Review

HIV/AIDS vaccines for Africa: scientific opportunities, challenges and strategies

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Abstract

More than decades have already elapsed since human immunodeficiency virus (HIV) was identified as the causative agent of acquired immunodeficiency syndrome (AIDS). The HIV has since spread to all parts of the world with devastating effects. In sub-saharan Africa, the HIV/AIDS epidemic has reached unprecedented proportions. Safe, effective and affordable HIV/AIDS vaccines for Africans are therefore urgently needed to contain this public health problem. Although, there are challenges, there are also scientific opportunities and strategies that can be exploited in the development of HIV/AIDS vaccines for Africa. The recent RV144 Phase III trial in Thailand has demonstrated that it is possible to develop a vaccine that can potentially elicit modest protective immunity against HIV infection. The main objective of this review is to outline the key scientific opportunities, challenges and strategies in HIV/AIDS vaccine development in Africa.

Introduction

Since the human immunodeficiency virus (HIV) was previously identified as the etiologic agent for acquired immunodeficiency syndrome (AIDS) more than three decades ago, the virus has spread to almost all parts of the world [1]. Globally, more than 34 million people are infected and living with the virus [2]. In Sub-Saharan Africa, the HIV/AIDS pandemic has reached unprecedented levels in most countries especially in Southern Africa [2]. The HIV that causes AIDS is a lentivirus and has an RNA genome that encodes nine open reading frames. The nine proteins encoded are classified into four groups, structural proteins (Gag and Env), enzyme proteins (Pol), regulatory proteins (Tat and Rev) and (d) accessory (auxiliary) proteins (Vpu, Vpr, Vif and Nef). Most of these genes are targets for the development of vaccines to be used for the prevention and control of HIV infection in Sub-Saharan Africa. The recent Thailand's RV144 phase-III trial has shown that it is possible to develop a vaccine that can induce some protective immune responses against HIV acquisition [3].

Methods

This was not a systematic review. So medical literature related to HIV/AIDS vaccines and other aspects of HIV in Africa was searched unsystematically. From the database, PubMed, specific search approaches were used. We searched the literature using keywords such as "HIV vaccines", "HIV immune responses", "HIV vaccines in Africa", "Challenges HIV vaccines", "HIV genetic diversity" and "HIV vaccine approaches". Only articles with data or results of interest to HIV vaccines in Africa were included. The articles selected were all in English. Most of the publications we used were from 1983 to 2013. We also collected literature related to HIV vaccines and biology of HIV infection from sources such as books and reports from organizations such as UNAIDS (the Joint United Nations Programme on HIV/AIDS) and World Health Organization.

Current status of knowledge

Immunological expectations of HIV/AIDS vaccines

In Sub-Saharan Africa, the main mode of HIV infection is through heterosexual transmission. It is therefore critical that HIV/AIDS vaccines for the region provoke both mucosal and systemic innate and adaptive immune responses against the virus. Immune cells of the innate system play a fundamental role in preventing or control HIV infection especially in the early stages of infection [4]. These cells secrete chemokines and cytokines that have the potential to block viral transmission and replication at mucosal sites. Natural killer (NK) cells are important cells of the innate immune system and they are capable of killing virus-infected cells either directly or through antibody-dependent cell-mediated cytotoxicity and they can produce interferon gamma (IFN-γ) and β-chemokines, which have anti-HIV activities [5]. Beta-chemokine-specific responses can also inhibit HIV entry during infection [6]. The antiviral activities of NK cells soon after SIV infection have been demonstrated in rhesus macaques [7]. Apart from NK cells, other cells of the innate system such as macrophages and dendritic cells also secrete a number of chemokines and cytokines that contribute in controlling HIV infection during the early stages [8]. In many ways, innate immune response controls the disease and regulates the nature and quality of the subsequent adaptive immune responses [9]. Thus vaccines that provoke the innate immune system may suppress HIV replication during the early stages of infection. However, one of the greatest challenges is to develop the HIV/AIDS vaccines that can activate the innate immune system, thereby subsequently signaling the adaptive arm of the system.

