree of divergence between different HIV-1 subtypes [48,49*–51]. Alternatively, immunization with SIVsm-based immunogens and subsequent challenge with SIVmac viruses may be used. While SIVmac239 and SIVmac251 have a

degree of resistance to antibody-mediated neutralization comparable to or exceeding typical circulating HIV-1 strains [52–54**], the SIVsmE660 isolate is composed of viral clones most of which are relatively more neutralization sensitive [48], making it a more tractable target for antibody-based prevention studies. However, unlike the SIVmac viruses, SIVsmE660 and E543 are highly susceptible to restriction by specific rhesus macaque TRIM5α alleles [55–58], which can impact virus acquisition after mucosal challenge [57,58], confounding the interpretation of vaccine efficacy [59,60]. Thus, when using SIVsmE660 and related challenge viruses, it is critical to genotype study animals for their TRIM5α alleles and either exclude those with restrictive TRIM5α alleles or account for the distribution of restrictive and permissive alleles among the study arms.

SIV vs. SHIV

The extreme neutralization resistance of many of the best characterized SIVmac viruses [52–54**], the limited availability until quite recently [54**] of well-pedigreed antibody reagents specific for SIV Env epitopes of interest, and the growing collection of broadly reactive anti-HIV-1 Env-specific neutralizing antibodies that do not cross-react with SIV have led to renewed enthusiasm for the development of relevant simian-human immunodeficiency viruses (SHIVs). These chimeric viruses, which conceptually comprise an SIV backbone genome containing an inserted HIV-1 Env gene, allow for the direct evaluation of HIV-1 Env targeted prevention strategies. The first efforts toward generating pathogenic CCR5-using SHIVs focused on animal-to-animal serial virus passage to develop SHIVs capable of high level viral replication in macaques [1,61,62]. Although some of these viruses have been highly valuable research tools for proof-of-concept evaluations of antibody-mediated infection prevention [63**,64**,65*–68*,69–71], they represent limited genotypic breadth, were derived from Envs cloned from chronically-infected people, often after in vitro passage, and thus do not represent the most clinically-relevant Envs for transmission studies and vaccine assessment. More recent efforts have taken advantage of the greater availability of HIV-1 Env clones from transmitted/founder (T/F) viruses, which initiated individual human infections. Through the use of *in vivo* competition, subtype B and C T/F Envs capable of mediating mucosal SHIV transmission and robust acute viral replication in RMs without any animal-to-animal serial passage or alteration have been identified [2*,72], with one subtype B SHIV even demonstrating the ability to induce progressive disease with AIDS defining clinical endpoints in the absence of any Env adaptation or alteration [72]. Mucosally transmissible SHIVs bearing unpassaged T/F CRF01_AE Envs have also been identified [3], as have SHIVs bearing T/F subtype A, B, C, and D Envs containing specific mutations proposed to improve interaction with the RM CD4 receptor [4*], which may prove to be a critical determinant of SHIV replication capacity [73*].

Although sustained elevated chronic viral loads with authentic pathogenesis and a progressive infection course is not essential for most prevention studies, these would be preferred features for new viruses and additional characterization and development of such viruses will be important. Taken together, though, the current generation of new SHIVs represent substantial progress, providing a promising panel with broad viral diversity and

range of neutralization profiles for use in NHP studies assessing Env-targeting vaccine modalities.

Generation and Characterization of Challenge Virus Stocks

Once a challenge virus is selected, choice of an infectious stock of that virus is a critical aspect of NHP vaccine challenge studies (Fig. 1). The specific challenge virus stock and the method used to generate it can dictate feasible downstream assays and impact study results. Virus stocks used for vaccine studies can be clones or swarms, produced by transfection or by short term expansion in an infected culture of mitogen activated primary macaque CD4+ T lymphocytes or PBMC host cells, although human cells have also been used.

Viral swarms

The ability to distinguish transmitted variants by viral genome sequencing allows for verification that the virus challenge dose appropriately recapitulates the limited number of transmitted variants that initiate most human sexual transmissions [74–76]. It may also increase the statistical power of a study without an increase in animal numbers. Enumerating the number of distinct transmitted variants can serve as a minimum estimate of infection/transmission-events per animal [77*,78**–80], with reduction of this parameter interpreted as a measure of partial vaccine efficacy, even if acquisition was not completely prevented. This type of analysis has traditionally required the use of an uncloned viral isolate swarm, such as SIVmac251 or SIVsmE660, containing sufficient viral diversity for individual variants to be genetically distinguished through single genome amplification and sequencing techniques, typically over full length Env sequences [80–82].

