Review

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Exploring HIV Vaccine Progress in Pre-Clinical and Clinical Setting: From History to Future Prospects

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Abstract: The human immunodeficiency virus (HIV) continues to pose a significant global health challenge, with millions of people affected and new cases emerging each year. While various treatment and prevention methods exist, including antiretroviral therapy and non-vaccine approaches, developing an effective vaccine remains the most crucial and cost-effective solution to combat the HIV epidemic. Despite significant advancements in HIV research, the HIV vaccine field has faced numerous challenges, and only one clinical trial has demonstrated a modest level of efficacy. This review delves into the history of HIV vaccines and the current efforts in HIV prevention, emphasizing pre-clinical vaccine development using the non-human primate model (NHP) of HIV infection. NHP models offers valuable insights into potential preventive strategies for combating HIV, and it plays a vital role in informing and guiding the development of novel vaccine candidates before they can proceed to human clinical trials.

Keywords: HIV epidemic; vaccine development; non-human primate model (NHP)

1. Introduction

Human immunodeficiency virus (HIV) continues to impose a significant global health impact, affecting millions of individuals annually with new infections. progress in treatment and prevention approaches, the imperative to develop an effective HIV vaccine remains urgent. Vaccines have historically played a crucial role in controlling and eradicating infectious diseases, and an equally effective HIV vaccine could be transformative in curtailing the epidemic. However, HIV poses unique challenges due to its ability to target the immune system, rapidly mutate, establish latent reservoirs, and evade immune responses. Intensive research efforts have been devoted to understanding the intricate biology of HIV and developing safe and effective vaccine candidates. The evolution of HIV vaccine research spans almost four decades and aligns with an enhanced comprehension of HIV and host immune responses.

History of HIV Vaccine Development. The history of HIV vaccine development spans nearly 40 years and initially focused on the role of antibodies, based on the concept that neutralizing antibodies could protect against HIV infection by preventing its entry into target cells (1) (2) (3) (4) (5). However, the highly variable and mutable nature of HIV presented significant challenges in eliciting broadly effective neutralizing antibody responses (6). A subsequential wave of HIV vaccine development shifted the attention to CD8+ T cells, recognizing their significance in HIV infection and control. HIV-specific CD8+ T cells can kill target cells and respond to multiple HIV strains (7). The broad recognition capability of CD8+ T cells was shown to be critical in preventing viral escape and could be harnessed to develop a globally effective multi-clade HIV vaccine. T cell-inducing recombinant viral vectors, such as adenovirus and poxvirus-based vectors, as well as DNA-based HIV vaccines, were developed based on this concept (8). During this HIV-vaccine era, significant progress was made in understanding T cell responses and identifying T cell epitopes, however it was found that CD8+ T cells alone could not eliminate the virus, nor could they protect against HIV acquisition (9). Additional hurdles of T cell vector-based strategies were immune responses to the vector itself (as

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opposed to HIV inserts) blunting vaccine immunogenicity and generation of newly activated CD4+ T cells, the preferred target of HIV, accentuating infection.

The third wave of HIV vaccine development focused on using a dual approach to harness immune responses elicited by DNA or vector-based vaccines in combination with protein components in prime-boost strategies. One such strategy quickly progressed to clinical trials and resulted in the only successful HIV efficacy vaccine trial in humans to date, although the efficacy was modest. This era also emphasized the exploration of new and improved adjuvants for the protein components, as the currently licensed adjuvant, alum, was found to be less effective than non-licensed adjuvants in inducing high HIV-specific antibody titers. However, subsequent attempts to enhance vaccine efficacy, including modifying adjuvants, have not yielded significant results in clinical settings (10, 11) (12).

Recent HIV Vaccine Development. In recent years, there has been a shift towards reevaluating the types and functions of antibodies necessary for prevention, the quality of induced T-cell responses, and the importance of boosting innate immune responses alongside adaptive-specific responses. Current research predominantly focuses on broadly neutralizing antibodies (bNAbs), which can neutralize a wide range of HIV strains by targeting conserved regions of the virus. These bNAbs provide valuable insights into potential targets for vaccine-induced antibody responses. Researchers are actively investigating strategies to elicit bNAbs through vaccination, either by administering bNAbs directly or designing immunogens to stimulate their production. Additionally, RNA vaccines have shown promise in infectious diseases, including the successful development of mRNA vaccines against COVID-19. Ongoing research and development efforts are exploring the use of RNA-based vaccines for HIV, incorporating conserved regions of the virus in vaccine designs to enhance effectiveness against a broader range of HIV strains.

The evolution of HIV vaccine research reflects the dynamic nature of the field and the adaptive response to the challenges posed by the virus. The shifting focus in the HIV vaccine field underscores the need for a multidimensional approach to developing an effective vaccine. It is now recognized that harnessing multiple aspects of the immune response, including neutralizing antibodies, non-neutralizing antibody effector function, T cell immunity, and potentially other immune mechanisms such as different arms of innate immunity, will be required for comprehensive protection against HIV infection. This review provides an overview of the current landscape of HIV vaccine research, highlighting key advancements, challenges, and promising strategies on the path towards developing an effective preventive HIV vaccine.

2. HIV Vaccines: Clinical trials

Protein-based vaccines. The first Phase 3 HIV vaccine efficacy trials ever conducted in humans were the VAX003 and VAX004; the vaccine was a bivalent gp120 envelope protein formulation with alum adjuvant to induce anti-HIV envelope antibodies (5, 13). VAX003 enrolled people who inject drugs in Thailand and used bivalent envelope subtype B and AE proteins. In contrast, VAX004 enrolled men who have sex with men and women at risk for heterosexual acquisition of HIV in the Americas and used bivalent subtype B proteins. Neither trial was successful in preventing HIV infection or decreasing viral replication or slowing disease progression. These results suggested that bivalent protein vaccination alone cannot provide protective efficacy against HIV (13).

Adenovirus Vector-based Vaccines. The disappointing results of the VAX003 and VAX004 trials led to a change in strategy towards developing vector-based vaccines that elicit strong HIV-specific T-cell responses (7). The STEP trial was designed to evaluate a replication-defective adenovirus serotype 5 (Ad5) vectored vaccine expressing HIV Gag, Pol, and Nef antigens, and enrolled men having sex with men (MSM), sex workers, and participants with elevated heterosexual risk in the Americas and Australia (14). The vaccine induced robust cellular immune responses, particularly HIV-specific CD8+ T cell responses. These responses were measured by analyzing the production of interferon-gamma (IFN-g), a cytokine involved in antiviral immune responses. Despite the induction of strong cellular immune responses, the STEP trial did not demonstrate efficacy in preventing HIV infection or reducing viral replication in those who became infected. In fact, an unexpected finding

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from the trial was an increased risk of HIV acquisition in a specific subset of participants who were uncircumcised and had pre-existing immunity to the Ad5 vector used in the vaccine.

After the lack of efficacy of the STEP trial, the Phambili trial HVTN 503 using the same Ad5 HIV-1 vaccine (15) was also terminated early because of the results of the STEP trial.

In vitro experiments suggested that Ad5-specific CD4+ T cells are highly susceptible to HIV infection and that these cells are preferentially lost in HIV-1-positive individuals. These findings raised important questions about the effect of pre-existing anti-vector immunity and using Ad5-vectored vaccines where Ad5 is prevalent. As a follow-up the HVTN 505 trial, a phase IIb clinical trial was conducted by the HIV Vaccine Trials Network (HVTN) to evaluate a DNA prime /rAd5 boost regimen that included immunogens targeting HIV Env, Gag, Pol, and Nef. The trial enrolled 2,504 Ad5 seronegative participants who were at high risk of HIV infection including men and transgender females who have sex with men. However, the trial was halted early in April 2013 due to the lack of efficacy observed in interim analyses.

The Ad26 “mosaic” vaccine was developed by Janssen Pharmaceuticals and utilizes viral vectors (Ad26 or modified vaccinia Ankara - MVA), protein boosts, and specially optimized immunogen sequences to create polyvalent "mosaic" antigens. These antigens aim to elicit both T cell responses and neutralizing antibodies, incorporating Env into their design. Mosaic antigens are generated from natural sequences, including common B and T cell epitopes while excluding rare ones (16). Clinical use of these mosaic antigens draws insights from NHP studies (discussed below) and the APPROACH study, which evaluated various regimens containing Ad26 or MVA vectors expressing mosaic antigens, some administered together with gp140 boosts. All regimens have proved safe and well-tolerated, with strong antibody responses detected. The mosaic antigens elicited binding IgG responses to cross-clade transmitted/founder Envs and other variants, similar to vaccine homologous responses. ADCP responses were found to be increased in the gp140-boosted groups, and serum neutralizing activity was observed against difficult to neutralize - tier-1 HIV variants. Subsequent clinical trials (TRAVERSE, ASCENT, IMBOKODO, and MOSAICO) expanded on these findings, testing various formulations and regimens (17). These trials have shown promising results in terms of safety and immunogenicity, with some formulations advancing to larger Phase 3 trials. However, phase 3 trials IMBOKODO did not prevent HIV infection in a population of young women in sub-Saharan Africa and reached a vaccine efficacy of only 25%. The phase 3 trial MOSAICO (or HPX3002/HVTN 706) was tested among men who have sex with men (MSM) and transgender people, involving 3,900 volunteers ages 18 to 60 years in Europe, North America, and South America. This trial also proved ineffective and was discontinued in 2023. Currently other Ad-based vectors are under investigation, including Ad35 (NCT01264445, phase I) (18) and Ad4 (NCT01989533, phase I) (19).

ALVAC Vector-based Vaccines. The RV144 Phase III HIV-1 vaccine trial was conducted in Thailand from 2003 to 2009 (20) (21). Enrolling over 16,000 participants from the general population, the trial was a collaborative effort between the Thai Ministry of Public Health, the US Army Surgeon General, the US Military HIV Research Program (MHRP), and various Thai and US government agencies, private companies, and nonprofit organizations (22). The vaccine regimen employed in the RV144 trial involved a prime-boost strategy, combining two vaccines: ALVAC-HIV from Sanofi Pasteur and AIDSVAX from VaxGene. These vaccines were designed based on HIV-1 B and E clades prevalent in Thailand. The primary objective of the trial was to evaluate the efficacy of this combination in preventing HIV infection and reducing viral RNA levels in infected individuals. Despite initial doubts and debates surrounding the immune response generated by this vaccine combination, the RV144 trial demonstrated its safety showed 60% vaccine efficacy (VE) at 12 months post-immunization, which decreased to 31% at 3.5 years (20). While the level of efficacy, was modest the results provided encouraging evidence for the feasibility of an HIV vaccine and indicates that further research is necessary to develop a vaccine capable of effectively safeguarding the general population against HIV acquisition. Notably, the RV144 vaccine did not generate neutralizing antibodies, nor CD8+ T cell responses, prompting researchers to investigate alternative protective mechanisms. Surprisingly, post-hoc analyses unveiled significant correlations between binding

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antibody responses and CD4+ T cell responses affecting the rate of HIV acquisition (23) (24) (25). Specifically, IgG antibody binding to the V1V2 region of the envelope demonstrated an inverse correlation with infection rate, suggesting a potential protective effect (26) (27). In contrast, the binding of plasma IgA antibodies to the envelope showed a direct correlation with the rate of infection, indicating a potential detrimental impact on vaccine efficacy. These findings underscore the significance of evaluating both the quality and specificity of antibody responses in HIV prevention (28). Furthermore, the analysis revealed additional correlates of protection. High avidity of IgG antibodies for the envelope, as well as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) activities, were inversely correlated with the risk of infection (29, 30) (31) (32) (33) (34). These data suggest that non-neutralizing effector functions of antibodies play a role in preventing HIV acquisition. Additionally, the presence of Env-specific CD4+ T cells was found to be inversely correlated with the risk of infection, further emphasizing the importance of cellular immune responses in vaccine-induced protection (23). Polyfunctional response in Env-specific CD4+ T cells expressing CD154 (or CD40 ligand), and secreting cytokines such as IL-2, IL-4, IFN-g, and TNF-a demonstrated the most robust correlation, resulting in a lower infection rate compared to individuals who did not generate such a multifaceted immune response (35) (23). Moreover, the analysis demonstrated selective effects of the vaccine on the V2 region of breakthrough viruses, suggesting a potential impact on viral evolution and the development of escape mutations. Transcriptomic analysis of RV144 trial samples identified the interferon regulatory factor 7 or IRF7 as a mediator of protection and the activation of mTORC1 as a correlate of the risk of HIV-1 acquisition (25, 36).