CD8+ T cell responses are an important arm of adaptive immunity and can play a major role in preventing or controlling of viral infections. In HIV infection, CD8+ cytotoxic T lymphocytes (CTLs) have the potential to destroy virally-infected cells by a number of ways [10]. First, the binding of HIV-specific CD8+ T cells to viral peptides presented by human leukocyte antigens (HLAs) on the surface of infected cells have the capacity to trigger lysis of HIV-infected cells [10, 11]. The lysis of the virus-infected cells occurs through the production of perforin and granzymes, which penetrate into the cells and induce apoptosis [12]. Second, HIV-specific CD8+ T cells produce cytokines such as IFN-γ and tumour necrosis factor alpha (TNF-α), which all have antiviral activities that can suppress replication of HIV [13]. Third, CD8+ T cells produce chemokines

that block HIV entry by binding to HIV coreceptors [13]. The cytolytic activities of CD8+ T cells can further be enhanced by these chemokines [11,13]. Scientific evidence are available that show that CD8+ T cell responses play an important role in controlling HIV infection or eliminating infected cells. In primary infection, HIV-specific CD8+ T-cells have been found to suppress or reduce viral replication [10,14]. After infection, it has also been found that the emergence of HIV-specific CD8+ T-cells is always associated with a decreased viral load [15]. Potent HIV-specific CD8+ T cell responses in chronic infection are also always correlated with low viraemia and reduced disease progression [15]. In animal models of HIV infection, it has been shown that depletion of CD8+ T lymphocytes would lead to failure to control viral replication [16]. In vitro studies have further demonstrated that HIV-specific CTLs can efficiently kill HIV-infected cells and inhibit viral replication [17]. It has also been noted that highly HIV-exposed seronegative African sex-workers had detectable HIV-specific lysis [18]. The emergence of CD8+ T cell epitope escape mutants has also been shown to be associated with rapid disease progression and this remains a great challenge to vaccine development [19]. Furthermore, vaccine-induced CD8+ T cell responses have been shown to protect macaques from developing AIDS after a challenge with simian-human immunodeficiency virus [20]. These studies clearly demonstrate that protection or control of HIV infection requires CD8+ T cell responses and vaccines targeted for Africans should induce this type of immune response.

There is also mounting evidence to support that CD4+ T helper (Th) responses play critical roles in prevention or control of HIV-1 infection and replication [21]. HIV-infected individuals who are long-term non-progressors have been found to have strong CD4+ T cell responses to HIV antigens [21]. CD4+ T cell responses were also associated with control of HIV viremia as it was noted that patients with the highest CD4+ T cell responses had the lowest viral loads, whereas patients with the lowest CD4+ T cell responses had the highest viral loads [22]. In some patients, strong CD4+ T helper responses were found to be associated with strong CD8+ CTL responses [21]. Recent studies have further suggested that HIV-specific CD4+ Th1 cells producing INF-β and IL-2, together with IgG2 were important in long-term control of HIV infection and reduced viraemia [23]. Thus, CD4+ T cells provide immunological help to CD8+ T cell responses. CD4+ T cells also produce cytokines such as IFN-γ and TNF-α that have antiviral activities [24]. CD4+ T cells further provide help for antibody responses that may be critical for neutralization of the virus. There have been recent reports of the cytotoxic CD4+ T-cells detected in HIV infection [25]. Therefore, HIV/AIDS vaccines for Africa need to induce specific CD4+ T cell responses that would enhance both CD8+ T cell and humoral immune responses.