Challenge with phenotypically heterogeneous viral isolate swarms may also enable the elucidation of vaccine efficacy mechanisms through “sieve analyses”. Evaluating the genotypes and associated phenotypes of “breakthrough” viral variants in vaccinated animals may provide insights into vaccine mechanism of action and viral evasion [49*,78**]. However, to identify genotypic signatures of breakthrough virus, the combination of genotype frequency within the stock, virus dose, and numbers of animals and challenges must be such that the specific genotype will be significantly selected for in vaccinees relative to control animals. Thus this type of analysis is critically dependent upon the genetic composition of the challenge stock used, and the presence and relative proportion of the pertinent variants can vary dramatically in unpredictable and uncontrolled ways from stock to stock [83].

There are other potential pitfalls to consider when utilizing uncloned virus swarms as challenge stocks, including variability in viral diversity and non-viral stock constituents from stock to stock [83]. This may confound comparisons between studies using different stocks of nominally the same virus. In addition, when animals are challenged with virus at limiting dilutions, resulting in the transmission of only one or a few viral variants from within the swarm, the specific transmitted variants infecting each animal are highly subject to stochastic selection, and thus each animal may be infected with one or several viral variants that are phenotypically distinct from the other study animals. While the complete range of phenotypic breadth within a given stock is difficult to capture, recent studies have

shown that there are considerable variant-to-variant phenotypic differences within such swarm stocks [48,83]. In addition, discriminating variants drawn from an uncloned swarm often requires the sequencing of large genomic regions, thereby limiting the depth of sequence sampling or dramatically increasing the costs of sequencing.

Viral clones

The use of clonal virus stocks, produced by transfecting non-permissive cells, typically 293T cells, with a molecularly cloned viral genome can obviate many of these issues while potentially creating others. By using a non-permissive cell type to produce the virus, viral replication in culture is avoided, resulting in a virus population that is genetically homogeneous, allowing greater stock-to-stock reproducibility. As a result, each animal will be challenged with and infected by a genetically identical virus, making comparisons between study groups and individual study animals more straightforward. However, potential drawbacks to consider for the use of clonal, transfection produced virus stocks include increased sensitivity to antibody-mediated neutralization [48,84–87] and lower apparent mucosal infectivity than matched viruses generated in infected PBMCs, despite comparable *in vitro* infectivity titers, two features which may be related to the relatively lower virion-associated Env glycoprotein content for virus produced in 293T cells [83,84]. Moreover, the clonal genetic composition of unmodified transfection-produced virus stocks precludes the discrimination and enumeration of transmitted variants following challenge.

Sequence tagged synthetic swarms

To allow for discrimination and enumeration of transmitted variants while minimizing the phenotypic differences from variant-to-variant, we recently described the generation of a synthetic viral swarm, generated by incorporating a series of sequence-identifiable synonymous mutations into a small region of the otherwise isogenic SIVmac239 viral genome [88]. This approach, which can be applied to other molecularly-cloned viruses such as SHIVs, allows distinct variants to be easily identified and enumerated after transmission, with increased feasibility for deep sequencing due to the short unique sequence region. If made by transfection, stocks of this synthetic swarm containing equivalent relative proportions of each of the genetic tags can be easily generated, though many of the other limitations of transfection-derived viruses still apply. Short term expansion in infected PBMCs of such molecularly tagged-virus populations would likely obviate many of these issues, and, unlike standard infectious molecular-clones that would not acquire sufficient genetic diversity for T/F virus discrimination following short term expansion in infected cells [83*], genetically-tagged viruses would already possess the needed diversity.

Challenge route, study design and interpretation

NHP/AIDS models have been used to varying degrees to evaluate virus transmission and preventative measures for most of the routes relevant to HIV-1 transmission, including intravenous (IV), intrarectal (IR), intravaginal (Ivag), penile, oral, and intrauterine transmission (Fig. 1). While some of these approaches have become routine for the field, they often rely on historical precedent and are still poorly understood, in part reflecting a limited understanding of the human transmission scenarios that scientists hope to model in

NHPs. For example, the vast majority of transmission and vaccine challenge studies performed in NHPs to date have utilized cell-free virus stocks that can be conveniently characterized, titered in vitro, and stored, and that behave reproducibly in animals. However, a role for the transmission of infected cells remains unclear, and may merit further consideration [89], but is currently omitted from most NHP studies.

Challenge route, dose, and schema

IV virus transmission represents the most consistent and straightforward transmission route in NHPs, but it also represents one of the least clinically relevant and most stringent routes for HIV vaccine research. Though there may be some utility for evaluating vaccine-mediated prevention of IV transmission in NHPs to model protection against transmission through needle sharing, standard NHP IV infection and challenge protocols involve the IV delivery of relatively large volumes of fluid containing relatively large numbers of virions, which does not ideally model this transmission route in humans.

