Tackling HIV and AIDS: contributions by non-human primate models

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During the past three decades, non-human primate (NHP) models have gained an increasing importance in HIV basic and translational research. In contrast to natural host models, infection of macaques with virulent simian or simian-human immunodeficiency viruses (SIV, SHIV) results in a disease that closely resembles HIV infection and AIDS. Although there is no perfect animal model, and each of the available models has its benefits and limitations, carefully designed NHP studies with selection of experimental variables have unraveled important questions of basic pathogenesis and have provided the tools to explore and screen intervention strategies. For example, NHP studies have advanced our understanding of the crucial events during early infection, and have provided proof-of-concept of antiretroviral drug treatment and prevention strategies such as pre-exposure prophylaxis (PrEP) regimes that are increasingly used worldwide, and upon overcoming further barriers of implementation, have the potential to make the next generation AIDS-free. Remaining goals include the pursuit of an effective HIV vaccine, and HIV cure strategies that would allow HIV-infected people to ultimately stop taking antiretroviral drugs. Through a reiterative process with feed-back from results of human studies, NHP models can be further validated and strengthened to advance our scientific knowledge and guide clinical trials.

Despite recent progress in curtailing new infections, the human immunodeficiency virus (HIV) pandemic continues to be one of the big public health challenges of our time. In 2015, the estimated numbers of new HIV infections were 2.1 million, and 36.7 million people were living with HIV[1].

Since the discovery of HIV in 1983 as the causative agent of the acquired immune deficiency syndrome (AIDS), a plethora of studies have provided us with a better understanding of the many aspects of HIV biology, including genetic structure, transmission, and the virus-host cell interactions that determine pathogenesis[2,3]. Although behavioral interventions (such as promotion of ABC: Abstinence, Be faithful and Condoms) significantly reduce transmission, they are insufficient to halt the epidemic. Accordingly, there has been an enormous global effort to develop biomedical strategies to prevent and/or treat infection, of which the success rates have been highly variable.

Despite the current absence of an effective HIV vaccine, the success in developing a growing arsenal of antiviral drugs has been astounding. Antiretroviral therapy (ART), a combination of antiretroviral drugs (ARVs), has transformed an HIV diagnosis from what was once an automatic death sentence, into a chronic, manageable disease dependent on sustained treatment. An additional benefit of effective ART with sustained virus suppression is that it severely reduces the chances of HIV transmission from the treated patient to their uninfected partners[4]. Recent studies documenting the high efficacy of pre-exposure prophylaxis (PrEP) regimens to reduce infection rates among uninfected people with high-risk behavior have also generated high enthusiasm[5]. Altogether, science has already given the world the tools sufficient to make the next generation free of AIDS; reaching that goal depends on our ability to scale up implementation of proven strategies by mobilizing resources and breaking down barriers of violated human rights, poverty, stigma and discrimination.

In this review, we will reflect back on how the gradual development and proper use of nonhuman primate (NHP) models of HIV infection has contributed to our current status, and how they can guide us into the future to tackle the remaining challenges, particularly, our quests for the two holy grails: an effective HIV vaccine, and an HIV cure (**Fig. 1**).

The need for animal models

Despite all best efforts, there are currently no good *in vitro* systems, mathematical or computer models to recapitulate the full spectrum of HIV pathogenesis, the effects of antiviral strategies, and the many interactions among them. This is where animal models can be beneficial (**Fig. 2**). Animal models can be

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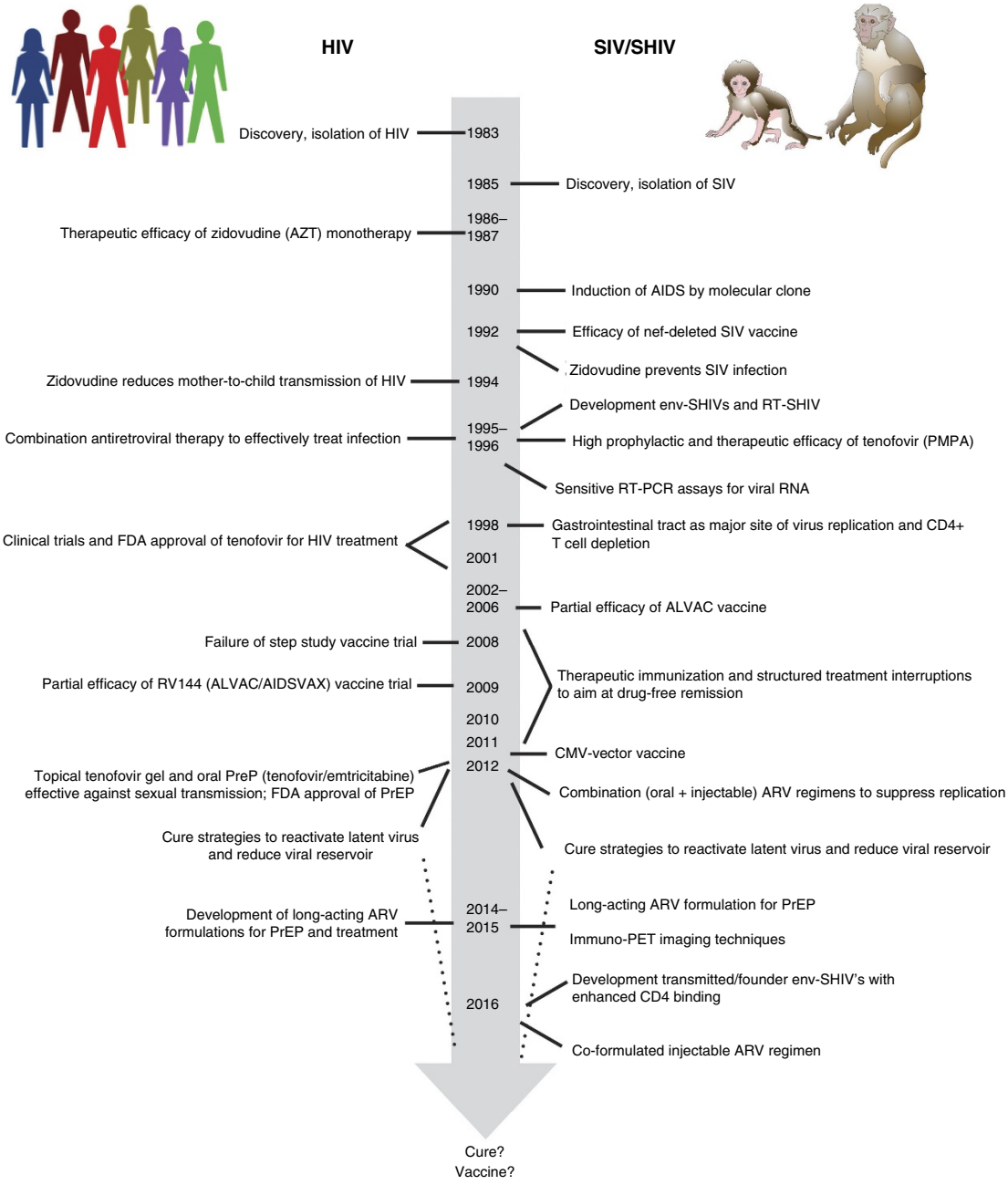