Humoral immune response that is mediated by antibodies produced by B cell lymphocytes plays a protective role against many viral pathogens including HIV [26]. The antibodies offer protection by neutralizing pathogen antigens, thereby preventing infection. Infection with HIV induces virus-specific antibody responses [26]. It has been demonstrated that HIV-infected individuals elicit high levels of antibodies against different viral antigens [27]. The antibodies that have the capacity to neutralize HIV (neutralizing antibodies) are mainly directed against the viral Env protein and they can potentially block HIV replication or infection by virus neutralization or antibody-dependent cellular cytotoxicity of HIV-infected cells [28]. Neutralizing antibodies are normally correlated with significant decline of the primary viremia [29]. Furthermore, some infected individuals with strong neutralizing antibody responses control their viraemia for a long time [30]. Studies in animal models have demonstrated that passive immunization with monoclonal antibodies generated from HIV-infected individuals such as 2F5, 2G12, and 4E10 could protect monkeys from challenge infections [31,32]. Studies done on RV144 trial follow-up also demonstrated that antibodies to variable loops of HIV-1 Env were associated with reduction in the acquisition of the virus [3]. Non-neutralizing antibodies through antibody-dependent cell-mediated-ADCC and antibody-dependent cell-mediated viral inhibition also play a key role in preventing or controlling HIV infection [33]. Therefore, HIV/AIDS vaccines developed for Africans should be capable of eliciting both neutralizing and non-neutralizing antibodies that can prevent establishment of a new HIV infection or reduce the replication of genetically diverse HIV viruses.

Most human pathogens such as HIV infect their hosts through the mucosal surfaces. These mucosal surfaces are found in the gastro-intestinal, urogenital and respiratory tracts and play important role in the uptake and transport of pathogens or antigens [34,35]. In Africa, the majority of HIV infections occur through mucosal exposure to seminal fluid or vaginal secretions of infected individuals. On top of mucosal transmission, HIV replicates in the mucosal lymphoid tissue before systemic spread [36]. The CD4+ T cells of mucosal lymphoid tissues are also the targets of HIV throughout infection, leading to their depletion [37]. Mucosal plasma cells synthesize secretory immunoglobulin A (IgA) that has

the potential to neutralize HIV [38]. Mucosal surfaces are rich in immune cells such as dendritic cells, macrophages, CD4+ and CD8+ T cells which can play important roles in provoking immunity to a variety of pathogens including HIV [39]. Studies have demonstrated the presence of SIV- and HIV-specific CD8+ T cell responses in the genital tracts of infected macaques [40]. Inducing humoral, CD8+ and CD4+ T cell responses at mucosal surfaces with vaccines can potentially prevent or control HIV replication in the mucosal lymphoid tissue. One of the key advantages of mucosal vaccination against HIV is that mucosal immunity protects systemic infection, whereas systemic immunity poorly protects against mucosal infection [41]. The other advantage of mucosal vaccination is that antigenic exposure at one mucosal site activates B and T cells to emigrate and home to other mucosal surfaces, thereby conferring protection at these sites [42]. Protective vaccine-induced mucosal immunity against HIV has been demonstrated in animal models [43]. Therefore the challenge is to develop HIV vaccines for Africa that can induce both B and T cell responses in mucosal tissues.

Immune correlates of HIV protection in Africa

To date, the exact immune correlates of protection against natural HIV infection are poorly defined. However, it is generally regarded that HIV protective immune responses should comprise of CD8+ T, CD4+ T cell and humoral immune responses. Different components of these immune responses have different effectiveness in preventing or controlling HIV infection. Whereas neutralizing antibodies can potentially block HIV infection, they are not effective against cells that are already infected [44]. Cellular immune responses can control HIV infection, but cannot prevent infection. The key challenge is to develop vaccines that can induce multiple forms of immune responses against HIV. Mucosal immune responses may potentially be required to control the early stages of HIV infection and replication. Mucosal immunity may also delay systemic spread of the virus. To date, we do not clearly know the exact nature, quality and magnitude of immune responses that should be elicited by the HIV or HIV/AIDS vaccine. Nevertheless, recent animal studies have suggested that vaccine-induced SIV-specific memory CD8+ T and CD4+ T cells may correlate with protection against simian AIDS disease in monkeys [45,46]. Although CD8+ T cell and neutralizing antibodies are considered important for HIV protection or control of infection, the RV144 trial has demonstrated that non-neutralizing antibodies are also crucial [3]. Non-neutralizing antibodies can mediate antibody-dependent cellular cytotoxicity (ADCC), thereby protecting against HIV infection

[47]. ADCC is important because it is associated with reduced HIV/AIDS diseases progression and prevention of cell-to-cell spread by the virus [48]. However, the rise of ADCC-escape HIV variants can be a scientific challenge to HIV/AIDS vaccine development [49]. Another key challenge is that antibodies mediated via complement system or Fc receptors can unfortunately facilitate the infectivity of the HIV [50].