Two early-phase trials, RV305 and RV306, were conducted to explore strategies for improving the durability of immune responses observed in the RV144 trial. RV305 enrolled individuals who had previously received the RV144 vaccine and evaluated the effects of boosting with ALVAC-HIV and AIDSVAX B/E. The results showed that the priming vaccination series in RV144 evoked memory responses, as evidenced by higher levels of IgG responses against gp120 and gp70-V1V2 compared to peak immunogenicity in RV144. Repeated booster vaccination led to the development of antibodies with characteristics of broadly neutralizing antibodies, such as increased somatic hypermutation and longer immunoglobulin heavy-chain complementarity-determining region 3 (HCDR3) length. However, it was observed that repeated boosting skewed the responses towards the IgG4 subclass, which is associated with reduced non-neutralizing function and did not improve durability of antibody responses (37) (38) (39) (40).

HVTN 097 was conducted in South Africa and utilized the same vaccine formulation and schedule as the RV144 trial. The study aimed to assess the overall response rates of plasma IgG and Env-specific CD4+ T cells expressing IFN-γ and/or IL-2. The results of HVTN 097 showed that the response rates were similar to those observed in RV144. HVTN 100 was also conducted in South Africa and employed a pox-protein vaccine regimen specifically designed for the local subtype C epidemic. The vaccine regimen consisted of the ALVAC-HIV vCP2438, which expressed HIV subtype C gp120, subtype B gp41, gag, and protease, followed by a boost with a bivalent subtype C (TV1/1086) gp120. Additionally, an alternative adjuvant, the MF59 oil-in-water emulsion was used instead of the aluminum hydroxide adjuvant used in RV144. The primary objectives of HVTN 100 were to evaluate the safety and tolerability of the vaccine regimen and assess the immune responses elicited by the vaccine (41). The study found that all vaccine recipients developed gp120 binding antibodies, and these antibody levels were significantly increased compared to RV144. Furthermore, the vaccine regimen induced higher CD4+ T cell responses to the corresponding envelope protein. Although the IgG antibody responses directed at 1086_V1V2 were lower in HVTN 100 compared to the RV144 regimen in HVTN 097(28) (42), the results showed that the vaccine met the criteria for advancing to the next phase, which was the HVTN 702 trial. The HVTN 702 Uhambo efficacy trial began in 2016 and enrolled individuals at risk for HIV in South Africa. However, interim analysis results revealed no significant evidence of decreased or increased infection rates associated with the vaccine regimen. Consequently, the trial was halted in February 2020 by the NIH US Data and Safety Monitoring Board due to the lack of efficacy (12) (43) (44). Considerations of the many distinctions between the South African and Thai trials are important to prevent any mistaken inference that the results of the former

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trial undermine those of the latter (45). Finally, the HVTN111 trial employed a DNA-prime strategy (subtype C DNA-HIV-PT123) and the same gp120 boost used in HVTN 100. This trial resulted in increased immune responses when compared HVTN100, including CD4+ T cells, and binding and neutralizing antibodies (46).

Broadly neutralizing antibodies. In the early days of the HIV epidemic, studies found a link between high neutralizing antibody levels and delayed disease progression in people with HIV (PWH) (47) (48). This discovery led to experimental transfers of hyperimmune plasma to individuals with active virus replication. Advances in antibody isolation and cloning techniques, including improved antigen design and B-cell receptor amplification, have enabled the identification of highly potent antibodies capable of neutralizing a wide range of HIV strains. Over the past decade, more than 60 clinical trials have explored the pharmacokinetics and immunological effects of these broadly neutralizing antibodies (bNAbs) in humans. Currently researchers are actively investigating strategies to elicit bNAbs through vaccination, either by administering bNAbs directly or designing immunogens to stimulate their production (49). The Assessing Antibody-Mediated Protection AMP trial investigated the potential of long-term administration of the passively infused bnAb VRC01, targeting the CD4 binding site (CD4bs), to prevent HIV-1 acquisition in humans (HVTN 704/HPTN 085 and HVTN 703/HPTN 081) (50). It involved 4,600 at-risk participants from diverse geographical regions, including Sub-Saharan Africa, the Americas, and Europe. The results revealed that VRC01 could prevent HIV-1 infection, with a high prevention efficacy of 75% observed against viruses sensitive to VRC01 (IC80 <1μg/ml). However, there was no efficacy against the majority of circulating strains (with IC80 values >1μg/ml), resulting in no significant overall protection. Interestingly, the outcome was reminiscent of what was observed with first-generation antiretroviral therapy, where innate resistance and the emergence of resistant isolates over time compromised the effectiveness of single therapeutic agents for prevention, thus suggesting the necessity of evaluating the efficacy of a more comprehensive and potent combination of antibodies (50).

Efforts to achieve bNAbs through active immunization are also ongoing. Indeed, while the first generation of gp120 protein-based vaccines were safe and generated neutralizing antibodies in clinical trials, they did not effectively prevent HIV-1 infection. The failure to elicit protective bnAbs can be attributed to various HIV-1 immune evasion strategies, including antigenic diversification during replication and the dense glycan shield on Env that hides critical antigenic epitopes from the immune system (51). The structural dynamics of the Env trimer, with its distinct conformations, trigger different antibody responses. The closed prefusion conformation is recognized by potent bnAbs, while antibodies targeting regions exposed in the open conformation induced by CD4 binding are weak or non-neutralizing and ineffective at preventing infection. Using structure-based vaccine design, stabilized viral immunogens have been developed that remain in the closed prefusion conformation, and can generate protective antibodies. SOSIPs are uniform, soluble, stable, trimeric forms of the HIV-1 envelope spike that closely resemble the native viral spike in terms of antigenicity and structure. They achieve stability through a disulfide bond called "SOS" between gp120 and gp41 and a specific point mutation named "IP" at residue 559, which helps maintain their trimeric structure (52, 53). This approach has been successful in creating vaccines against other viruses (54). For HIV-1, a soluble protein trimer immunogen was designed based on the clade A HIV strain BG505 (BG505 SOSIP.664) (55) (52). Prior studies showed that this construct, although containing stabilizing mutations, could still be recognized by non-neutralizing, CD4-induced antibodies. An additional disulfide mutation (DS) was introduced within gp120 to prevent any CD4-induced conformational change. This modified prefusion-closed conformation immunogen, Trimer 4571 (BG505 DS-SOSIP.664), exhibited the desired antigenic profile and was resistant to CD4-induced conformational changes (56). A phase 1 small sample size clinical trial concluded with encouraging results (56).

3. Preclinical evaluation of HIV vaccines in NHPs

The use of macaque models of HIV infection has played a crucial role in developing and evaluating HIV vaccines. Macaques, specifically Indian-origin rhesus macaques (*Macaca mulatta*) and pig-tailed macaques (*Macaca nemestrina*), have been widely employed due to their close genetic and

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immunological similarities to humans, making them valuable surrogate models for studying HIV infection and vaccine responses. The history of macaque models in HIV vaccine research can be traced back to the early 1980s when scientists began searching for an animal model that could mimic the immunopathogenesis of HIV infection. Gibbons and chimpanzee were not deemed reasonable hosts (57), while Asian macaques, including *Macaca mulatta* (rhesus macaques, RM) and *M. fascicularis* (cynomolgus monkeys, CM) and cells from these species appeared to be resistant to HIV-1 (58) (58). In 1984, a breakthrough was the discovery of a related lentivirus, simian immunodeficiency virus (SIV), in captive macaques. SIV naturally infects various African non-human primates, such as sooty mangabeys, African green monkeys without inducing disease but leads to immunodeficiency in Asian macaques that are experimentally infected (59) (60) (61 {Klatt, 2012 #37). The similarity between SIV and HIV, as well as the ability of SIV to cause an AIDS-like disease in macaques, led to the establishment of SIV infection models in macaques to study the pathogenesis and immune responses associated with HIV infection. Since then, macaque models of SIV infection have been extensively utilized to assess the safety and immunogenicity of potential HIV vaccine candidates before moving to human clinical trials (62).

Preferred NHP models for HIV vaccines. There are currently multiple NHP models which differ in species, challenge route, virus doses, and strain. RMs, particularly Indian-origin (as opposed to Chinese-origin) are the most used macaques in HIV vaccine studies in the US. It is worth noting that CO RMs often display lower viral loads in comparison to Indian-origin (IO) RMs, both during the acute and chronic stages of infection (63) (64). While this difference may be seen as a potential limitation, researchers have successfully addressed this challenge by creating alternative viruses that are specifically adapted for CO RMs. Pig-tailed macaques (PTMs) have also been used; however, they have higher baseline immune activation levels than RM even without infection and differ in HIV-1 restriction factors (TRIM5alpha) (65) (66). Nonetheless studies in pigtails offer a good model for intravaginal challenge because females possess menstrual cycles like humans, making them valuable for studying factors affecting susceptibility to vaginal infection and evaluating interventions for preventing vaginal virus transmission.

Virus challenge. An important focus in vaccine studies involving macaques is the selection of the right virus for challenging NHPs. Choosing a virus with high virulence and strong replication can lead to excessive pathology, overwhelming the host's immune responses after vaccination and resulting in an underestimate of vaccine efficacy. Conversely, using a virus with low virulence and weak replication might be easily controlled by the vaccine-induced immune response, leading to an overestimate of efficacy (67) (68) (69). Additionally, some viral strains are highly sensitive to neutralizing antibodies, making them unsuitable for evaluating mucosal transmissions. Fortunately, many challenge viruses have been developed, offering a range of options for preclinical vaccine studies using NHP models, and these viruses have been well-reviewed and summarized elsewhere (70). SIVmac251 and SIVmac239 are among the most widely used viruses in early NHP vaccine studies. SIVmac251 is a swarm first isolated from rhesus macaque “251” at the New England Primate research Center (71). Over time, various SIVmac251 stocks have been generated through *in vitro* passage or by isolating new viral populations from infected animals. These stocks consist of heterogeneous swarms of viruses that can transmit multiple variants across mucosal tissues (72-74). This diversity is important in NHP vaccine studies, as it reflects the complexity of viral populations encountered in natural infections and provides a more realistic model for evaluating vaccine efficacy. When infected with pathogenic SIV strains, RMs exhibit consistent disease progression and high viral loads during the acute phase of infection, allowing for studying the impact of vaccination on viral replication, disease progression, and immune system dynamics. The genetic variations observed in the reverse transcriptase and protease of HIV-1 and SIVmac pose challenges in assessing the effectiveness of antiretroviral drugs that specifically target these proteins within the SIVmac-RM model. Additionally, evaluating vaccines against HIV-1 using this model becomes impractical due to differences in cytotoxic T cell epitopes and the lack of cross-reactivity in neutralizing antibodies. To address these limitations, researchers have developed chimeric viruses known as SHIVs, which incorporate specific HIV genes into the SIV backbone (75) (76) (77) (12). Recent initiatives have

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shifted towards employing transmitted/founder (T/F) HIV-1 Env clones, which are highly pertinent Envs for transmission research and vaccine evaluations (72) (76). These T/F SHIVs can facilitate mucosal transmission and trigger strong viral replication in rhesus macaques without the need for consecutive modifications. Ongoing endeavors are directed at creating SHIVs with greater viral diversity and neutralization characteristics, as well as sustaining consistently elevated chronic viral loads and progressive infection patterns, to more accurately replicate HIV pathogenesis in specific prevention studies.