FIGURE 1 | Time line of HIV and SIV research. Without being able to be comprehensive, some of the milestones in SIV/SHIV research are presented with special emphasis on antiviral strategies for which proof-of-concept was first demonstrated in NHP models and of which efficacy was subsequently confirmed in clinical trials[22,31,32,44,45,71,78,92,94,98,99,104,105,107,129,134–146].

useful to explore novel hypotheses that are logistically difficult or unethical to explore in humans. Advantages of animal models include our ability to control the input variables (for example, characteristics of host factors, virus inoculum; antiviral intervention), and to do procedures that are difficult to do in humans (for example, administration of specific cell-depleting antibodies; access to tissue samples via biopsy or euthanasia). Accordingly, experiments can be performed to address specific questions, using a limited number of animals and in a defined time frame (**Fig. 2**).

The ideal animal model of HIV infection would be one that involves HIV infection of a small laboratory animal that closely mimics HIV transmission and disease pathogenesis. But such small animal models do not yet exist, and it is important to acknowledge that each of the currently available small or large animal models has its limitations.

Although several lentiviruses cause slowly progressive degenerative diseases of certain farm animals, a major difference with HIV infection of humans—and thus limitation—is that these lentiviruses infect only the macrophages and monocytes and not CD4+ T helper

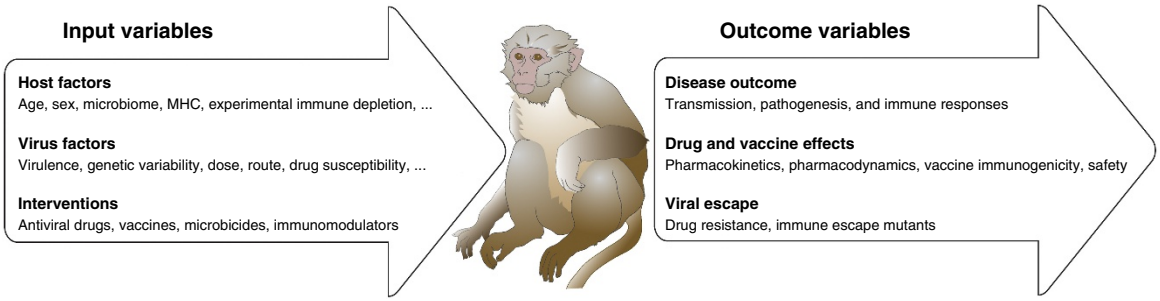

FIGURE 2 | The ultimate goal of antiviral strategies is to improve or maintain the overall health of the host by preventing infection or disease progression. Animal models allow us to control and manipulate many input variables through experimental approaches that are not feasible or ethical in humans, but that give us a better idea into pathogenesis and mechanisms of antiviral activity. The outcome is determined by many variables and interactions between the virus, the host and the antiviral drugs, most of which cannot be mimicked appropriately by *in vitro* studies, but which together determine the overall outcome of disease or health.

cells and, therefore do not cause immunosuppression in their hosts (reviewed in ref. 6). A variety of feline and rodent models of HIV infection have been developed; some of the rodent models have been engineered to be permissive to HIV-1 replication. While useful for initial screening (**Fig. 3**), each of these models has its own limitations (reviewed in refs. 7,8). Further testing is best done in NHP models. NHPs are phylogenetically the closest to humans, and have very similar physiology. In addition, as outlined below, NHP models that closely resemble HIV infection of humans have been developed.

The development of antiviral strategies can be accelerated by efficient and predictive *in vitro* systems, complemented with animal models, capable of screening and selecting the best products at each decision point (**Fig. 3**). An opportunity to discard ineffective products early in the development process (and thus save much time and resources), however, is lost whenever an available appropriate animal model is bypassed[9]. In addition to directly testing efficacy, animal models can be very useful to define surrogate markers of efficacy, which can help understand the mechanisms of action, guide clinical trials and accelerate future product development.

Key features of the NHP model: considerations on the species, virus, and infection method

Since the discovery of HIV and simian immunodeficiency virus (SIV) in the 1980s, and a better understanding of their origins and evolution[6,10,11], scientific progress has led to an increased availability of resources, reagents and NHP models. But there is no 'one-formula-fits-all' model. Instead, with so many choices, the craftsmanship of a research team resides in using the available knowledge to prudently select and balance the many host and viral input variables to target the experimental question at stake, while maintaining biological relevance and translational linkage to HIV infection in humans[12]. While there is merit in efforts for standardization, premature standardization for its own sake needs to be avoided[12].