Scientific challenges to HIV/AIDS vaccine development for Africa

Current efforts in development of HIV vaccines for Sub-Saharan Africans are hampered by a number of scientific challenges. A key characteristic feature of HIV subtypes in Sub-Saharan Africa is their high genetic variability [51]. The genetic diversity and high mutation rate emanate from the failure of the HIV reverse transcriptase (RT) enzyme to proof-read the viral genome during replication [52]. The RT can further facilitate the generation of new viral genetic recombinants due to its recombinogenic properties [53]. These two factors contribute to the generation of high genetic diversity of HIV in Africa. The HIV has been divided into groups, sub-types and circulating recombinant forms (CRF) and unique recombinants [54]. Three distinct HIV-1 groups of viruses exist [54]. They are M (main), O (outlier) and N (new or non-M/non-O). Group M viruses are responsible for the majority of HIV-1 infections worldwide including Africa. Group O isolates are highly divergent from group M, their prevalence is low compared to other viruses and infection is confined to West African countries. Phylogenetic analysis of the env and gag genes of the group M has established 9 distinct subtypes (subtypes A, B, C, D, F, G, H, J and K) [55]. Different parts of Africa have different HIV-1 subtypes being prevalent. The genetic diversity of HIV-1 subtypes and emergence of new recombinants remains one of the key challenges in vaccine development in Africa. It is therefore remains a great challenge to develop HIV/AIDS vaccines that can target all the diverse HIV-1 subtypes and recombinants circulating in Africa. Another strategy will be to develop vaccines specific for specific regions of Africa that include only antigens of the circulating subtypes.

As stated above, the correlates of protection after HIV infection or vaccination are very complex and poorly understood [56]. Our poor understanding of the correlates of HIV protection makes the development of vaccines for Africans a great challenge. However, studies in Africa have given us some clues of the requirements or expectations of an AIDS vaccine. Some sex workers in Africa were

found to be resistant to HIV infection despite them being exposed to the risk [18]. The high prevalence of HIV discordance in African couples also suggests that the correlates of protection exist in the African populations [57]. Further research on HIV discordance should bring a deeper understanding of some of the correlates of protection in Africans and can subsequently lead to the development of vaccines.

Currently, there are no good animal models to test HIV/AIDS vaccines. Chimpanzees and macaques are most commonly used to study the HIV pathogenesis as well as vaccines [58]. Mice are also used in pre-clinical evaluation of vaccines, but results in mice do not normally predict what will be found in humans. The use of monkey or baboon primate models in studying HIV/AIDS vaccines has also its drawbacks. Data generated from mouse, monkey or baboon models do not normally translate to what will be found in human clinical trials. Humanized mice can also be used in testing HIV/AIDS vaccines, but they do not normally elicit strong immune responses [59].

Bantu-speaking African populations of Sub-Saharan Africa are highly diverse genetically [60]. The high prevalence of high-risk exposed HIV-seronegative individuals in Africa has suggested the role of genetic factors in influencing HIV infection or immune responses to infection [61]. Some of the genetic factors include the genes of HIV coreceptors and their natural ligands, the human leukocyte antigen (HLA), apolipoprotein B mRNA-editing, enzyme-catalytic (APOBEC), tripartite motif-containing protein 5 (TRIM5) and killer cell immunoglobulin-like receptor (KIR) genes [62,63]. The geographic and genetic variations in some of these host genes especially the HLA haplotypes in Africa are likely to affect immune responses to HIV infection or vaccinations in Africans. It is therefore important to bear this in mind when developing HIV/AIDS vaccines for Africans that genetic diversity may cause variation in immune responses.