Challenge route. NHP models have been employed to study various routes of virus transmission related to HIV-1, including intravenous (i.v.), intrarectal, intravaginal (i.vag), penile, oral, and intrauterine transmission, mimicking various modes of HIV transmission. However, these models have limitations in accurately mirroring human transmission scenarios. The i.v. route is the most reliable in NHPs but lacks clinical relevance for HIV vaccine research. In preclinical studies involving macaques, a significant focus has been on mucosal challenges since HIV infection is often acquired through heterosexual transmission via mucosal exposures. Historically and currently, intrarectal (IR) challenges are commonly used because they provide a relatively easy means of infection and allow for the use of both male and female NHP. In contrast, intravaginal (i.vag) challenges were initially less frequently employed, mainly because of the limited availability of female macaques (sustaining the breeding colonies in Non-Human Primate Centers across the US). Moreover, the menstrual cycle, vaginal mucosal structure, and microbial composition all play roles in influencing susceptibility to SIV or SHIV infection. Despite the challenges and limitations associated with Ivag challenges, preclinical trials using female macaques have increased in number in recent years. This method is considered the best way to simulate the male-to-female HIV transmission route, which accounts for the majority of HIV transmissions in humans. Penile challenges have also been developed in macaques, however, similarly to Ivag, they require higher virus doses and exhibit more variability between animals compared to intrarectal transmission (72) (78) (79) (74) (80).

Virus dose. In the past, researchers used higher virus doses, approximating the minimum amount required to infect the majority of unvaccinated control animals with a single challenge. This approach allows for assessing vaccine effectiveness through sequencing techniques, along with evaluating infection rates, the number of transmitted variants per animal, and sieving analysis. However, the HIV transmission rate per coital act is estimated to be very low in humans (81) (82). It was also discovered that in humans, infection results from a limited range of viral variants responsible for systemic infection following sexual transmission of HIV-1, typically involving 1 to 5 T/F (72) (74) (83). To better replicate human transmission, preclinical studies in NHPs have shifted their focus towards utilizing repeated low-dose challenge paradigms (84) (85-87). This involves determining a challenge dose that infects only a portion of unvaccinated control animals per exposure and subjecting animals to repeated exposures until signs of infection emerge or a predetermined number of challenges is reached. While the inoculum size used in these studies may still exceed typical human exposures, it helps simulate human mucosal transmission by emulating a restricted set of initial viral variants. It's important to note that variations in challenge modalities, including the dose, can significantly impact vaccine efficacy (84, 88). Nonetheless, studies, using different macaque species, are vital for HIV research, shedding light on pathogenesis, immune responses, and vaccine development. Each species has unique strengths and weaknesses, enabling researchers to choose the best model for specific goals. These varied models provide valuable insights, driving progress in HIV research and efforts to develop effective preventive and therapeutic measures.

4. Correlates of protection in NHP HIV vaccine studies

Macaques are vital for HIV vaccine development, with preclinical studies using chimeric viruses in rhesus macaques as benchmarks. The similarity between the immune systems of humans and macaques makes macaque models invaluable for immunogenicity studies in vaccine development. These studies have proven to be highly reliable in this model for assessing various aspects of vaccine performance. Specifically, they provide insights into the safety profile of the vaccine and its ability to induce vaccine-induced immune responses, irrespective of the specific type of vaccine being tested.

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However, disparities in vaccine efficacy between preclinical and clinical trials emphasize the need to reevaluate the reliability of macaque data for advancing candidate vaccines to clinical trials (89).

NHP prediction of Vaccine Efficacy. Early vaccines studies involving NHPs offered a glimmer of hope for a preventive vaccine to be able to control viral replication and acquisition against high dose mucosal challenges. Studies conducted in the late 1980s and early 1990s initially generated excitement as recombinant live and DNA vaccines demonstrated measurable CD8+ T lymphocyte cytotoxic responses (CTL) in both rhesus macaques and humans (90) (91). Additionally, several studies have demonstrated that passively administered antibodies can protect non-human primates (NHP) from Simian-human immunodeficiency virus (SHIV) infection (92) (93) (94) (95). In monkeys, recombinant protein- and peptide-based vaccines elicited measurable levels of neutralizing antibodies. However, Phase III clinical trials revealed a stark contrast: recombinant HIV envelope (Env)-expressing vaccines failed to stimulate broad-spectrum protective antibodies, even against closely related viruses (13). This discrepancy highlighted a significant disparity between results in macaques and humans, leading to a fading hope of finding a quick solution to the HIV vaccine challenge through neutralizing antibodies.

Broadly Nabs/SOSIPS in NHP. In preclinical studies, it was found to be safe and induced neutralizing antibodies in rhesus macaques when administered with an adjuvant. Furthermore, when used in combination vaccine regimens with an HIV-1 fusion peptide-coupled carrier, Trimer 4571 resulted in cross-clade neutralizing antibodies in mice, guinea pigs, and rhesus macaques. (96) (97) (98). Native-like SOSIP trimers have been successful in eliciting antibodies capable of neutralizing autologous tier 2 strains in animal models and rhesus macaques (99, 100) (101) (102). A recent study suggested that high serum neutralizing antibody (nAb) titers elicited by the BG505 SOSIP trimer were linked to protection against repeated, low-dose rectal challenges with SHIV.BG505. However, the study had limitations, and the protective efficacy was not conclusively demonstrated. In response, a preclinical efficacy trial was conducted using rhesus macaques. The macaques were immunized with BG505 SOSIP in 3M-052 adjuvant alone or in combination with three different heterologous viral vectors expressing SIVmac239 Gag. These viral vectors did not express Env and were included to investigate whether anti-Gag T cell responses played a role in protection. A control group received only the 3M-052 adjuvant. The results showed significant and robust protection against repeated low-dose intravaginal challenges with SHIV.BG505 in both vaccination groups compared to the control group. Specifically, a serum neutralizing antibody titer greater than 1:319, measured two weeks after the final immunization, was found to be a reliable predictor of protection. This finding prompted further examination of the specificities and characteristics of these neutralizing antibodies associated with preventing SHIV acquisition (103). Another study investigated the targets of neutralizing antibodies (nAb) in rhesus macaques immunized with BG505 SOSIP and found that these nAbs predominantly targeted the 465-glycan hole cluster. Longitudinal analysis revealed that N611 antibodies emerged before nAb in some macaques, and when nAb remained focused on the 465 glycan hole, it led to an increase in nAb titer. Monoclonal antibodies from a protected macaque showed potent neutralization of BG505 Env and BG505.SHIV, providing valuable insights into the immunogenicity of the C3/465 glycan hole cluster in BG505 SOSIP (104).

Ad5 – based vaccines in NHP. Consequently, many researchers shifted their focus toward developing immunization approaches centered on harnessing antiviral T cell responses (105) (106) (107). Early evidence in non-human primates suggested that such responses might partially limit infections with distinct SIV strains. Researchers also concentrated on creating potent vector systems for inducing HIV-specific CTL responses, with the aim of reducing disease progression rather than achieving sterilizing immunity. While these second-generation vaccines, including adenovirus-based vectors, demonstrated promise in preclinical studies in macaques (105) (106) (107), concerns emerged regarding their efficacy against pathogenic SIV challenges in outbred genetic haplotypes (106). Despite debates about the relevance of various SIV and SHIV challenge models to human HIV infection, Ad5 studies advanced into clinical trials based on the assumption that protection in macaques would translate to humans, and non-protection would likewise correlate. However, as previously discussed, the STEP Phase IIb clinical trial lacked efficacy and unexpected increased HIV

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transmission rates in certain Ad5-seropositive vaccine recipients, in stark contrast to earlier macaque studies (108).

Ad26 – based and mosaic vaccines in NHP. Ad26-based vectors were developed and evaluated in macaques based on the assumption that humans had not been previously exposed to Ad26, unlike Ad5. Preclinical testing in macaques involved high-dose challenges with SIVs and SHIVs, and it demonstrated protection against high viral replication and mucosal acquisition when administered alone or in combination with DNA or gp140 protein in prime-boost regimens (109) (110). However, clinical trials in humans using similar approaches did not achieve significant protection

Polyvalent mosaic antigens expressed by the recombinant, replication-incompetent adenovirus serotype 26 vectors were also tested in rhesus monkeys and informed the clinical trials that followed (111, 112). Indeed, they showed that adding mosaic antigens could markedly augment both the breadth and depth without compromising the magnitude of antigen-specific T cell responses as compared with consensus or natural sequence HIV-1 antigens (111) (112). Contrary to what was later observed in humans, Ad26 mosaic vaccines protected macaques from acquisition against heterologous SHIV challenges (86) (100). Parallel to the APPROACH study, an NHP study was conducted, observing similar immunogenicity (113). The Ad26.Mos.HIV/gp140 vaccine (adjuvanted in aluminum phosphate) showed significant protection against intrarectal challenges with SHIV-SF162P3, with an impressive 94% reduction in per acquisition risk and 66% complete protection.

ALVAC – based vaccines in NHP. Together with other poxviruses, the canarypox ALVAC vector has been extensively studies in macaques, against SIV, SHIV, and HIV isolates (114-119). Results showed variable levels of cellular immune responses and prevention of infection against HIV-2 and other attenuated SIV viruses. As with the Ad based vectored vaccines, poxviruses also significantly reduced peak viral loads during acute infection (116, 118-120). Among the pox vector-based vaccines, only ALVAC-based HIV-1 vaccines have been tested in phase 3 clinical trials and have been shown to be safe and immunogenic in humans and partially effective. The reduced protection against HIV acquisition provided by the ALVAC–SIV + gp120 alum regimen was both limited and temporary, indicating a need for enhancement. In macaques, vaccination with a comparable SIV-based vaccine regimen also notably reduced the risk of acquiring SIVmac251 (with 44% efficacy), and this effect was linked to the quantity of mucosal antibodies targeting V2, similarly to humans (11). The substitution of the alum with the MF59 adjuvant resulted in loss of vaccine efficacy, similarly to what observed in the RV144 follow up trials in Africa (12).

Taken together these results suggest that some NHP seemed to be potentially more likely than others to be able to predict vaccine efficacy; however, it is difficult to understand the reasons. One possibility could be different virus stocks used, or study design. There are considerable variations in the dosages of uncloned SIV or SHIV used in these studies. Variation includes viral stocks from different laboratories with different passage history and *in vitro* production methods. Another consideration is that, given that around 80% of HIV transmissions are caused by a single highly virulent virion, using a virus stock with either high or low variance can compromise the accuracy of modeling natural HIV infection (121). However, side by side comparison between viral stock and same vaccines have never been done because they are too costly. Another possible consideration for macaques to humans' discrepancies is how vaccine efficacy (VE) is calculated. There has been an increased attention given to the specific methodologies and considerations involved in assessing VE, highlighting the need for more comprehensive research and discussion in the field to establish standardized protocols and guidelines for evaluating vaccine efficacy in NHP models. Survival analyses, particularly employing Cox's proportional hazard models and likelihood ratio tests, are the preferred methods for assessing vaccine efficacy in macaque models, comparing the risk of SIV/SHIV infection between vaccinated and unvaccinated groups over time. These analyses help define vaccine efficacy as the relative reduction in per-contact transmission probability when comparing vaccinated and unvaccinated macaques (87, 122).

What is clear is that the evaluation of vaccine efficacy is significantly influenced by the design of challenge experiments. The design of challenge experiments significantly impacts efficacy assessment, including endpoints, sample size, unvaccinated macaque infection rates, susceptible

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macaque proportions, and statistical methods. Precise sample size calculation to achieve at least 80% statistical power is essential. These NHP models can be further refined for evaluating HIV-1 vaccine candidates and guiding clinical trials. Overall, the macaque model's ability to replicate human-like immune responses and safety profiles in immunogenicity studies plays a pivotal role in the development and evaluation of vaccines, helping to identify promising candidates for further clinical testing in human trials. Lastly, when possible, it is important to bridge preclinical and clinical data.