Given the central role that sexual transmission plays in the HIV-1 pandemic, much of the HIV vaccine research currently conducted in NHPs focuses on the use of mucosal challenge studies to model sexual transmission. Of the available mucosal challenge routes in NHP study animals, IR challenges represent the most consistent and practically feasible. However, even for IR challenges, there are likely refinements to consider, as demonstrated by a recent study highlighting the impact of simple parameters such as inoculum volume and the presence of rectal/colonic feces on inoculum distribution along the mucosal surface [90]. Ivag and penile challenge models represent somewhat more demanding systems. Consistent with human transmission rates, successful Ivag and penile challenges in NHPs require relatively higher doses of virus than IR transmission and are more prone to animal-to-animal variability [81,82,91]. Efforts to improve the consistency of virus infection rates in unvaccinated control animals via the penile and Ivag routes have included, respectively, the use of two sequential penile challenges per day [82,92] and the administration of the progestin drug Depo Provera [93,94], which has been shown to thin the vaginal epithelium and increase susceptibility to infection [95]. While the relevance of either practice may be limited, avoiding Depo Provera creates logistical challenges as it becomes critical to monitor and control for animal menstrual cycles, which can substantially impact susceptibility to vaginal infection [30,35,36*–38,96]. Although efforts are usually made to perform mucosal challenges atraumatically, to avoid harm to study animals and confounding effects from transmission inconsistencies due to mucosal damage, it is also worth considering whether this practice best models mucosal transmission associated with coital acts in humans.

Following the discovery that the majority of sexual HIV-1 transmission in humans results in only one or a few viral variants establishing systemic infection [75,76], and the demonstration that this phenomena could be recapitulated through the use of titered inocula doses in NHPs [80,81,91], much of the HIV prevention research conducted in NHPs has moved in recent years to the use of repeated limiting dose challenge studies. In a repeated limiting dose challenge paradigm, the titer of the challenge stock must be determined in animals to identify a dose that infects only a fraction of unvaccinated control animals per exposure (typically half or fewer), with animals repeatedly exposed until evidence of

infection, or through a predetermined number of challenges. While the size of the inoculum used even in limiting-dose challenge studies may exceed that of typical human mucosal HIV exposures, this model paradigm allows for the approximation of limited numbers of founder variants following transmission, simulating typical human mucosal transmission, while satisfying practical considerations, such as study duration and costs associated with numbers of exposures required to infect control animals. Use of a repeat challenge model impacts statistical analysis and interpretation of results, and while calculation of “per challenge vaccine efficacy” may provide a measure of partial vaccine efficacy, this would seem of limited value in a study where all vaccinees ultimately become infected after a small number of challenges. Between labs, there is considerable variability in the challenge frequency, challenge stock used, and whether or not virus doses escalate throughout the study, and the potential impact of these parameters on subsequent challenges remains unclear, although most data argue against immunological sensitization from challenges not resulting in a take of infection. An alternative approach involves the use of somewhat higher doses of viruses, approximating a minimal dose required to infect the majority of unvaccinated control animals with a single challenge. In this type of study design, researchers can utilize sequencing approaches as described above to assess vaccine efficacy by combining evaluations of infection rates, number of transmitted variants per animal, and sieving analysis [49*,78**,97].

Conclusions

In recent years, substantial advances have been made toward the development of additional NHP/AIDS model options, with the potential for greater model relevance and translatability. However, with this increase in the number of existing model parameters and the increasing availability of assay technologies comes a greater need and opportunity for additional model development and characterization. Given the many NHP/AIDS models available for use in HIV vaccine studies, it is important that the most appropriate model, based on relevance and feasibility, be selected for each specific experimental question and that premature standardization for its own sake is avoided. Through the use of relevant, well-characterized parameters, in NHP/AIDS models HIV vaccine researchers have robust *in vivo* experimental systems that may provide key insights toward the preclinical development of a successful HIV vaccine.

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Key Points

- Multiple challenge models, each consisting of a unique combination of a NHP host species, a challenge virus prepared by a particular method, and a specific challenge route and schema, are available for vaccine and other prevention studies.
- Each of these factors can significantly impact study logistics, feasible analyses, outcomes and interpretations.
- Investigators should thoughtfully select the model whose features best address the specific experimental question of interest.
- Recent developments, including new SHIVs incorporating more clinically relevant HIV-1 Env sequences, generation of transmitted SIV clones, and sequence tagged “synthetic swarms” of challenge viruses offer new possibilities for conducting more sophisticated and potentially clinically relevant studies of vaccines and other prevention approaches.