Scientific strategies for HIV/AIDS vaccine development for Africa

To date, there is no licensed HIV/AIDS vaccine for Africa. Several rational and empirical strategies to HIV vaccine development have so far failed dismally. However, a variety of these strategies need to be refined if we are to develop potential vaccine candidates for Africa. It has been a classical approach to use inactivated viruses as vaccines. It is possible to inactivate HIV and this strategy has been explored [64,65]. Although the inactivated HIV vaccine candidates

may be safe for use, even in immunocompromised people, the strategy is not advocated for due to poor immunogenicity elicited by these vaccines. Such HIV vaccines would not be very helpful for Africa, given their poor immunogenicity. The great challenge is to generate inactivated vaccines that are highly immunogenic in Africans. It is easy to genetically attenuate viruses such as HIV using modern tools of genetic engineering whereby mutations or deletions are introduced in specific viral genes. This generates genetically attenuated HIV vaccines, which are expected to confer immunogenicity without causing AIDS. Although some of the live attenuated HIV vaccines have shown a degree of protective efficacy in animal models this approach has not attracted much attention because of safety concerns in humans [66-68]. To date, no live attenuated HIV/AIDS vaccine candidates specifically developed for Africa have been tested in clinical trials. Subunit vaccine candidates against HIV/AIDS have been developed [69,70]. These vaccines are based on purified HIV antigens such as Envelope and Gag. Most of the early HIV/AIDS vaccines that entered Phase I trials were based on Envelope subunit. HIV virus-like particles (VLPs) are also subunit vaccines generated from the expression of Gag alone and can be used as immunogens. They have been found to be immunogenic in animal trials especially when used in prime-boost strategies with other vaccines [70,71]. Although HIV/AIDS subunit vaccines have been found to be immunogenic in animals, human trials have shown disappointing results.

Recombinant plasmids, when used as DNA vaccines induce immune responses specific to the antigen genes carried [72]. Most HIV DNA vaccines have been shown to be safe and to induce protective immune responses in animal models [73,74]. Induction of both CD8+ CTL and humoral immune responses were demonstrated in animals primed with gp120 DNA vaccine and boosted with gp120 subunit vaccine [75]. A number of candidate DNA vaccines for HIV-1 have already been developed and some tested in Africa for immunogenicity [76-78]. Such vaccines are likely to be useful in Africa since they induce strong immune responses especially if they are used in prime-boost strategies. Recombinant live viruses can be exploited as vaccine vectors for heterologous antigens [79,80]. The key advantage of viral vectors is that they can generate very strong antigen-specific CD8+ and CD4+ T cell as well as humoral immune responses [79-81]. Most viral vectors can target both the innate and adaptive immune responses at both the mucosal and systemic compartments. A number of viral vaccine vectors for HIV antigens have so far been developed and tested. These viral vaccine vectors have been genetically engineered to express different HIV antigens.

Effective anti-HIV immunity, sometimes protective, has been observed in a number of animal studies in which vectors such as adenovirus, alphavirus, sendai virus, herpes simplex virus, human rhinovirus and polio virus were used to express HIV antigens [82-86]. Recombinant viral vectors therefore seem to offer great opportunities for vaccine development for Africans because of their ability to induce strong HIV-specific immune responses. Recombinant bacteria can also be used to deliver heterologous antigens to the host's immune system [87]. Their potential use as candidate HIV vaccine vectors to deliver either HIV antigens or HIV DNA vaccines is currently being increasingly studied. Recombinant Bacillus Calmette-Guerin (BCG) expressing HIV antigens has been shown to induce antigen-specific immune responses in vaccinated animals [88]. BCG is generally a good vaccine vector for HIV/AIDS because of a number of reasons such as its known safety record [89]. Another attractive bacterial vaccine vector for HIV/AIDS is *Listeria monocytogenes* [90]. The key advantage of *Listeria* as a vaccine vector is that it replicates in the cytosol, thereby inducing both strong CD8+ and CD4+ T cell responses. *Shigella* is also an attractive vector that is capable of replicating in the cytosol thereby inducing strong cellular immune responses [91]. Attenuated *Shigella* strains have already been successfully used to deliver HIV DNA vaccines, resulting in induction of HIV-specific CD8+ T responses [92]. Recombinant *Salmonella* has also a great potential as a vaccine vector for HIV [93-95]. Therefore, recombinant bacterial vaccine vectors can be harnessed in the development of HIV/AIDS vaccines for Africa.