NHP species used in HIV research

A common theme among the different NHP models is that a disease, which resembles AIDS in humans, is most consistently seen during infection of non-natural hosts. Chimpanzees in the wild are the source of SIVcpz, the immediate precursor of HIV-1[10]. Although SIVcpz infection of chimpanzees in the wild is associated with an increased mortality rate[13], very few animals that have been experimentally infected with SIVcpz or HIV-1 in captivity have developed disease; in addition, low availability, high price and ethical issues pose major constraints[14,15]. HIV-1 infection could be induced in young pigtailed macaques, but virus replication was not sustained and no disease was observed[16]. HIV-2 infection models have been developed with hamadryas baboons (*Papio hamadryas*) and several macaque species; depending on the HIV-2 isolates, the outcome varied from an AIDS-like disease with CD4+ T cell decline to no disease[17–19]; currently, these models are rarely used.

Many NHP species in Africa are naturally infected with SIV strains; examples are African green monkeys (SIVagm) and sooty mangabeys (SIVsm). These viruses are more closely related to HIV-2 than to HIV-1. Despite persistent virus replication, these natural hosts rarely develop diseases. Accordingly, these natural, nonprogressive SIV infections represent an evolutionary adaptation that allows a balanced coexistence of the virus and the host immune system, associated with phenotypic changes to CD4+ T cell subsets, limited immune activation and apoptosis, and preserved mucosal immunity[20,21].

In contrast, it was discovered in the 1980s that SIV infection of non-natural hosts, such as Asian macaques, results in a disease that recapitulates many features of human AIDS[22]. While limitations of the SIV-macaque models include their high cost, relative availability, and the subtle differences between HIV-1 and SIV (SIV resembles HIV-2 more than HIV-1), the many similarities in virus, host and disease pathogenesis, and the availability of many cross-reactive laboratory reagents to monitor immunological markers, have made macaques the most relevant NHP model in HIV research. Accordingly, they are the focus of the remainder of this review.

The most commonly used macaque species are rhesus (*Macaca mulatta*), cynomolgus (*M. Fascicularis*), and pigtailed macaques (*M. nemestrina*). Additional host factors within a species that have been observed to affect transmission and disease pathogenesis of certain virus isolates include origin (Indian or Chinese), MHC I alleles (for example, Mamu-A*01, Mamu-B*08) and restriction alleles (for example, TRIM5α)[23–27].

Considerations while selecting the virus

With the availability of multiple challenge viruses with different properties, the selection of the virus needs to be done carefully,

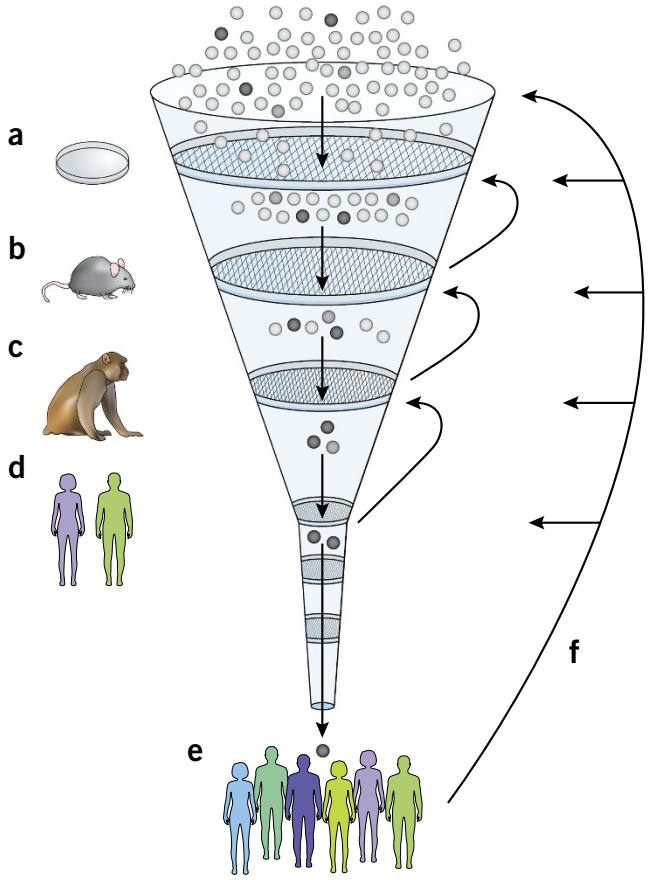

FIGURE 3 | Role of animal models in the development of anti-HIV strategies. Because human trials are very expensive, time-consuming and logistically complicated, only a limited number of antiviral strategies can be evaluated. Progress can be accelerated by an efficient and reliable pipeline process with defined decision points, to filter out non-effective or toxic compounds (open circles) and guide effective products (black circles) more rapidly through toward clinical testing. High-throughput systems with relatively low cost, such as *in vitro* testing (**a**) can be used as an initial screening method. While small animal models such as rodents (**b**) are useful for initial *in vivo* screening, NHP models (**c**) that resemble more closely HIV infection of humans are recommended as a final pre-clinical evaluation step, so the most promising compounds can enter sequential human clinical trials (**d**) and the best compound(s) can become approved for widespread use (**e**). Results down the pipeline need to feed back higher up into animal models and *in vitro* test systems for further validation and optimization to enhance their predictability (**f**).

because it can affect the outcome of the NHP studies. Initially only uncloned SIV isolates were available. The SIV isolates commonly used belong to a few groups, in particular SIVmac, SIVsm and SIVmne. While CD4 is their main cell receptor, most SIVs use CCR5 as a co-receptor. SIV variants with varying degrees of virulence have been developed, ranging from very virulent isolates (*e.g.*, SIVmac251) that induce persistently high viremia and rapid disease progression, to avirulent isolates which induce transient or low-level viremia and no disease, even in newborn macaques, and therefore have been explored as live-attenuated vaccines[28]. This spectrum of infection outcomes makes the NHP model also suitable to assess how genetic changes in the virus (*e.g.*, drug or immune escape mutations) affect virulence.