Other HIV vaccine strategies tested in NHP

Numerous studies have been conducted to date involving other attenuated recombinant poxvirus vectors expressing HIV/SIV antigens in particular, modified Vaccinia Ankara (MVA) and New York Vaccinia (NYVAC) (123, 124) (125) (126) (127) .These strategies are reviewed elsewhere and are currently at various stages in clinical trials with the aim to establish their efficacy (128).

The utilization of the human cytomegalovirus (CMV) vector represents a promising and innovative approach in the development of HIV vaccines. Immunization of non-human primates (NHPs) with CMV/SIV vectors has shown persistent and high-frequency SIV-specific memory T-cell responses at potential SIV replication sites. This resulted in sustained control of SIV infection in 50% of the NHPs, and this protective effect was associated with the elicitation of unconventional MHC-E-restricted CD8+ T-cell responses (129). Currently, the initial clinical trial is underway to evaluate the safety and immunogenicity of a CMV vector-based vaccine named VIR-1111, with recruitment targeting healthy individuals who are CMV seropositive (130) (130, 131). HIV-RNA based vaccines have also been tested and following the success of vaccination for COVID these affords have been expanding with different messenger (m)RNA vaccines being tested (132). Currently, mRNA HIV vaccine candidates developed by Scripps Research Institute and Moderna are ben tested a Phase 1 clinical trials(133).

5. Vaccine induced Correlates of HIV in humans and NHP

Correlates of Protection serve as critical immune biomarkers, evaluating vaccination response and predicting the anticipated level of vaccine efficacy for a specific clinical outcome (134). Whether mechanistic or non-mechanistic, a Correlate of Protection is valuable as a surrogate endpoint in this context. The identification of immunological markers associated with the risk of transmission in both preclinical and clinical trials for HIV-1 vaccines has significantly propelled the field of HIV-1 vaccine development, guiding the exploration of new vaccine candidates (135). Studies on immune correlates have spawned innovative hypotheses about the immunological processes that may contribute to averting HIV-1 acquisition. Recent research on HIV-1 immune correlates reveals that various types of immune responses collectively constitute an immune correlate, highlighting the role of polyfunctional immune control in preventing HIV-1 transmission. Consideration of the study population and species is crucial in understanding vaccine correlates. Although various non-human primate (NHP) challenge studies, employing diverse vaccine approaches, have shown partial protection against SIV or SHIV acquisition through CD8 T cell responses and neutralizing antibodies, the only partially effective trial against HIV did not yield similar results. The discussion on the role of adaptive immune responses in protecting against HIV has been extensive; hence, we discuss the contribution of innate immune responses and preexisting immunity.

Innate immunity. Indubitably, a sharp paradigm shift that has opened new avenues in HIV vaccinology was the finding that protection was correlated with responses that are non-specific to HIV (e.g., monocytes and NKs), suggesting that balancing the activity of innate and adaptive (virus-specific) responses may be a winning strategy (136). These finding were corroborated in three independent candidate HIV vaccines (including RV144) in macaques (11, 136) and more recently, in DNA/ MVA- based + protein vaccines (125). By using systems vaccinology, we reported the activation of both hypoxia and inflammasome pathways within protective monocytes. Our data suggest that the RV144-like vaccine in monkeys was effective because it “trained” monocytes that in turn affected adaptive responses via the monocyte/ T-cell crosstalk. Unlike conventional vaccines that aim to elicit only specific responses to vaccine-related antigens, trained immunity-based vaccines

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may offer greater protection by stimulating general long-term boosting of innate immune mechanisms (e.g., monocytes/macrophages) against pathogens and by harnessing the activation of T cell responses to the virus and even non-related antigens.

Other arms of the innate immune system such as natural killer (NK) and natural killer T (NKT) cells may also act as a bridge between the innate and adaptive immune response to shape the quality and magnitude of the vaccine response. Natural killer (NK) cells may be important to improved vaccine immunogenicity, as shown in our RV144 macaque model (10). Although NK cells are a part of the innate immune system and lack clonal antigen receptors, they are now known to be unique in having adaptive properties of immunologic memory such as antigen-specific recall responses to a variety of pathogens, most notably to cytomegalovirus (CMV) infection (137, 138). Conclusive evidence of the presence of adaptive memory NK cells was first demonstrated against murine CMV, where NK cells expanded and cleared CMV through a memory-like response (139). Subsequent studies have affirmed the dominant role of human and rhesus CMV in inducing adaptive memory-like NK cells with epigenetic imprinting, a unique receptor repertoire, and diverse function in humans and macaques (140-145). Additionally, memory-like NK cells without antigen specificity can be induced after cytokine activation with IL-12, IL-15, or IL-18 (146, 147). This raises the question of whether NK memory influences trained immunity by monocytes and whether it can be harnessed to improve vaccine efficacy(148) (147).

NKT are unique immunomodulatory innate T-cells with an invariant TCR recognizing glycolipids presented on MHC class-I-like CD1d molecules. Activated iNKT rapidly secrete pro-and anti-inflammatory cytokines, potentiate immunity, and modulate inflammation. Due to their rapid response and broad functional potential, iNKT bridge the gap between innate and adaptive immunity (149). Once activated, iNKT can be directly cytolytic (through perforin and granzyme B) and display Th1, Th2 and Th17 effector functions. Additionally, iNKT rapidly influence the function of multiple immune subsets. Bidirectional interactions between iNKT and dendritic cells (DC) enhances DC maturation and facilitates antigen cross-presentation and priming of antigen-specific T-lymphocyte responses, IFNγ production by iNKT rapidly activates NK cells improving cytolysis, iNKT are also known to recruit and provide help to B-cells, improving B-cell maturation, antibody class-switching and overall humoral immunity(150) (151). iNKT activation was shown to enhance antigen specific CD4+ and CD8+ T cell responses to HIV DNA vaccine in mice with the effect being observed during DNA priming (152). We demonstrated the effects of iNKT activation in the NHP model of Mauritian-origin cynomolgus macaques and showed downstream activation effects on CD4+ T-lymphocytes, monocytes, dendritic cells and B cells (153). Harnessing the immunotherapeutic potential of iNKT activation may be another useful tool for potentiating HIV vaccine efficacy which can be tested in the NHP model.

Preexisting immunity effect on HIV vaccines. Preexisting immunity to HIV can have both positive and negative effects on the efficacy of HIV vaccines. This preexisting immunity might include specific antibodies, immune responses, or immune memory cells. While this immunity can be beneficial in controlling the virus in infected individuals, it can also complicate vaccine development. If a vaccine induces immune responses that are too similar to those of preexisting immunity, it may not provide additional protection. Interesting results were obtained by Campion *et al* In a study conducted as part of the HIV Vaccine Trial Network (HVTN) 106 phase I trial, the role of cross-reactive memory CD4+ T cells in the primary immune response to HIV-1 gp160 envelope (Env) was investigated. The study utilized ultrasensitive quantification and epitope mapping, revealing the presence of both naive and memory CD4+ T cells specific to Env in individuals who had not been previously exposed to the virus. Surprisingly, the primary immune responses triggered by the vaccine were primarily derived from the preexisting memory CD4+ T cell pool. This finding underscores the phenomenon known as "original antigenic sin" within the context of early vaccine-induced T cell responses, highlighting the significance of preexisting memory T cells in shaping the immune response to novel pathogen (154, 155). In the context of HIV vaccine development, it has also been observed that even the most potent and broadly neutralizing antibodies, when reverted to their inferred germline versions representing naive B cell receptors, often fail to bind to the HIV envelope.

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This implies that the initial B cell response is not exclusively composed of naive B cells but also includes a pre-existing pool of cross-reactive, antigen-experienced B cells that expand upon exposure to Env. As part of the HIV Vaccine Trial Network (HVTN) 105 trial, researchers isolated gp120-reactive monoclonal antibodies (mAbs) from participants. Through deep sequencing and lineage tracking, it was discovered that several of these antibody lineages were present in the participants' pre-immune peripheral blood. Furthermore, these lineages persisted in the post-vaccination bone marrow, particularly within the long-lived plasma cell compartment. Interestingly, the pre-immune lineage members included not only immunoglobulin (Ig)M but also IgG and IgA, and they exhibited somatic hypermutation. These findings suggest that vaccine-induced gp120-specific antibody lineages originate from both naive and cross-reactive memory B cells, underscoring the complex interplay of B cell populations in the immune response to HIV (156).

BCG and HIV vaccines. Preexisting immunity to HIV that arises from other vaccinations or coinfections against/with pathogens is an important area of research in the context of HIV vaccine development(157-160). This phenomenon (e.g.: heterologous immunity" or "cross-reactive immunity" or HIV epitope mimicry) (161, 162). For example, Bacillus Calmette-Guérin (BCG) is a vaccine primarily used for the prevention of tuberculosis (TB) in humans and RM (160, 163-165). Currently, the BCG vaccine is licensed for intradermal delivery. However, this strategy does not protect adults from pulmonary TB nor can be utilized in people living with HIV. Thus, intravenous administration of BCG has been explored with promising results in RM (160, 166).

BCG vaccination has also been explored for its potential role in enhancing the immune response to HIV in the context of HIV vaccine development. Indeed, BCG is known to have a non-specific immunomodulatory effect on the immune system. It can activate various components of the immune system, including innate immune cells and it is therefore referred to as the gold standard for trained immunity(167, 168). In this context BCG "trains" the myeloid monocyte/macrophage lineage to be protective against TB and unrelated pathogens(169, 170). BCG functions as a "self-adjuvanted" vaccine, engaging multiple pattern recognition receptors (PRRs) like Toll-like receptors (TLR2, TLR4, TLR8) to enhance vaccine-induced immunity (171, 172). BCG also induces epigenetic reprogramming in bone marrow myeloid precursors, leading to protection against various unrelated pathogens.

BCG-trained monocytes boost responses to different exposures through non-antigen-specific mechanisms, including increased cytokine and chemokine release and the support of memory adaptive responses (173). Recent findings also suggest that BCG induces sustained changes in T cell repertoire, potentially contributing to long term protection (174). Applying trained immunity to enhance specific HIV responses across multiple clades is an intriguing concept, with trained monocytes initiating the response, followed by effective adaptive cytotoxic responses against conserved HIV regions. BCG's potential role in HIV vaccine development remains an area of active research. So far various studies involving BCG based HIV/SIV vaccines have been tested in non-human primates (175, 176) (177) (178). Results show promising immune responses and are summarized in **Table 1**. Recombinant (r)BCG (-Tokyo) and Vaccinia Virus (DIs) were tested in combination in cynomolgus macaques (177) .The rBCG expressed full -length Gag was used as prime and was followed by non-replicating vaccinia virus. High levels IFNg responses were detected, and vaccinated monkeys were protected from high viral replication and CD4+ T cell depletion for a year after intrarectal exposures to a pathogenic SHIV clone, compared to controls. rBCG and Ad5 combination strategy was tested in RM (175). Strong polyfunctional CD8+ T cells were induced by rBCG expressing SIV Gag and Pol and rAd5 expressing SIV antigens. BCG strain AERAS-401 expressing HIVA immunogen as a prime, followed by MVA.HIVA and OAdV.HIVA vaccines were tested in RM (176). Recombinant Mtb strain mc26435 expressing SIV Gag was evaluated for TB- and SIV-specific immune responses in infant macaques. Results show low levels of SIV-specific immunity observed following oral and intradermal priming, that were enhanced after boosts (176) (179). BCG-SIVgag constructs acted as a strong SIV-specific prime for cellular immune responses, inducing SIV-specific CD8+ and CD4+ T-cell responses after the prime. Maintenance of immunogenicity was observed more than 2 years following prime-boost administration, though no protective effect was measured against repeated SIVmac251 rectal mucosal challenge (178).