Candidate HIV/AIDS vaccines for Africa can be used in prime-boost strategies in order to improve their potency and effectiveness. In these strategies, one vaccine is used to prime the immune system and the second vaccine is used to boost the response [96,97]. A number of candidate HIV/AIDS vaccines have already been tested in a prime-boost strategy and have been shown to induce good responses [98,99]. DNA vaccines have already been shown to be best at priming and recombinant viral vectors such as poxvirus vectors are good at boosting the immune responses [100]. Even the Thailand's RV144 phase-III trial used the prime-boost strategy in which a recombinant canarypox vector-based candidate was used in combination with an engineered HIV-1 gp120 protein [3].

Conclusion

The HIV/AIDS remains a serious public health problem in Africa and development of safe, effective and affordable vaccines for the region remains a daunting challenge. However, a number of efforts have already been done to develop candidate vaccines.

Competing interests

The authors declare no competing interest.

Authors' contributions

All the authors gathered data and wrote the manuscript. All authors read and agreed to the final manuscript.

Acknowledgments

References

1. Gallo RC, Sarin PS, Gelmann EP et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome. Science. 1983; 220(4599):865-867. **PubMed**| **Google Scholar**

2. UNAIDS Global Report on Global AIDS Epidemic. 2012; World Health Organization, Geneva. **Google Scholar**

3. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009; 361(23):2209–2220. **PubMed** | **Google Scholar**

4. Lehner T. Innate and adaptive mucosal immunity in protection against HIV infection. Vaccine. 2003; 21 (S2):S68-S76. **PubMed** | **Google Scholar**

5. Forthal DN, Landucci G, Daar ES. Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. J Virol. 2001; 75(15):6953-6961. **PubMed** |**Google Scholar**

6. Cocchi F, DeVico AL, Lu W et al. Soluble factors from T cells inhibiting X4 strains of HIV are a mixture of ? chemokines and RNases. Proc Natl Acad Sci U S A. 2012; 109(14):5411-5416. **PubMed** | **Google Scholar**

7. Giavedoni LD, Velasquillo MC, Parodi LM, et al. Cytokine expression, natural killer cell activation, and phenotypic changes in lymphoid cells from rhesus macaques during acute infection with pathogenic simian immunodeficiency virus. J Virol. 2000; 74(4):1648-1657. **PubMed** | **Google Scholar**

8. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol. 2001; 19:65-91. **PubMed** | **Google Scholar**

9. Pulendran B, Ahmed R. Translating innate immunity into immunological memory: implications for vaccine development. Cell. 2006; 124(4):849-863. **PubMed** |**Google Scholar**

10. Yang OO, Walker BD. CD8+ cells in human immunodeficiency virus type I pathogenesis: cytolytic and noncytolytic inhibition of viral replication. Adv Immunol. 1997; 66:273-311. **PubMed** | **Google Scholar**

11. Hadida F, Vieillard V, Mollet L, et al. Cutting edge: RANTES regulates Fas ligand expression and killing by HIV-specific CD8 cytotoxic T cells. J Immunol. 1999; 163(3):1105-1109. **PubMed** | **Google Scholar**

12. McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. Nature. 2001; 410(6831):980-987. **PubMed** | **Google Scholar**

13. Gulzar N, Copeland KF. CD8+ T-cells: function and response to HIV infection. Curr HIV Res. 2004; 2(1):23-37. **PubMed** | **Google Scholar**

14. Walker BD, Chakrabarti S, Moss B, et al. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature. 1987; 328(6128): 345-348. **PubMed** | **Google Scholar**

15. Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. Annu Rev Immunol. 2003; 21:265-304. **PubMed** | **Google Scholar**

16. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood. 2006; 107(12):4781-4789.**PubMed** | **Google Scholar**