In experimental designs, the selection of viruses with high diversity ("viral swarms") or viral clones needs to be weighed carefully, as either one has benefits and limitations. In addition, once a challenge virus has been selected, the choice of a particular virus stock needs careful consideration, because the history and *in vitro* production methods affect viral genetic diversity and other nonviral components in the stock, which can impact the outcome of *in vivo* studies[12]. To allow for discrimination and enumeration of transmitted variants while minimizing phenotypic differences between variants, sequence-tagged synthetic swarms have been generated, by incorporating a series of identifiable synonymous mutations into a small region of an otherwise isogenic SIV or SHIV clone[29].

Although SIV is related to HIV-1, there are still differences that affect its susceptibility to certain ARV- or immune-based strategies. Preclinical testing of antiviral strategies in the SIV-macaque model is most relevant if the viral target and its susceptibility to inhibition naturally resemble, or can be engineered to resemble, that of HIV-1. While SIVs are susceptible to most ARVs, an exception are non-nucleoside reverse transcriptase inhibitors (NNRTI), such as nevirapine and efavirenz, which are active only against HIV-1 and not against HIV-2 or SIV[30]. Accordingly, the construction of infectious SIV/HIV-1 chimeric viruses, in which the reverse transcriptase (RT) gene of SIV was replaced by its counterpart of HIV-1 (so called RT-SHIVs) has allowed evaluation of NNRTI-containing regimens in primate models[31,32].

Similarly, because there are significant differences between SIV and HIV envelope, env-SHIVs that contain the HIV-1 envelope region into an SIV backbone have been constructed to allow direct testing of strategies that target the HIV-1 envelope region. Although high chronic viremia and the development of disease is not essential for prevention studies, such features are preferable for pathogenesis and treatment studies[12]. First generation env-SHIVs had the shortcomings that they were mostly derived from subtype B *env* genes cloned from chronically infected people, often after *in vitro* or *in vivo* serial passage, which affected their neutralization profiles. Some of these early SHIVs, because of CXCR4 co-receptor usage, had a disease pathogenesis (including very rapid CD4+ cell depletion) that differed from the typical course seen with HIV and SIV infection. Other early SHIVs that used the CCR5 co-receptor were either non-virulent, or did not give consistently persistent viremia[33]. In recent years, efforts to improve the env-SHIV model have focused on generating virulent env-SHIVs that encode *env* sequences from circulating transmitted/founder (T/F) viruses from a more diverse genetic background, and don't require serial passage[33]. Recently, introduction of specific mutations that improve interaction with the rhesus macaque CD4 receptor has led to enhanced replication fitness in macaques[34]. Further evaluation and optimization of these T/F SHIVs is likely to lead to improved value of the macaque model for testing vaccine-based strategies.

Considerations on how to infect

Similar to the low risk-per-exposure in humans[35], the rates of natural transmission from infected macaques to others animals (for example, through biting) in group-housed settings were slow and difficult to control[36], which makes it impractical to do timely studies with limited animal numbers. Therefore, to mimic the common

routes of HIV transmission in humans, NHP models have been developed that directly inoculate animals using different routes (i.e., intravenous, vaginal, penile, intrarectal, or oral)[37–40]. In addition, pediatric HIV infection models have been developed by using pregnant and infant macaques[41].

Using the direct inoculation routes, two main exposure models have been developed to achieve high infection rates; single high-dose, and repeated low-dose inoculation models. While earlier studies generally used a single high-dose inoculation, more recently, models with repeated low-dose inoculations have been developed[42]. The repeated low-dose mucosal inoculation models recapitulate many of the features of mucosal HIV transmission—such as transmission of a limited number of variants across the mucosal barrier and early diversification—better than the high-dose models[43]. The repeated low-dose inoculation models are particularly relevant to test prophylactic strategies where efficacy may be missed with the single high-dose inoculation models[44].

Contributions of NHPs to HIV science and pathogenesis

From the mid-80s to mid-90s, progress in gaining scientific knowledge from NHP studies was crippled by a relatively poor understanding of basic pathogenesis, the absence of sensitive, quantitative assays to monitor virus replication (especially during the asymptomatic stage), and a lack of potent, safe and easily administrable antiviral drugs to perturb the system and validate the NHP model for testing antiviral strategies. Because infection of macaques with very virulent SIVs results in more fulminant virus replication and faster disease course than typical HIV infection in humans, this also created a higher barrier to detect a beneficial effect of a relatively weak or mediocre antiviral strategy. The scarcity of promising intervention results, not surprisingly, instilled some skepticism about the value of the NHP models.

All of this changed in the mid-90s, with several breakthroughs, including the availability of quantitative and highly sensitive assays to measure viral RNA, the observations that plasma RNA levels were predictive of disease progression, and the discovery of potent antiviral drugs, such as tenofovir (**Fig. 1**). NHP studies could use the same markers (such as plasma RNA levels and CD4+ T lymphocyte counts) that were used in humans to monitor disease progression and the effect of antiviral strategies. Since then, the number of assays and reagents to monitor viral and immune markers during infection, and to disturb the system (such as monoclonal antibodies to deplete or block specific cell types) has grown rapidly and enhanced the value of NHP models. An exciting development in recent years is the development of real-time *in vivo* imaging techniques, such as immunoPET, to study viral kinetics and the impact of intervention strategies, which can reduce the need for invasive procedures to investigate tissues[45].

Because of the unique opportunities to control variables and collect samples, studies in macaques have provided a much better understanding of many host and viral factors that contribute to pathogenesis; from transmission with initial infection, to the role of humoral and cellular immunity in attempting to control virus replication, to the development of progressive disease[46–50]. For example, studies of early SIV infection have demonstrated rapid viral dissemination[51], and an important role of gut-associated lymphoid tissues, where acute infection leads to a rapid and persistent depletion of memory CD4+ T cells, loss of mucosal function and integrity, and microbial dysbiosis and translocation, which leads to chronic inflammation and immune activation that further drives disease progression.

HIV vaccine development

Since the initial discovery of SIV, the macaque model has been used extensively to explore different HIV vaccine approaches. Despite progress, we don't have an effective HIV vaccine yet. Many excellent review articles on the use of NHP models for preclinical HIV vaccine development have been published[48,49,52,53]. Accordingly, only a few key themes will be summarized.