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Table 1. Summary of In Vivo Studies on BCG-Based HIV-1/SIV Vaccines in Nonhuman Primates.

Study	Vaccine	Animal	Result	Reference
Cynomolgus Macaques	rBCG (full-length SIV Gag) + Vaccinia virus boost	Cynomolgus Macaques	High IFN-γ secretion, protection from viral challenge, observed for a year; No protection with separate vaccine modalities	(177)
Rhesus Macaques	rBCG (SIV Gag and Pol) + rAd5 boost	Rhesus Macaques	Induced polyfunctional CD8+ T-cell profile	(175)
Rhesus Macaques	AERAS-401 prime + MVA.HIVA and OAdV.HIVA boost	Rhesus Macaques	High-frequency HIV-1-specific T-cell responses; Safety demonstrated, lower T-cell immunogenicity in infants	(180)
Infant Macaque Model	rMtb mc26435 expressing SIV Gag + MVA boost	Infant Macaque Model	Low levels of SIV-specific immunity, enhanced after boosts Mucosal SIV-specific IgA in saliva and intestinal IgA and IgG	(176) (179)
Chacma Baboons	rBCGpan-Gag prime + Gag VLP boost	Chacma Baboons	Gag-specific responses after two primes, enhanced by Gag VLP boost	(181)
Rhesus Macaques	Minigenes + rBCG, rDNA, rYF17D, rAd5 combinations	Rhesus Macaques (Mamu-A*01+ MHC-1)	Modest reduction in viral set point following SIVmac239 challenge; Need for strategies to overcome immunodominance	(182)
Rhesus Macaques	rBCG-SIVgag constructs	Rhesus Macaques	Strong SIV-specific prime for cellular immune responses; Maintenance of immunogenicity over 2 years, no protective effect	(178)

The studies collectively demonstrate the potential of mycobacterium-based HIV-1/SIV vaccines in inducing specific immune responses in nonhuman primates. Some approaches show promising results in terms of immunogenicity and protection against viral challenges, while others highlight the need for further strategies to overcome challenges such as immunodominance. Long-term immunogenicity is observed in several cases, but the quest for a fully protective vaccine continues (183).

CMV natural infection in HIV vaccines. Human CMV (HCMV) impacts almost every part of the host immune system. Studies in identical twins discordant for CMV infection differ in >50% of about 200 immune parameters, providing strong evidence for its influence in shaping the immune landscape (184). HCMV profoundly impacts NK cells and is a major driver of NK memory (185). NK cells play a significant role in the defense against herpesviruses and have a particularly unique

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relationship with CMV. Indeed, the co-evolution of CMV and the human immune system has led to the expansion of a unique memory-like NK cell subset that is not found in naïve hosts. CMVs are highly species-specific and have co-evolved with their respective host species (186). The CMV species that is most closely related to HCMV, and that can be experimentally studied, is infection of rhesus macaques with RhCMV (187) (188) (189) (190). RhCMV is widely prevalent in group-housed captive rhesus macaques in the SPF colony at the Tulane Primate Research Center (TNPRC) and recapitulates many of the known features of HCMV, including natural history and its effect on the immune system (190) (189) (140, 188, 191, 192). CMV's effect on shaping the immune system could therefore have consequences on the host response to vaccines including preclinical AIDS vaccine testing. There are conflicting data on the effect of CMV co-infection on vaccination (148). One study reported both higher and lower anti-influenza antibody responses depending on age (188), and another observed increased influenza vaccination-induced antibody responses in CMV+ compared to CMV– macaques (193). Because of the effect of CMV seropositivity on alterations in the T cell repertoire and immunosenescence, its impact on vaccine responses remains an important, albeit unresolved consideration (194) (195).

6. Future Directions

Extensive preclinical and clinical testing has highlighted that solely targeting one facet of the immune system is not an effective strategy for achieving protection against HIV. Lessons learned from the T cell vaccine era (second wave) emphasize that adopting a "more is better" approach, such as aiming for strong CD8+ T cell responses or higher levels of interferon-gamma (IFNg) as an efficacy marker, has proven inadequate. Intriguingly, the only vaccine to achieve partial protection against HIV acquisition did not induce anti-viral CD8+ T cells. Furthermore, this same vaccine failed to reduce HIV replication levels in individuals who became infected. It appears that safeguarding against viral replication and acquisition may require distinct immune responses. This notion finds support in observations that successful viral load control in macaques did not translate into protection against acquisition in humans, and in some instances, it even heightened the risk of viral acquisition. It is therefore worthwhile to reconsider the strategy by emphasizing a short-term vaccine approach focused on preventing acquisition rather than striving for long-lasting protection (like bNabs approaches). Alternatively, new adjuvants could be developed to circumvent the necessity of inducing CD4+ T cells or to redirect CD4+ T cells away from transmission sites, possibly using innovative strategies, such as chemokines (196) heat shock proteins (197) or other strategies (198).

We find ourselves in what could be considered a new era of vaccine development, although it can be challenging to accurately define waves while actively experiencing them. Undoubtedly, the emergence of the COVID-19 pandemic has transformed how we disseminate scientific information and, most importantly, has underscored the urgency of expediting vaccine testing in human subjects. Current challenges in the preclinical field include the availability of specific NHP models in the post-COVID era and cost constraints. National Primate Centers are actively expanding their colonies due to disruptions in supplies from other countries. Cost limitations are particularly pertinent in low-dose repeated challenge models, where achieving statistical power necessitates larger animal groups. Furthermore, conducting side-by-side comparisons of multiple vaccines can be financially burdensome and often impractical unless we have identified clear indicators of protection and thoroughly studied them. Despite these considerations and challenges, the NHP model remains an indispensable tool in vaccine research, offering a crucial bridge between preclinical studies and the complex human clinical trial phase.

Finally, by examining the ongoing efforts and advancements in HIV prevention research, we hope to contribute to the collective knowledge and foster new ideas that can pave the way for the development of an effective HIV vaccine.

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References

1. Esparza J. A brief history of the global effort to develop a preventive HIV vaccine. Vaccine. 2013;31(35):3502-18.
2. Plotkin SA. Immunologic correlates of protection induced by vaccination. Pediatr Infect Dis J. 2001;20(1):63-75.
3. Merz B. HIV vaccine approved for clinical trials. JAMA. 1987;258(11):1433-4.
4. Hu SL, Abrams K, Barber GN, Moran P, Zarling JM, Langlois AJ, et al. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. Science. 1992;255(5043):456-9.
5. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis. 2005;191(5):654-65.
6. Cohen J. The Hiv Vaccine Paradox. Science. 1994;264(5162):1072-4.
7. McMichael A, Hanke T. The quest for an AIDS vaccine: is the CD8+ T-cell approach feasible? Nature Reviews Immunology. 2002;2(4):283-91.
8. Lu S. Immunogenicity of DNA vaccines in humans It takes two to tango. Hum Vaccines. 2008;4(6):449-52.
9. McFann K, Baxter BA, LaVergne SM, Stromberg S, Berry K, Tipton M, et al. Quality of Life (QoL) Is Reduced in Those with Severe COVID-19 Disease, Post-Acute Sequelae of COVID-19, and Hospitalization in United States Adults from Northern Colorado. Int J Environ Res Public Health. 2021;18(21).
10. Vaccari M, Fourati S, Gordon SN, Brown DR, Bissa M, Schifanella L, et al. HIV vaccine candidate activation of hypoxia and the inflammasome in CD14(+) monocytes is associated with a decreased risk of SIVmac251 acquisition. Nat Med. 2018;24(6):847-56.
11. Vaccari M, Gordon SN, Fourati S, Schifanella L, Liyanage NP, Cameron M, et al. Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. Nat Med. 2016;22(7):762-70.
12. Gray GE, Bekker LG, Laher F, Malahleha M, Allen M, Moodie Z, et al. Vaccine Efficacy of ALVAC-HIV and Bivalent Subtype C gp120-MF59 in Adults. N Engl J Med. 2021;384(12):1089-100.
13. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis. 2006;194(12):1661-71.
14. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008;372(9653):1881-93.
15. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis. 2011;11(7):507-15.
16. Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusim K, Funkhouser R, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med. 2007;13(1):100-6.
17. Baden LR, Stieh DJ, Sarnecki M, Walsh SR, Tomaras GD, Kublin JG, et al. Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. Lancet HIV. 2020;7(10):e688-e98.
18. Leroux-Roels I, Koutsoukos M, Clement F, Steyaert S, Janssens M, Bourguignon P, et al. Strong and persistent CD4+ T-cell response in healthy adults immunized with a candidate HIV-1 vaccine containing gp120, Nef and Tat antigens formulated in three Adjuvant Systems. Vaccine. 2010;28(43):7016-24.
19. Keefer MC, Gilmour J, Hayes P, Gill D, Kopycinski J, Cheeseman H, et al. A phase I double blind, placebo-controlled, randomized study of a multigenic HIV-1 adenovirus subtype 35 vector vaccine in healthy uninfected adults. PLoS One. 2012;7(8):e41936.
20. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009;361(23):2209-20.
21. Pitisuttithum P. HIV vaccine research in Thailand: lessons learned. Expert Rev Vaccines. 2008;7(3):311-7.
22. Vaccari M, Poonam P, Franchini G. Phase III HIV vaccine trial in Thailand: a step toward a protective vaccine for HIV. Expert Rev Vaccines. 2010;9(9):997-1005.
23. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. Nat Biotechnol. 2015;33(6):610-6.

24. de Souza MS, Ratto-Kim S, Chuenarom W, Schuetz A, Chantakulkij S, Nuntapinit B, et al. The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope. J Immunol. 2012;188(10):5166-76.
25. Tomalka JA, Pelletier AN, Fourati S, Latif MB, Sharma A, Furr K, et al. The transcription factor CREB1 is a mechanistic driver of immunogenicity and reduced HIV-1 acquisition following ALVAC vaccination. Nat Immunol. 2021;22(10):1294-305.
26. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012;366(14):1275-86.
27. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. PLoS One. 2014;9(2):e87572.
28. Shen X, Laher F, Moodie Z, McMillan AS, Spreng RL, Gilbert PB, et al. HIV-1 Vaccine Sequences Impact V1V2 Antibody Responses: A Comparison of Two Poxvirus Prime gp120 Boost Vaccine Regimens. Sci Rep. 2020;10(1):2093.
29. Shangguan S, Ehrenberg PK, Geretz A, Yum L, Kundu G, May K, et al. Monocyte-derived transcriptome signature indicates antibody-dependent cellular phagocytosis as a potential mechanism of vaccine-induced protection against HIV-1. Elife. 2021;10.
30. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol. 2012;86(21):11521-32.
31. Pollara J, Bonsignori M, Moody MA, Liu P, Alam SM, Hwang KK, et al. HIV-1 vaccine-induced C1 and V2 Env-specific antibodies synergize for increased antiviral activities. J Virol. 2014;88(14):7715-26.
32. Ferrari G, Pollara J, Kozink D, Harms T, Drinker M, Freel S, et al. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. J Virol. 2011;85(14):7029-36.
33. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PLoS One. 2013;8(9):e75665.
34. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc Natl Acad Sci U S A. 2013;110(22):9019-24.
35. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. Annu Rev Med. 2015;66:423-37.
36. Fourati S, Ribeiro SP, Blasco Tavares Pereira Lopes F, Talla A, Lefebvre F, Cameron M, et al. Integrated systems approach defines the antiviral pathways conferring protection by the RV144 HIV vaccine. Nat Commun. 2019;10(1):863.
37. Easterhoff D, Pollara J, Luo K, Janus B, Gohain N, Williams LD, et al. HIV vaccine delayed boosting increases Env variable region 2-specific antibody effector functions. JCI Insight. 2020;5(2).
38. Pitisuttithum P, Nitayaphan S, Chariyalertsak S, Kaewkungwal J, Dawson P, Dhitavat J, et al. Late boosting of the RV144 regimen with AIDSVAX B/E and ALVAC-HIV in HIV-uninfected Thai volunteers: a double-blind, randomised controlled trial. Lancet HIV. 2020;7(4):e238-e48.
39. Costanzo MC, Paquin-Proulx D, Schuetz A, Akapirat S, Shubin Z, Kim D, et al. ALVAC-HIV and AIDSVAX B/E vaccination induce improved immune responses compared with AIDSVAX B/E vaccination alone. JCI Insight. 2023;8(9).
40. Shubin Z, Stanfield-Oakley S, Puangkaew J, Pitisutthithum P, Nitayaphan S, Gurunathan S, et al. Additional boosting to the RV144 vaccine regimen increased Fc-mediated effector function magnitude but not durability. AIDS. 2023;37(10):1519-24.
41. Laher F, Moodie Z, Cohen KW, Grunenberg N, Bekker LG, Allen M, et al. Safety and immune responses after a 12-month booster in healthy HIV-uninfected adults in HVTN 100 in South Africa: A randomized double-blind placebo-controlled trial of ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59 vaccines. PLoS Med. 2020;17(2):e1003038.

42. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin Immunol. 2020;214:108393.
43. Bekker LG, Moodie Z, Grunenberg N, Laher F, Tomaras GD, Cohen KW, et al. Subtype C ALVAC-HIV and bivalent subtype C gp120/MF59 HIV-1 vaccine in low-risk, HIV-uninfected, South African adults: a phase 1/2 trial. Lancet HIV. 2018;5(7):e366-e78.
44. Cohen J. Combo of two HIV vaccines fails its big test. Science. 2020;367(6478):611-2.
45. Zolla-Pazner S, deCamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, et al. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. PLoS One. 2013;8(1):e53629.
46. Moodie Z, Walsh SR, Laher F, Maganga L, Herce ME, Naidoo S, et al. Antibody and cellular responses to HIV vaccine regimens with DNA plasmid as compared with ALVAC priming: An analysis of two randomized controlled trials. PLoS Med. 2020;17(5):e1003117.
47. Karpas A, Hill F, Youle M, Cullen V, Gray J, Byron N, et al. Effects of passive immunization in patients with the acquired immunodeficiency syndrome-related complex and acquired immunodeficiency syndrome. Proc Natl Acad Sci U S A. 1988;85(23):9234-7.
48. Jackson GG, Perkins JT, Rubenis M, Paul DA, Knigge M, Despotes JC, et al. Passive immunoneutralization of human immunodeficiency virus in patients with advanced AIDS. Lancet. 1988;2(8612):647-52.
49. Frattari GS, Caskey M, Sogaard OS. Broadly neutralizing antibodies for HIV treatment and cure approaches. Curr Opin HIV AIDS. 2023;18(4):157-63.
50. Corey L, Gilbert PB, Juraska M, Montefiori DC, Morris L, Karuna ST, et al. Two Randomized Trials of Neutralizing Antibodies to Prevent HIV-1 Acquisition. N Engl J Med. 2021;384(11):1003-14.
51. Burton DR, Desrosiers RC, Doms RW, Feinberg MB, Gallo RC, Hahn B, et al. Public health. A sound rationale needed for phase III HIV-1 vaccine trials. Science. 2004;303(5656):316.
52. Binley JM, Sanders RW, Clas B, Schuelke N, Master A, Guo Y, et al. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J Virol. 2000;74(2):627-43.
53. Sanders RW, Derking R, Cupo A, Julien JP, Yasmeen A, de Val N, et al. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog. 2013;9(9):e1003618.
54. Crank MC, Ruckwardt TJ, Chen M, Morabito KM, Phung E, Costner PJ, et al. A proof of concept for structure-based vaccine design targeting RSV in humans. Science. 2019;365(6452):505-9.
55. Kwon YD, Pancera M, Acharya P, Georgiev IS, Crooks ET, Gorman J, et al. Crystal structure, conformational fixation and entry-related interactions of mature ligand-free HIV-1 Env. Nat Struct Mol Biol. 2015;22(7):522-31.
56. Houser KV, Gaudinski MR, Happe M, Narpala S, Verardi R, Sarfo EK, et al. Safety and immunogenicity of an HIV-1 prefusion-stabilized envelope trimer (Trimer 4571) vaccine in healthy adults: A first-in-human open-label, randomized, dose-escalation, phase 1 clinical trial. EClinicalMedicine. 2022;48:101477.
57. Gardner MB, Luciw PA. Animal models of AIDS. FASEB J. 1989;3(14):2593-606.
58. Agy MB, Frumkin LR, Corey L, Coombs RW, Wolinsky SM, Koehler J, et al. Infection of Macaca nemestrina by human immunodeficiency virus type-1. Science. 1992;257(5066):103-6.
59. Gardner MB. The history of simian AIDS. J Med Primatol. 1996;25(3):148-57.
60. Klatt NR, Silvestri G, Hirsch V. Nonpathogenic simian immunodeficiency virus infections. Cold Spring Harb Perspect Med. 2012;2(1):a007153.
61. Apetrei C, Kaur A, Lerche NW, Metzger M, Pandrea I, Hardcastle J, et al. Molecular epidemiology of simian immunodeficiency virus SIVsm in U.S. primate centers unravels the origin of SIVmac and SIVstm. J Virol. 2005;79(14):8991-9005.
62. Weiss R. Monkey-Business over Aids Vaccine. Brit Med J. 1991;303(6807):872-.
63. Joag SV, Stephens EB, Adams RJ, Foresman L, Narayan O. Pathogenesis of SIVmac infection in Chinese and Indian rhesus macaques: effects of splenectomy on virus burden. Virology. 1994;200(2):436-46.
64. Trichel AM, Rajakumar PA, Murphey-Corb M. Species-specific variation in SIV disease progression between Chinese and Indian subspecies of rhesus macaque. J Med Primatol. 2002;31(4-5):171-8.

65. Brennan G, Kozyrev Y, Hu SL. TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis. Proc Natl Acad Sci U S A. 2008;105(9):3569-74.
66. Wilson SJ, Webb BL, Ylinen LM, Verschoor E, Heeney JL, Towers GJ. Independent evolution of an antiviral TRIMCyp in rhesus macaques. Proc Natl Acad Sci U S A. 2008;105(9):3557-62.
67. Feinberg MB, Moore JP. AIDS vaccine models: challenging challenge viruses. Nat Med. 2002;8(3):207-10.
68. Fischer W, Apetrei C, Santiago ML, Li Y, Gautam R, Pandrea I, et al. Distinct evolutionary pressures underlie diversity in simian immunodeficiency virus and human immunodeficiency virus lineages. J Virol. 2012;86(24):13217-31.
69. Hirsch VM, Johnson PR. Pathogenic diversity of simian immunodeficiency viruses. Virus Res. 1994;32(2):183-203.
70. Del Prete GQ, Lifson JD, Keele BF. Nonhuman primate models for the evaluation of HIV-1 preventive vaccine strategies: model parameter considerations and consequences. Curr Opin HIV AIDS. 2016;11(6):546-54.
71. Lewis MG, Bellah S, McKinnon K, Yalley-Ogunro J, Zack PM, Elkins WR, et al. Titration and characterization of two rhesus-derived SIVmac challenge stocks. AIDS Res Hum Retroviruses. 1994;10(2):213-20.
72. Keele BF, Li H, Learn GH, Hraber P, Giorgi EE, Grayson T, et al. Low-dose rectal inoculation of rhesus macaques by SIVsmE660 or SIVmac251 recapitulates human mucosal infection by HIV-1. J Exp Med. 2009;206(5):1117-34.
73. Bixby JG, Laur O, Johnson WE, Desrosiers RC. Diversity of envelope genes from an uncloned stock of SIVmac251. AIDS Res Hum Retroviruses. 2010;26(10):1115-31.
74. Stone M, Keele BF, Ma ZM, Bailes E, Dutra J, Hahn BH, et al. A limited number of simian immunodeficiency virus (SIV) env variants are transmitted to rhesus macaques vaginally inoculated with SIVmac251. J Virol. 2010;84(14):7083-95.
75. Song RJ, Chenine AL, Rasmussen RA, Ruprecht CR, Mirshahidi S, Grisson RD, et al. Molecularly cloned SHIV-1157ipd3N4: a highly replication- competent, mucosally transmissible R5 simian-human immunodeficiency virus encoding HIV clade C Env. J Virol. 2006;80(17):8729-38.
76. Asmal M, Luedemann C, Lavine CL, Mach LV, Balachandran H, Brinkley C, et al. Infection of monkeys by simian-human immunodeficiency viruses with transmitted/founder clade C HIV-1 envelopes. Virology. 2015;475:37-45.
77. Tartaglia LJ, Chang HW, Lee BC, Abbink P, Ng'ang'a D, Boyd M, et al. Production of Mucosally Transmissible SHIV Challenge Stocks from HIV-1 Circulating Recombinant Form 01_AE env Sequences. PLoS Pathog. 2016;12(2):e1005431.
78. Ma ZM, Keele BF, Qureshi H, Stone M, Desilva V, Fritts L, et al. SIVmac251 is inefficiently transmitted to rhesus macaques by penile inoculation with a single SIVenv variant found in ramp-up phase plasma. AIDS Res Hum Retroviruses. 2011;27(12):1259-69.
79. Ma ZM, Dutra J, Fritts L, Miller CJ. Lymphatic Dissemination of Simian Immunodeficiency Virus after Penile Inoculation. J Virol. 2016;90(8):4093-104.
80. Qureshi H, Ma ZM, Huang Y, Hodge G, Thomas MA, DiPasquale J, et al. Low-Dose Penile SIVmac251 Exposure of Rhesus Macaques Infected with Adenovirus Type 5 (Ad5) and Then Immunized with a Replication-Defective Ad5-Based SIV gag/pol/nef Vaccine Recapitulates the Results of the Phase IIb Step Trial of a Similar HIV-1 Vaccine. Journal of Virology. 2012;86(4):2239-50.
81. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis. 2005;191(9):1403-9.
82. Gray RH, Wawer MJ, Brookmeyer R, Sewankambo NK, Serwadda D, Wabwire-Mangen F, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet. 2001;357(9263):1149-53.
83. Liu J, Keele BF, Li H, Keating S, Norris PJ, Carville A, et al. Low-dose mucosal simian immunodeficiency virus infection restricts early replication kinetics and transmitted virus variants in rhesus monkeys. J Virol. 2010;84(19):10406-12.
84. Vaccari M, Keele BF, Bosinger SE, Doster MN, Ma ZM, Pollara J, et al. Protection afforded by an HIV vaccine candidate in macaques depends on the dose of SIVmac251 at challenge exposure. J Virol. 2013;87(6):3538-48.