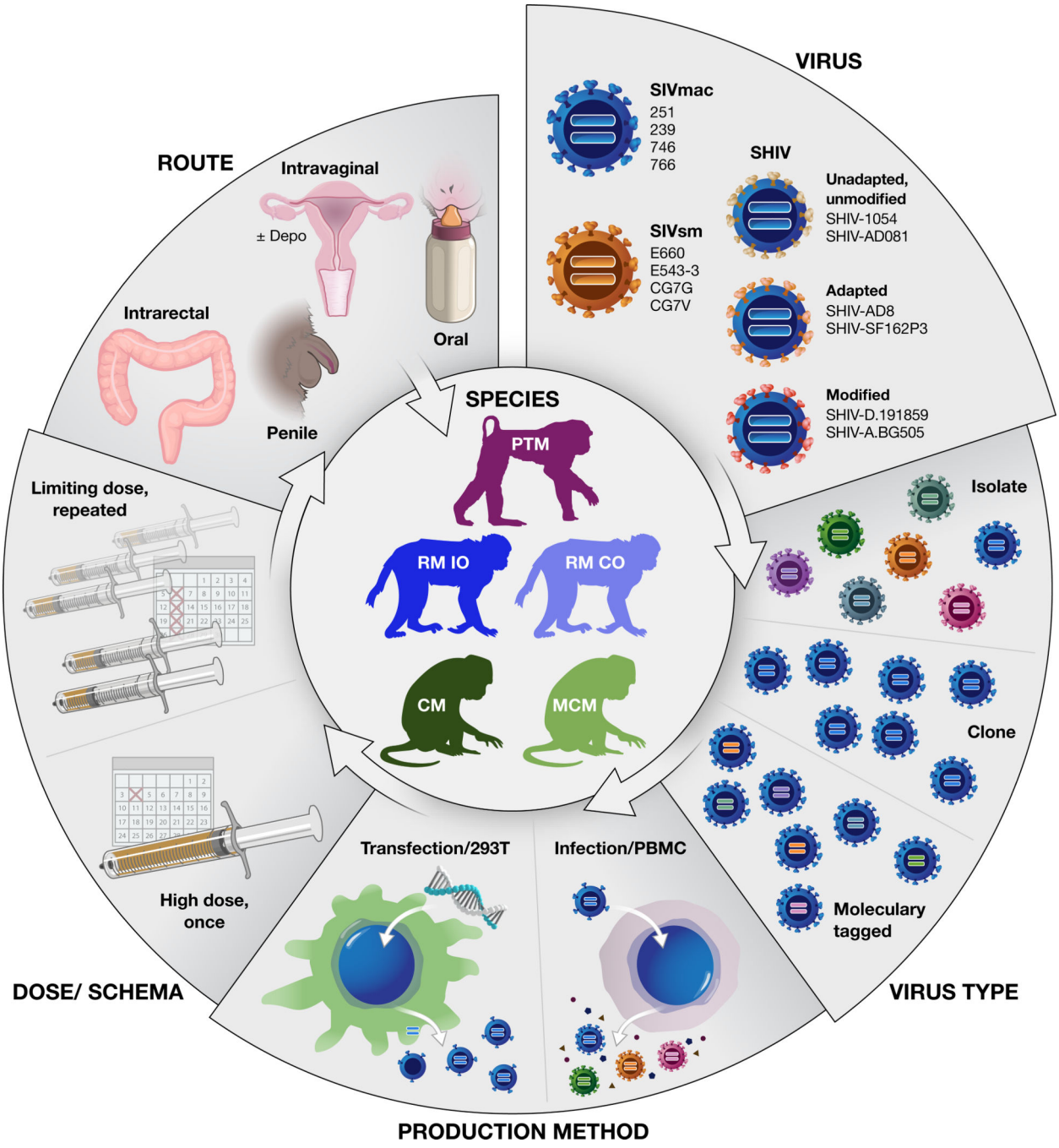

Figure 1.

Schematic representation of NHP/AIDS virus challenge study design considerations. Example SIV challenge viruses shown include those of the SIVmac and SIVsm lineages, including recently described transmitted/founder clones (SIVmac746, 766, SIVsmCG7G, CG7V) derived from these lineages [5]. Representative SHIV challenge viruses are also shown; additional SHIVs have also been recently described [1,2*–4*]. Depo, Depo-Provera.

PTM, pig-tailed macaque. RM IO, rhesus macaque Indian-origin. RM CO, rhesus macaque Chinese-origin. CM, cynomolgus macaque. MCM, Mauritian cynomolgus macaque.