While the ideal HIV vaccine provides sterilizing immunity (i.e., complete prevention of infection), a more realistic goal—consistent with the mode of action of other viral vaccines—is that vaccine-induced immune responses may not prevent initial infection, but would block or reduce systemic dissemination and permanently protect against disease progression. A challenge for vaccine development is that immunologic mechanisms that mediate protection against initial infection may be different from those that control virus replication after initial infection.

Using NHP models, a large variety of vaccine strategies, using different immunogens, adjuvants and prime-boost combinations, have been tested and found to have variable levels of efficacy. Progress has been intermittent, rather than steady, because of the extreme difficulty to identify a single *in vitro* immune effector function or combination of such markers that is consistently associated with efficacy[54]. Likewise, a comparison of studies suggest that different vaccine strategies may achieve some level of efficacy through different mechanisms, and the outcome can also depend on host genetics (such as MHC alleles or restrictive TRIM5 alleles) and the selection of input variables of the model (including viral strain, dose and route of inoculation)[55–57]. As a result, the pendulum of focus has often swung back and forth between vaccines that induce antibodies, cell-mediated immune responses, or a combination of both.

Support for the protective role of antibodies comes from many passive immunization studies in macaques that demonstrated efficacy of antibodies with high *in vitro* antiviral activity (either through direct neutralization, or via antibody-dependent cell mediated inhibitory mechanisms)[58–63]. However, the challenge is that it has been very difficult to induce potent antiviral antibodies through active immunization[64], likely because cross-reactivity between HIV Env gp41 and gut microbiota diverts immune responses away from that pathway[65].

Numerous other macaque studies have demonstrated an important role of CD8+ and CD4+ T cell-mediated immune responses[48]. While initial attention was largely focused on the induction of central memory T cell (Tcm) responses, recent studies using a rhesus cytomegalovirus vector that induces effective memory T cell (Tem) responses have demonstrated high efficacy in approximately half of the animals, that, despite initial blip(s) of viremia, clear SIV then to undetectable levels[56,66]. A better understanding of the mechanisms of protection of this vaccine is likely to provide clues to further improve vaccine development.

Despite our relatively limited understanding of antiviral immunity, one theme that has emerged over recent years is that

'the more the better' does not apply to HIV vaccine development. Macaque studies have demonstrated the need for an intricate balance between quantity, quality, timing and location of the different antiviral immune responses—both adaptive and innate—as too much immune activation and inflammation can be harmful by promoting virus replication and dissemination[67–69]. The need for such balance is also corroborated by results of phase III human clinical trials, such as the STEP Study, which—despite being first believed to have good cell-mediated immunogenicity—failed to protect and even enhanced infection in certain subgroups; and the Thai ALVAC/AIDSVAX trial, which, despite relatively low immune responses, had a 30% reduced infection rate[70,71]. Accordingly, circular feedback between animal studies and human clinical vaccine trials will be important for further optimization of (i) *in vitro* assays to measure immune correlates of protection, and (ii) the input variables of *in vivo* NHP vaccine studies, to enhance their predictability for subsequent HIV vaccine trials.

NHP models and microbicide development

In the absence of an effective HIV vaccine, there has been considerable interest in the development of microbicides, or compounds that after topical application onto mucosal surfaces can prevent infection. As more detailed reviews on the use and value of NHP models for microbicide development have been published[72,73], a few highlights are discussed here.

NHP studies have demonstrated that short-term topical high-dose administration of a relatively large number of compounds, targeted near the time of a single atraumatic intravaginal or intrarectal SIV or SHIV exposure, protected macaques against infection. However, when tested in clinical trials, most microbicides have fared poorly, and sometimes increased susceptibility[74–78]. The most likely reason of this discrepancy is that first-generation microbicides were mostly compounds that disrupted the viral membrane. In human clinical trials, and subsequently confirmed in macaque studies, the repeated administration of these first-generation microbicides led to a disruption of the epithelial barrier or irritation of the vaginal mucosa with recruitment of target cells; such local inflammation and immune activation, likely aggravated by coitus-induced mechanical trauma, enhanced susceptibility to infection[72–75].

Since then, the focus of microbicide research has gradually shifted to so-called 'second-generation microbicides', which include virus-specific inhibitors. Particular attention was given to those ARVs, such as tenofovir, that have a long intracellular half-life and were proven to be effective in animal models when systemic drug levels were also effective. Results of NHP studies demonstrating efficacy of vaginal gels containing tenofovir or other ARVs[79–81] were predictive of the partial efficacy of a 1% tenofovir gel in protecting women against HIV infection in the CAPRISA 004 trial in South-Africa[78]; efficacy correlated with adherence and mucosal drug levels[82]. To increase adherence, safety and efficacy, ongoing research efforts in NHP models and human trials include the use of combination microbicide vaginal rings, potentially combined with contraceptives[83,84].

NHP models and antiretroviral drug development

As touched upon earlier, NHP models for preclinical drug development had to gradually overcome many hurdles. In the mid-1990s, a sudden leap of progress was made with the discovery of tenofovir (previously called PMPA; 9-[2-(phosphonomethoxy)propyl]adenine). The demonstration of tenofovir's high efficacy in NHPs before human studies had multiple impacts. In addition to providing the scientific rationale to initiate human trials with this compound, it provided proof-of-concept of the validity of the NHP model, it raised the bar of expectations for subsequent strategies, and it established tenofovir as a cornerstone to build potent combination regimens which are now widely used.

Studies in macaque models have contributed significantly to our knowledge on the many aspects of antiretroviral drug administration.

Pharmacokinetics and safety

Because of their similar physiology and metabolism, non-human primates have been useful to study the toxicity and pharmacokinetics of ARVs, including the effects of pregnancy and drug transfer across the placenta and into breast milk[85]. Newly developed methods to measure tissue distribution and intracellular drug levels provide additional opportunities to correlate pharmacokinetics with efficacy and safety[86–88].