85. Morris MR, Byrareddy SN, Villinger F, Henning TC, Butler K, Ansari AA, et al. Relationship of menstrual cycle and vaginal infection in female rhesus macaques challenged with repeated, low doses of SIVmac251. J Med Primatol. 2015;44(5):301-5.
86. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013;155(3):531-9.
87. Hudgens MG, Gilbert PB, Mascola JR, Wu CD, Barouch DH, Self SG. Power to detect the effects of HIV vaccination in repeated low-dose challenge experiments. J Infect Dis. 2009;200(4):609-13.
88. Hudgens MG, Gilbert PB. Assessing vaccine effects in repeated low-dose challenge experiments. Biometrics. 2009;65(4):1223-32.
89. Shedlock DJ, Silvestri G, Weiner DB. Monkeying around with HIV vaccines: using rhesus macaques to define 'gatekeepers' for clinical trials. Nat Rev Immunol. 2009;9(10):717-28.
90. Lubeck MD, Natuk R, Myagkikh M, Kalyan N, Aldrich K, Sinangil F, et al. Long-term protection of chimpanzees against high-dose HIV-1 challenge induced by immunization. Nat Med. 1997;3(6):651-8.
91. Boyer JD, Ugen KE, Wang B, Agadjanyan M, Gilbert L, Bagarazzi ML, et al. Protection of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination. Nat Med. 1997;3(5):526-32.
92. Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, Weisgrau KL, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A. 2012;109(46):18921-5.
93. Pegu A, Yang ZY, Boyington JC, Wu L, Ko SY, Schmidt SD, et al. Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. Sci Transl Med. 2014;6(243):243ra88.
94. Gautam R, Nishimura Y, Pegu A, Nason MC, Klein F, Gazumyan A, et al. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. Nature. 2016;533(7601):105-9.
95. Saunders KO, Wang L, Joyce MG, Yang ZY, Balazs AB, Cheng C, et al. Broadly Neutralizing Human Immunodeficiency Virus Type 1 Antibody Gene Transfer Protects Nonhuman Primates from Mucosal Simian-Human Immunodeficiency Virus Infection. J Virol. 2015;89(16):8334-45.
96. Zhou T, Doria-Rose NA, Cheng C, Stewart-Jones GBE, Chuang GY, Chambers M, et al. Quantification of the Impact of the HIV-1-Glycan Shield on Antibody Elicitation. Cell Rep. 2017;19(4):719-32.
97. Chuang GY, Lai YT, Boyington JC, Cheng C, Geng H, Narpala S, et al. Development of a 3Mut-Apex-Stabilized Envelope Trimer That Expands HIV-1 Neutralization Breadth When Used To Boost Fusion Peptide-Directed Vaccine-Elicited Responses. J Virol. 2020;94(13).
98. Chuang GY, Geng H, Pancera M, Xu K, Cheng C, Acharya P, et al. Structure-Based Design of a Soluble Prefusion-Closed HIV-1 Env Trimer with Reduced CD4 Affinity and Improved Immunogenicity. J Virol. 2017;91(10).
99. Kumar S, Singh S, Chatterjee A, Bajpai P, Sharma S, Katpara S, et al. Recognition determinants of improved HIV-1 neutralization by a heavy chain matured pediatric antibody. iScience. 2023;26(9):107579.
100. Pauthner MG, Nkolola JP, Havenar-Daughton C, Murrell B, Reiss SM, Bastidas R, et al. Vaccine-Induced Protection from Homologous Tier 2 SHIV Challenge in Nonhuman Primates Depends on Serum-Neutralizing Antibody Titers. Immunity. 2019;50(1):241-52 e6.
101. Crooks ET, Tong T, Chakrabarti B, Narayan K, Georgiev IS, Menis S, et al. Vaccine-Elicited Tier 2 HIV-1 Neutralizing Antibodies Bind to Quaternary Epitopes Involving Glycan-Deficient Patches Proximal to the CD4 Binding Site. PLoS Pathog. 2015;11(5):e1004932.
102. Klasse PJ, Ketas TJ, Cottrell CA, Ozorowski G, Debnath G, Camara D, et al. Epitopes for neutralizing antibodies induced by HIV-1 envelope glycoprotein BG505 SOSIP trimers in rabbits and macaques. PLoS Pathog. 2018;14(2):e1006913.
103. Arunachalam PS, Charles TP, Joag V, Bollimpelli VS, Scott MKD, Wimmers F, et al. T cell-inducing vaccine durably prevents mucosal SHIV infection even with lower neutralizing antibody titers. Nat Med. 2020;26(6):932-40.
104. Charles TP, Burton SL, Arunachalam PS, Cottrell CA, Sewall LM, Bollimpelli VS, et al. The C3/465 glycan hole cluster in BG505 HIV-1 envelope is the major neutralizing target involved in preventing mucosal SHIV infection. PLoS Pathog. 2021;17(2):e1009257.
105. Shiver JW, Fu TM, Chen L, Casimiro DR, Davies ME, Evans RK, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature. 2002;415(6869):331-5.

106. Casimiro DR, Wang F, Schleif WA, Liang X, Zhang ZQ, Tobery TW, et al. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. J Virol. 2005;79(24):15547-55.
107. Liang X, Casimiro DR, Schleif WA, Wang F, Davies ME, Zhang ZQ, et al. Vectored Gag and Env but not Tat show efficacy against simian-human immunodeficiency virus 89.6P challenge in Mamu-A*01-negative rhesus monkeys. J Virol. 2005;79(19):12321-31.
108. Robb ML. Failure of the Merck HIV vaccine: an uncertain step forward. Lancet. 2008;372(9653):1857-8.
109. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, et al. Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. Science. 2015;349(6245):320-4.
110. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature. 2012;482(7383):89-93.
111. Barouch DH, O'Brien KL, Simmons NL, King SL, Abbink P, Maxfield LF, et al. Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nat Med. 2010;16(3):319-23.
112. Santra S, Liao HX, Zhang RJ, Muldoon M, Watson S, Fischer W, et al. Mosaic vaccines elicit CD8(+) T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys. Nature Medicine. 2010;16(3):324-U122.
113. Barouch DH, Tomaka FL, Wegmann F, Stieh DJ, Alter G, Robb ML, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet. 2018;392(10143):232-43.
114. Abimiku AG, Franchini G, Tartaglia J, Aldrich K, Myagkikh M, Markham PD, et al. HIV-1 recombinant poxvirus vaccine induces cross-protection against HIV-2 challenge in rhesus macaques. Nat Med. 1995;1(4):321-9.
115. Andersson S, Makitalo B, Thorstensson R, Franchini G, Tartaglia J, Limbach K, et al. Immunogenicity and protective efficacy of a human immunodeficiency virus type 2 recombinant canarypox (ALVAC) vaccine candidate in cynomolgus monkeys. J Infect Dis. 1996;174(5):977-85.
116. Benson J, Chougnet C, Robert-Guroff M, Montefiori D, Markham P, Shearer G, et al. Recombinant vaccine-induced protection against the highly pathogenic simian immunodeficiency virus SIV(mac251): dependence on route of challenge exposure. J Virol. 1998;72(5):4170-82.
117. Franchini G, Markham P, Gard E, Fargnoli K, Keubaruwa S, Jagodzinski L, et al. Persistent infection of rhesus macaques with a molecular clone of human immunodeficiency virus type 2: evidence of minimal genetic drift and low pathogenetic effects. J Virol. 1990;64(9):4462-7.
118. Hanke T, Samuel RV, Blanchard TJ, Neumann VC, Allen TM, Boyson JE, et al. Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. J Virol. 1999;73(9):7524-32.
119. Ourmanov I, Brown CR, Moss B, Carroll M, Wyatt L, Pletneva L, et al. Comparative efficacy of recombinant modified vaccinia virus Ankara expressing simian immunodeficiency virus (SIV) Gag-Pol and/or Env in macaques challenged with pathogenic SIV. J Virol. 2000;74(6):2740-51.
120. Hel Z, Venzon D, Poudyal M, Tsai WP, Giuliani L, Woodward R, et al. Viremia control following antiretroviral treatment and therapeutic immunization during primary SIV251 infection of macaques. Nat Med. 2000;6(10):1140-6.
121. Walker LM, Sok D, Nishimura Y, Donau O, Sadjadpour R, Gautam R, et al. Rapid development of glycan-specific, broad, and potent anti-HIV-1 gp120 neutralizing antibodies in an R5 SIV/HIV chimeric virus infected macaque. Proc Natl Acad Sci U S A. 2011;108(50):20125-9.
122. Nolen TL, Hudgens MG, Senb PK, Koch GG. Analysis of repeated low-dose challenge studies. Stat Med. 2015;34(12):1981-92.
123. Valentin A, McKinnon K, Li J, Rosati M, Kulkarni V, Pilkington GR, et al. Comparative analysis of SIV-specific cellular immune responses induced by different vaccine platforms in rhesus macaques. Clin Immunol. 2014;155(1):91-107.
124. Vaccari M, Mattapallil J, Song K, Tsai WP, Hryniewicz A, Venzon D, et al. Reduced protection from simian immunodeficiency virus SIVmac251 infection afforded by memory CD8+ T cells induced by vaccination during CD4+ T-cell deficiency. J Virol. 2008;82(19):9629-38.

125. Bollimpelli VS, Reddy PBJ, Gangadhara S, Charles TP, Burton SL, Tharp GK, et al. Intradermal but not intramuscular modified vaccinia Ankara immunizations protect against intravaginal tier2 simian-human immunodeficiency virus challenges in female macaques. Nat Commun. 2023;14(1):4789.
126. Perdiguero B, Hauser A, Gomez CE, Peterhoff D, Sideris E, Sorzano COS, et al. Potency and durability of T and B cell immune responses after homologous and heterologous vector delivery of a trimer-stabilized, membrane-displayed HIV-1 clade ConC Env protein. Front Immunol. 2023;14:1270908.
127. Gomez CE, Perdiguero B, Garcia-Arriaza J, Esteban M. Poxvirus vectors as HIV/AIDS vaccines in humans. Hum Vaccin Immunother. 2012;8(9):1192-207.
128. Garcia-Arriaza J, Esteban M. Enhancing poxvirus vectors vaccine immunogenicity. Hum Vaccin Immunother. 2014;10(8):2235-44.
129. Malouli D, Hansen SG, Hancock MH, Hughes CM, Ford JC, Gilbride RM, et al. Cytomegaloviral determinants of CD8(+) T cell programming and RhCMV/SIV vaccine efficacy. Sci Immunol. 2021;6(57).
130. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature. 2011;473(7348):523-7.
131. Picker LJ, Lifson JD, Gale M, Jr., Hansen SG, Fruh K. Programming cytomegalovirus as an HIV vaccine. Trends Immunol. 2023;44(4):287-304.
132. Valentin A, Bergamaschi C, Rosati M, Angel M, Burns R, Agarwal M, et al. Comparative immunogenicity of an mRNA/LNP and a DNA vaccine targeting HIV gag conserved elements in macaques. Front Immunol. 2022;13:945706.
133. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, et al. Rational HIV immunogen design to target specific germline B cell receptors. Science. 2013;340(6133):711-6.
134. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010;17(7):1055-65.
135. Tomaras GD, Plotkin SA. Complex immune correlates of protection in HIV-1 vaccine efficacy trials. Immunol Rev. 2017;275(1):245-61.
136. Vaccari M, Fourati S, Gordon SN, Brown DR, Bissa M, Schifanella L, et al. HIV vaccine candidate activation of hypoxia and the inflammasome in CD14(+) monocytes is associated with a decreased risk of SIV(mac251) acquisition. Nat Med. 2018;24(6):847-56.
137. Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL. NK cells and immune "memory". J Immunol. 2011;186(4):1891-7.
138. Paust S, von Andrian UH. Natural killer cell memory. Nat Immunol. 2011;12(6):500-8.
139. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature. 2009;457(7229):557-61.
140. Ram DR, Manickam C, Hueber B, Itell HL, Permar SR, Varner V, et al. Tracking KLRC2 (NKG2C)+ memory-like NK cells in SIV+ and rhCMV+ rhesus macaques. PLoS Pathog. 2018;14(5):e1007104.
141. Luetke-Eversloh M, Hammer Q, Durek P, Nordstrom K, Gasparoni G, Pink M, et al. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. PLoS Pathog. 2014;10(10):e1004441.
142. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood. 2004;104(12):3664-71.
143. Beziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Bjorklund AT, et al. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. Blood. 2013;121(14):2678-88.
144. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity. 2015;42(3):443-56.
145. Lee J, Zhang T, Hwang I, Kim A, Nitschke L, Kim M, et al. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. Immunity. 2015;42(3):431-42.
146. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. Proc Natl Acad Sci U S A. 2009;106(6):1915-9.
147. Sun JC, Lanier LL. Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination? NK Cell Memory and Immunization Strategies against Infectious Diseases and Cancer. Cold Spring Harb Perspect Biol. 2018;10(10).

148. Goodier MR, Jonjic S, Riley EM, Juranic Lisnic V. CMV and natural killer cells: shaping the response to vaccination. Eur J Immunol. 2018;48(1):50-65.
149. Brennan PJ, Brigl M, Brenner MB. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. Nat Rev Immunol. 2013;13(2):101-17.
150. Fujii S, Shimizu K, Smith C, Bonifaz L, Steinman RM. Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. J Exp Med. 2003;198(2):267-79.
151. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, et al. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol. 1999;163(9):4647-50.
152. Huang Y, Chen A, Li X, Chen Z, Zhang W, Song Y, et al. Enhancement of HIV DNA vaccine immunogenicity by the NKT cell ligand, alpha-galactosylceramide. Vaccine. 2008;26(15):1807-16.
153. Bond NG, Fahlberg MD, Yu S, Rout N, Tran D, Fitzpatrick-Schmidt T, et al. Immunomodulatory potential of in vivo natural killer T (NKT) activation by NKTT320 in Mauritian-origin cynomolgus macaques. iScience. 2022;25(3):103889.
154. Campion SL, Brenna E, Thomson E, Fischer W, Ladell K, McLaren JE, et al. Preexisting memory CD4+ T cells contribute to the primary response in an HIV-1 vaccine trial. J Clin Invest. 2021;131(23).
155. Fazekas de St G, Webster RG. Disquisitions on Original Antigenic Sin. II. Proof in lower creatures. J Exp Med. 1966;124(3):347-61.
156. Basu M, Fucile C, Piepenbrink MS, Bunce CA, Man LX, Liesveld J, et al. Mixed Origins: HIV gp120-Specific Memory Develops from Pre-Existing Memory and Naive B Cells Following Vaccination in Humans. AIDS Res Hum Retroviruses. 2023;39(7):350-66.
157. Pasco ST, Anguita J. Lessons from Bacillus Calmette-Guerin: Harnessing Trained Immunity for Vaccine Development. Cells. 2020;9(9).
158. Arts RJW, Moorlag S, Novakovic B, Li Y, Wang SY, Oosting M, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. Cell Host Microbe. 2018;23(1):89-100 e5.
159. Bonavida V, Frame M, Nguyen KH, Rajurkar S, Venketaraman V. Mycobacterium tuberculosis: Implications of Ageing on Infection and Maintaining Protection in the Elderly. Vaccines (Basel). 2022;10(11).
160. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, 2nd, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature. 2020;577(7788):95-102.
161. Uthayakumar D, Paris S, Chapat L, Freyburger L, Poulet H, De Luca K. Non-specific Effects of Vaccines Illustrated Through the BCG Example: From Observations to Demonstrations. Front Immunol. 2018;9:2869.
162. Spencer JC, Ganguly R, Waldman RH. Nonspecific protection of mice against influenza virus infection by local or systemic immunization with Bacille Calmette-Guerin. J Infect Dis. 1977;136(2):171-5.
163. Stensballe LG, Nante E, Jensen IP, Kofoed PE, Poulsen A, Jensen H, et al. Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls community based case-control study. Vaccine. 2005;23(10):1251-7.
164. Larsen MH, Biermann K, Chen B, Hsu T, Sambandamurthy VK, Lackner AA, et al. Efficacy and safety of live attenuated persistent and rapidly cleared Mycobacterium tuberculosis vaccine candidates in non-human primates. Vaccine. 2009;27(34):4709-17.
165. Dijkman K, Sombroek CC, Vervenne RAW, Hofman SO, Boot C, Remarque EJ, et al. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat Med. 2019;25(2):255-62.
166. Larson EC, Ellis-Connell AL, Rodgers MA, Gubernat AK, Gleim JL, Moriarty RV, et al. Vaccination with intravenous BCG protects macaques with pre-existing SIV infection from tuberculosis. Res Sq. 2023.
167. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LAB, Jacobs C, et al. Long-Lasting Effects of BCG Vaccination on Both Heterologous Th1/Th17 Responses and Innate Trained Immunity. J Innate Immun. 2014;6(2):152-8.
168. Madura Larsen J, Benn CS, Fillie Y, van der Kleij D, Aaby P, Yazdanbakhsh M. BCG stimulated dendritic cells induce an interleukin-10 producing T-cell population with no T helper 1 or T helper 2 bias in vitro. Immunology. 2007;121(2):276-82.

169. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonca LE, Pacis A, et al. BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. Cell. 2018;172(1-2):176-90 e19.
170. Vierboom MPM, Dijkman K, Sombroek CC, Hofman SO, Boot C, Vervenne RAW, et al. Stronger induction of trained immunity by mucosal BCG or MTBVAC vaccination compared to standard intradermal vaccination. Cell Rep Med. 2021;2(1):100185.
171. Heldwein KA, Liang MD, Andresen TK, Thomas KE, Marty AM, Cuesta N, et al. TLR2 and TLR4 serve distinct roles in the host immune response against Mycobacterium bovis BCG. J Leukoc Biol. 2003;74(2):277-86.
172. Dowling DJ, Scott EA, Scheid A, Bergelson I, Joshi S, Pietrasanta C, et al. Toll-like receptor 8 agonist nanoparticles mimic immunomodulating effects of the live BCG vaccine and enhance neonatal innate and adaptive immune responses. J Allergy Clin Immunol. 2017;140(5):1339-50.
173. Bekkering S, Blok BA, Joosten LA, Riksen NP, van Crevel R, Netea MG. In Vitro Experimental Model of Trained Innate Immunity in Human Primary Monocytes. Clin Vaccine Immunol. 2016;23(12):926-33.
174. James CA, Yu KKQ, Mayer-Blackwell K, Fiore-Gartland A, Smith MT, Layton ED, et al. Durable Expansion of TCR-delta Meta-Clonotypes After BCG Revaccination in Humans. Front Immunol. 2022;13:834757.
175. Cayabyab MJ, Korioth-Schmitz B, Sun Y, Carville A, Balachandran H, Miura A, et al. Recombinant Mycobacterium bovis BCG prime-recombinant adenovirus boost vaccination in rhesus monkeys elicits robust polyfunctional simian immunodeficiency virus-specific T-cell responses. J Virol. 2009;83(11):5505-13.
176. Rosario M, Fulkerson J, Soneji S, Parker J, Im EJ, Borthwick N, et al. Safety and immunogenicity of novel recombinant BCG and modified vaccinia virus Ankara vaccines in neonate rhesus macaques. J Virol. 2010;84(15):7815-21.
177. Ami Y, Izumi Y, Matsuo K, Someya K, Kanekiyo M, Horibata S, et al. Priming-boosting vaccination with recombinant Mycobacterium bovis bacillus Calmette-Guerin and a nonreplicating vaccinia virus recombinant leads to long-lasting and effective immunity. J Virol. 2005;79(20):12871-9.
178. Korioth-Schmitz B, Perley CC, Sixsmith JD, Click EM, Lee S, Letvin NL, et al. Rhesus immune responses to SIV Gag expressed by recombinant BCG vectors are independent from pre-existing mycobacterial immunity. Vaccine. 2015;33(42):5715-22.
179. Aghdasi HS, Bisadi P, Moghaddam ME, Abbaspour M. High-Resolution Images with Minimum Energy Dissipation and Maximum Field-of-View in Camera-Based Wireless Multimedia Sensor Networks. Sensors (Basel). 2009;9(8):6385-410.
180. Rosario M, Hopkins R, Fulkerson J, Borthwick N, Quigley MF, Joseph J, et al. Novel recombinant Mycobacterium bovis BCG, ovine atadenovirus, and modified vaccinia virus Ankara vaccines combine to induce robust human immunodeficiency virus-specific CD4 and CD8 T-cell responses in rhesus macaques. J Virol. 2010;84(12):5898-908.
181. Chege GK, Burgers WA, Stutz H, Meyers AE, Chapman R, Kiravu A, et al. Robust immunity to an auxotrophic Mycobacterium bovis BCG-VLP prime-boost HIV vaccine candidate in a nonhuman primate model. J Virol. 2013;87(9):5151-60.
182. Martins MA, Wilson NA, Piaskowski SM, Weisgrau KL, Furlott JR, Bonaldo MC, et al. Vaccination with Gag, Vif, and Nef gene fragments affords partial control of viral replication after mucosal challenge with SIVmac239. J Virol. 2014;88(13):7493-516.
183. Hopkins R, Bridgeman A, Joseph J, Gilbert SC, McShane H, Hanke T. Dual neonate vaccine platform against HIV-1 and M. tuberculosis. PLoS One. 2011;6(5):e20067.
184. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, et al. Variation in the human immune system is largely driven by non-heritable influences. Cell. 2015;160(1-2):37-47.
185. Rolle A, Brodin P. Immune Adaptation to Environmental Influence: The Case of NK Cells and HCMV. Trends Immunol. 2016;37(3):233-43.
186. McGeoch DJ, Rixon FJ, Davison AJ. Topics in herpesvirus genomics and evolution. Virus research. 2006;117(1):90-104.
187. Barry PA, Strelow L. Development of breeding populations of rhesus macaques (Macaca mulatta) that are specific pathogen-free for rhesus cytomegalovirus. Comp Med. 2008;58(1):43-6.
188. Oxford KL, Dela Pena-Ponce MGA, Jensen K, Eberhardt MK, Spinner A, Van Rompay KK, et al. The interplay between immune maturation, age, chronic viral infection and environment. Immun Ageing. 2015;12:3.

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189. Shah SV, Manickam C, Ram DR, Kroll K, Itell H, Permar SR, et al. CMV Primes Functional Alternative Signaling in Adaptive Deltag NK Cells but Is Subverted by Lentivirus Infection in Rhesus Macaques. Cell Rep. 2018;25(10):2766-74 e3.
190. Truitt LL, Yang D, Espinoza DA, Fan X, Ram DR, Mostrom MJ, et al. Impact of CMV Infection on Natural Killer Cell Clonal Repertoire in CMV-Naive Rhesus Macaques. Front Immunol. 2019;10:2381.
191. Kaur A, Kassis N, Hale CL, Simon M, Elliott M, Gomez-Yafal A, et al. Direct relationship between suppression of virus-specific immunity and emergence of cytomegalovirus disease in simian AIDS. J Virol. 2003;77(10):5749-58.
192. Mendez-Lagares G, Chin N, Chang WLW, Lee J, Rosas-Umbert M, Kieu HT, et al. Cytomegalovirus mediates expansion of IL-15-responsive innate-memory cells with SIV killing function. J Clin Invest. 2021;131(15).
193. Furman D, Jojic V, Sharma S, Shen-Orr SS, Angel CJ, Onengut-Gumuscu S, et al. Cytomegalovirus infection enhances the immune response to influenza. Sci Transl Med. 2015;7(281):281ra43.
194. Bowyer G, Sharpe H, Venkatraman N, Ndiaye PB, Wade D, Brenner N, et al. Reduced Ebola vaccine responses in CMV+ young adults is associated with expansion of CD57+KLRG1+ T cells. J Exp Med. 2020;217(7).
195. Sharpe HR, Provine NM, Bowyer GS, Moreira Folegatti P, Belij-Rammerstorfer S, Flaxman A, et al. CMV-associated T cell and NK cell terminal differentiation does not affect immunogenicity of ChAdOx1 vaccination. JCI Insight. 2022;7(6).
196. Mohan T, Zhu W, Wang Y, Wang BZ. Applications of chemokines as adjuvants for vaccine immunotherapy. Immunobiology. 2018;223(6-7):477-85.
197. Strbo N, Vaccari M, Pahwa S, Kolber MA, Doster MN, Fisher E, et al. Cutting edge: novel vaccination modality provides significant protection against mucosal infection by highly pathogenic simian immunodeficiency virus. J Immunol. 2013;190(6):2495-9.
198. Ratnapriya S, Perez-Greene E, Schifanella L, Herschhorn A. Adjuvant-mediated enhancement of the immune response to HIV vaccines. FEBS J. 2022;289(12):3317-34.