While most studies used short-term drug administration, studies with tenofovir have demonstrated the value of NHP to study the safety of prolonged treatment regimens (up to 14 years), starting at birth and continuing throughout adulthood, including pregnancy[89–91]; prolonged drug safety studies in NHP are especially relevant, considering, in the absence of a cure, the requirement of lifelong treatment and the risk of teratogenicity.

ARV-drug based strategies to prevent infection

Many NHP studies have investigated whether oral or parenteral administration of ARVs near the time of virus inoculation could prevent infection. Early studies were not very effective in preventing infection, but a likely reason for this was the combination of a high-dose virus inoculum, the direct intravenous route of virus inoculation, and the relatively weak potency of drugs at that time[85]. The proof-of-concept that ARVs could prevent infection was demonstrated with zidovudine, which protected infant macaques following a low-dose intravenous inoculation[92]. Subsequently, a growing series of studies which used low virus doses, sometimes combined with a mucosal route of virus inoculation, have demonstrated that administration of a number of ARVs starting before, at the time of virus inoculation, or shortly thereafter, were able to prevent infection at varying success rates[85]. General take-home messages are that (i) pre-exposure prophylaxis (PrEP) is more successful than post-exposure prophylaxis (PEP), (ii) with potent drugs, such as tenofovir and emtricitabine, short or intermittent regimens that target the timings of viral exposures can be effective, (iii) the intravenous route of virus infection is the most difficult one to protect against, (iv) for post-exposure prophylaxis (PEP), time is of the essence; even if PEP is started within the first 24 h, a combination of the timing and duration of drug administration determines the success rate, as a delay in the start, a shorter duration, or interruption of the treatment regimen all reduced efficacy, (v) in some PEP studies, efficacy was partially mediated through the induction of antiviral immune responses that helped to control infection, (vi) some drugs (for example, tenofovir) can still be (partially) effective against viral mutants with reduced *in vitro* drug susceptibility.

The abundance of evidence of the efficacy of ARVs in preventing infection in NHP models, coupled with the absence of an effective HIV vaccine, has provided the scientific impetus to test similar strategies in humans in several clinical settings. Early on drug regimens, particularly those containing zidovudine and nevirapine, were proven to be effective in reducing the rate of mother-to-infant transmission of HIV, including in developing countries[93–95]. In addition, PEP has been routinely recommended after occupational (for example, needle-stick accidents of health care workers) and non-occupational exposures to HIV[96].

In recent years, there has been a growing interest in developing PrEP regimens to prevent sexual transmission, and transmission through needle-sharing among injecting drug users. Based on the promising NHP data, tenofovir (with or without emtricitabine) became the leading candidate. During the past years, clinical trials have demonstrated prophylactic efficacy of a daily PrEP regimen in men who have sex with men, heterosexual men and women, and injecting drug users; efficacy correlated strongly with drug levels and adherence[97–101]. To overcome the barrier of poor adherence with daily PrEP regimens, several new strategies are being explored. Recent clinical studies have demonstrated high efficacy of "on-demand" PrEP (i.e., taking tablets shortly before and after each high-risk sexual act)[102]. An alternative approach to overcome poor adherence is the use of long-acting drug formulations where a single administration results in protective systemic drug levels for weeks or months. While proof-of-concept of this approach has been demonstrated with an integrase inhibitor in NHP models of intravaginal and intrarectal infection[103,104], clinical trials have been initiated with the non-nucleoside RT inhibitor (NNRIT) rilpivirine[105]. In conclusion, the high efficacy of PrEP with proper adherence has generated a lot of excitement, which drives the continued efforts to overcome the remaining practical, ethical and financial barriers needed to roll-out wider implementation of PrEP strategies in real-world settings[106].

ARV therapy and the effects of drug resistance

Well before it was fully recognized in HIV-infected patients and it became included in treatment guidelines, NHP studies provided important proof-of-concept of the benefits of early ARV therapy: early treatment with several ARVs available at that time (which in hindsight were not very potent), even when given as monotherapy, delayed or reduced the peak of acute viremia, enhanced antiviral immune responses and delayed disease progression[85].

For these earliest available ARVs, which had problems of administration (for example, macaques can refuse oral medicines with poor palatability), toxicity or relatively weak efficacy in HIV-infected people, it was difficult to detect therapeutic benefits in NHPs when treatment was started during chronic SIV infection, especially when virulent SIVs were used that induced high viremia and rapid immunosuppression. However, from 1995 onwards, more potent, safer and easily administrable drugs, like tenofovir, became available and allowed prolonged antiviral drug studies. However, even with relatively potent drugs, monotherapy was generally less effective for late therapy than for early therapy; with late therapy, the reduction in viremia was often transient or undetectable, and hindered by the faster emergence of viral mutants with reduced drug susceptibility[85].

In recent years, a growing arsenal of potent ARVs has become available to treat SIV or SHIV-infected macaques with combination regimens of which the efficacy mirrors that seen in humans. Co-formulated injectable combination regimens containing 3 or more antiretroviral drugs have been developed that are well tolerated and enabled to achieve sustained suppression of viremia in infected NHPs[107]. These drug combinations also provide powerful tools to gain further insights into disease pathogenesis and the effects of drug therapy, including studies to investigate viral reservoirs during therapy, with the aim of developing strategies aimed at a cure .

Although the emergence of drug-resistant viral mutants has become less of a problem with the newest ARV combinations, if proper adherence is followed, it was a big problem for the older drug regimens, and still continues to limit patients' options for 2nd and 3rd line regimens. Since the first description of a zidovudine-resistant SIV mutant 20 years ago[108,109], NHP models were used to gain insights in the emergence and clinical implications of such drug-resistant mutants, such as Q151M, K65R and M184V RT mutants[85]. In contrast to what is feasible in humans, an animal model allows the most direct and controlled approach to study the replication fitness, virulence and clinical implications of drug-resistant virus: animals can be inoculated with drug-resistant viral mutants or their wild-type counterparts, and viremia and clinical outcome can be compared in drug-treated versus untreated animals. NHP studies demonstrated that, depending on the drug and the individual host factors, the clinical implications of drug-resistant virus can range from loss of benefits to sustained benefits of antiretroviral therapy[108,110,111].

NHP experiments have also provided important proof-of-concept of the role of antiviral immune responses during antiretroviral drug treatment, because transient CD8+ cell depletion during suppressive monotherapy or combination therapy led to a transient increase of viremia[112–114]. Such observations helped us to understand that the success of antiviral drug therapy is more than just a combination of sufficient drug levels and susceptible virus, and have led to the development of a model of viral dynamics during drug therapy that incorporates the role of the immune system, and the replication fitness and virulence of drug-resistant virus. In this model (**Fig. 4**), antiviral immune responses contribute significantly to the antiviral efficacy of drugs by reducing the burst of virus replication in productively infected cells. In the absence of such antiviral immune responses, antiviral drugs face a more daunting task to control viremia as already infected cells can survive longer and produce more viral progeny[115].

This model also incorporates the effects of altered replication fitness or diversity of mutant virus, and/or residual drug activity. In particular, even a relatively minor decrease in replication fitness or viral heterogeneity, or a partial inhibition of virus replication by the drug regimen, can have a major impact on viremia if it provides more opportunity for antiviral immune responses to kill or inhibit productively infected cells before the major viral burst. In contrast, without effective antiviral immune responses (such as during late-stage disease), a small difference in replication fitness or residual drug activity may not translate into any significant difference in viremia and clinical outcome[114,116,117].

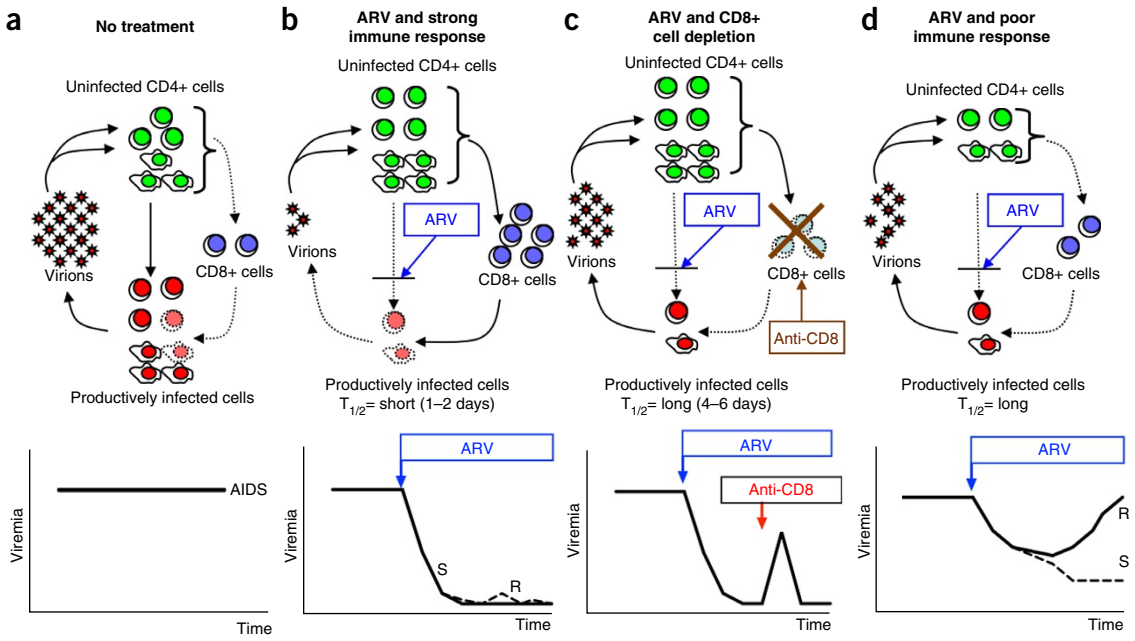

FIGURE 4 | Model of viral dynamics during antiretroviral drug therapy (modified from ref. 114). (**a**) Without drug treatment, virulent virus replicates to high titers because of high infection rates of CD4+ T helper cells and antigen presenting cells which are unable to provide sufficient assistance to CD8+ cell-mediated immune responses to contain virus replication. Progressive destruction of the immune system leads to AIDS. (**b**) A potent drug regimen reduces the number of CD4+ T helper cells and antigen presenting cells that become newly infected. Potent CD8+ cell-mediated immune responses reduce the half-life of and thus the burst size of viral progeny for those cells that already became infected. The combined antiviral activities of drug(s) and antiviral CD8+ cells are efficient to induce and maintain low or undetectable viremia, especially for susceptible (S) virus, but potentially even for drug-resistant viral mutants (R), with occasional blips, as shown for tenofovir in the macaque model[91,113,114]. (**c**) During artificial CD8+ cell depletion of ART-treated animals with low viremia, productively infected cells survive longer and produce more progeny virus, resulting in transiently higher viremia, which returns to baseline upon return of CD8+ cells; this has been shown to occur both in combination therapy as well as tenofovir monotherapy[112,114]. (**d**) During immunodeficiency, the reduced function of antigen presenting cells and CD4+ T helper cells results in insufficient assistance to antiviral CD8+ cells to remain active, especially at lower levels of viremia. Even when infection of new cells is reduced by an efficient drug regimen, the half-life of the productively infected cells is long, resulting in a less effective suppression of virus replication. If the virus remains susceptible (S), viremia can become low, but not necessarily undetectable. But without sufficient immune restoration, the emergence of drug-resistant mutants (R) is expected to lead to a rebound in viremia[111,113,148].

This model helps to explain the variability in the virologic responses that are observed among individuals and among study cohorts, including observations that certain drug regimens can still provide therapeutic virologic and/or immunologic benefits in the presence of drug-resistant virus[115,118–121]. This synergism between antiviral drugs and antiviral immune responses supports the exploration of immunotherapeutic strategies with the aim of restoring the immune system or enhancing antiviral immune responses to reduce the need for continued ART.

Beyond treatment: the ultimate goal of curing HIV

Considering the bleak prognosis for HIV-infected patients during the early years of the epidemic, our current ability to manage HIV infection with ARVs and other supportive interventions represents a triumph of research and modern medicine[122]. However, the need for proper adherence and regular hospital visits, the cost of the drugs, and the risk of toxicity (which may be cumulative after decades of treatment) continue to fuel the quest for an HIV cure, in which people would be able to stop taking ARVs.

In the so-called Berlin patient, radiation followed by an allogeneic Δ32 CCR5 bone marrow transplant to treat leukemia seems to have eradicated infection. While this sparked great excitement for the prospects of a sterilizing cure (i.e., where infection is totally eliminated), further studies on additional patients failed to see this effect, as after withdrawal of ARVs, viremia eventually rebounded. Because a sterilizing cure will be challenging to achieve, the focus has shifted to a long-term drug-free remission, also called functional cure, in which after ARV treatment interruption, viremia remains low or undetectable for a prolonged time due to immunological control[123].

NHP studies have made major contributions in our stepwise progress toward a cure, and many results from NHP studies have been corroborated by observations in humans. Early on, it was hypothesized that prolonged ARV treatment by itself could potentially lead to elimination of viral reservoirs[124]. However, numerous studies in macaques and humans have demonstrated that when administration of suppressive ARV regimens is initiated during chronic infection, there is insufficient immune restoration, and without sufficient antigenic stimulation, antiviral immune responses fade over time; accordingly viremia generally rebounded once ARVs were stopped[85,123].

Accordingly, as a logical next step, investigators explored combining ARVs with immunotherapeutic strategies with the aim of restoring the immune system or enhancing antiviral immune responses

so that when drug treatment was stopped, viremia was controlled better. Although a variety of strategies (including structured treatment interruption and active immunizations) showed benefits from the additional intervention, and those benefits were most prominent when ARVs were initiated early in infection. The benefits during later infection had more individual variability, and viremia generally still rebounded[125]. An exception was a study in which infected macaques received prolonged tenofovir monotherapy (>8-10 years), and upon tenofovir withdrawal, controlled replication for a follow-up period of 1 year. The likely reason for this result was that these animals had K65R RT viral mutants with reduced susceptibility; therefore, the ongoing low-level virus replication during tenofovir treatment acted as an autologous viral vaccine that induced a unique virus-host balance, characterized by potent viral immune responses that controlled virus replication after drug withdrawal[91]. Although this study provided hope for a functional cure, it also highlighted the need to develop strategies that would achieve such effect in a shorter time frame and in individuals who have maximal suppression of wild-type virus.

Because of these earlier results, it became clear that the next step would be to focus on strategies aimed at reducing viral reservoirs before withdrawal of ARVs. Viral persistence in reservoirs is complex and multifactorial. Because viral latency in CD4+ T cells and macrophages is likely an important mechanism of viral persistence, an approach to eliminating such cells is the "shock-and-kill" approach, based on the hypothesis that HIV latency can be reversed through latency-reactivating agents, which leads to the clearance of these cells through virus- or immune-mediated cytolysis. As viral gene expression and viral replication by themselves may not be sufficient to induce the death of the infected cell, additional immunotherapeutic strategies to selectively and effectively destroy these infected cells are likely needed[123]. Such strategies aimed at reactivating latent virus and eradicating viral reservoirs are likely to carry the risk of adverse effects, such as toxicity, or, in the case of incomplete eradication, a rebound of viremia upon withdrawal of ARVs, which may affect future treatment options. Without proof of concept, only a few people who fare well on a stable ARV regimen may be interested to volunteer for such clinical trials. This dilemma emphasizes the value of NHP models in the preclinical development and screening of such methods. As outlined earlier, while studies in NHPs offer the advantage to control many variables and have extra access to tissue samples to evaluate pharmacokinetics, tissue distribution, efficacy and safety, a limitation remains the high expense associated with prolonged infection and treatment studies. Therefore, animals are generally started earlier on ARVs, and kept on a suppressive ARV regimen for a shorter time before the intervention, in comparison to the situation of most HIV-infected people. It is currently unclear how this affects the outcome of the observations.

In recent years, several studies in NHPs have shown a promising effect on reactivating latent virus, reducing viral reservoirs and/or reducing viral rebound after ARV withdrawal. These studies have used a variety of strategies including protein kinase C activators, histone deacetylase inhibitors, TLR-7 agonists, or modulators of cell differentiation (auranofin) or trafficking (anti- $\alpha 4\beta 7$ antibody)[126–132]. Data from ongoing and pending human clinical trials will need to determine how well the observations of the NHP models translate into potential clinical applications for HIV-infected people. Although the ultimate goal would be to discover a relatively simple "magic bullet" regimen, for now it is more likely that a functional cure will consist of a combination of strategies, that will need to be fine-tuned and personalized for each patient. Reaching that goal depends on continuing stepwise progress, in which NHP models will continue to provide valuable contributions.

CONCLUSIONS

During the past 3 decades, since the initial discovery of HIV and SIV, NHP models of HIV infection and AIDS have evolved dramatically. Thanks to the persistence and audacity of researchers, initial obstacles were overcome, and the gradual development of better resources and reagents, coupled with a growing understanding of disease pathogenesis, have improved the usefulness of NHPs to test hypotheses that are difficult or impossible to explore otherwise, and to evaluate novel intervention strategies.

The relevance of NHP models has been clearly established, as studies in macaques played a crucial role in the development of several ARV-based strategies that are now increasingly used. While we still hope to see the icing on the cake—an HIV vaccine and a cure—that would make NHP models of HIV infection eventually become obsolete, it is important to be aware of the larger impact of this journey. Many of the resources, reagents and tools developed to study NHP models of HIV are now increasingly being applied to tackle other human and animal diseases, including emerging diseases such as Zika virus[133]. This extra return on investment emphasizes the need for a continued concerted effort to carefully design and use animal models to improve global health.

COMPETING FINANCIAL INTERESTS
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