17. Madden LJ, Zandonatti MA, Flynn CT et al. CD8+ cell depletion amplifies the acute retroviral syndrome. J Neurovirol. 2004; 10 (S1):58-66. **PubMed** | **Google Scholar**

18. Kaul R, Rowland-Jones SL, Kimani J et al. New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers. Immunol Lett. 2001; 79(1-2):3-13. **PubMed** | **Google Scholar**

19. Allen TM, Altfeld M, Yu XG, et al. Selection, transmission, and reversion of an antigen-processing cytotoxic T-lymphocyte escape mutation in human immunodeficiency virus type 1 infection. J Virol. 2004; 78(13):7069-7078. **PubMed** |**Google Scholar**

20. Amara RR, Villinger F, Altman JD et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science. 2001; 292(5514):69-74. **PubMed** | **Google Scholar**

21. Kalams SA, Buchbinder SP, Rosenberg ES, et al. Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. J Virol. 1999; 73(8):6715-6720. **PubMed** | **Google Scholar**

22. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science. 1997; 278(5342):1447-1450.**PubMed | Google Scholar**

23. Emu B, Sinclair E, Favre D et al. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. J Virol. 2005; 79(22):14169-14178. **PubMed | Google Scholar**

24. Porichis F, Kaufmann DE. HIV-specific CD4 T cells and immune control of viral replication. Curr Opin HIV AIDS. 2011; 6(3):174-80. **PubMed | Google Scholar**

25. Zaunders JJ, Dyer WB, Wang B et al. Identification of circulating antigen-specific CD4+ T lymphocytes with a CCR5+, cytotoxic phenotype in an HIV-1 long-term nonprogressor and in CMV infection. Blood. 2004; 103(6): 2238–2247. **PubMed |Google Scholar**

26. Letvin NL, Walker BD. Immunopathogenesis and immunotherapy in AIDS virus infections. Nat Med. 2003; 9(7):861-866. **PubMed | Google Scholar**

27. Nabel GJ, Sullivan NJ. Antibodies and resistance to natural HIV infection. N Engl J Med. 2000; 343(17): 1263-1265. **PubMed | Google Scholar**

28. Gómez-Román VR, Patterson LJ, Venzon D et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol. 2005; 174(4):2185-2189. **PubMed | Google Scholar**

29. Moore JP, Cao Y, Ho DD et al. Development of the anti-gp120 antibody response during seroconversion to human immunodeficiency virus type 1. J Virol. 1994; 68(8):5142-5155. **PubMed | Google Scholar**

30. Harrer T, Harrer E, Kalams SA, et al. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. AIDS Res Hum Retroviruses. 1996; 12(7):585-592. **PubMed | Google Scholar**

31. Mascola JR, Stiegler G, VanCott TC et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med. 2000; 6(2):207-210. **PubMed | Google Scholar**

32. Baba TW, Liska V, Hofmann-Lehmann R et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med. 2000; 6(2):200-206. **PubMed | Google Scholar**

33. Xiao P, Zhao J, Patterson LJ et al. Multiple vaccine-elicited nonneutralizing antienvelope antibody activities contribute to protective efficacy by reducing both acute and chronic viremia following simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. J Virol. 2010; 84(14):7161-7173. **PubMed | Google Scholar**

34. Neutra MR, Frey A and Kraehenbuhl JP. Epithelial M cells: gateways for mucosal infection and immunization. Cell. 1996; 86(3):345-348. **PubMed | Google Scholar**

35. Amerongen HM, Weltzin R, Farnet CM et al. Transepithelial transport of HIV-1 by intestinal M cells: the mechanism of transmission of AIDS. J Acquir Immune Defic Syndr. 1991; 4(8):760-765. **PubMed | Google Scholar**

36. Brenchley JM, Schacker TW, Ruff LE et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004; 200(6):749-759. **PubMed | Google Scholar**

37. Mehandru S, Tenner-Racz K, Racz P et al. The gastrointestinal tract is critical to the pathogenesis of acute HIV-1 infection. J Allergy Clin Immunol. 2005; 116(2):419-422.**PubMed | Google Scholar**

38. Devito C, Hinkula J, Kaul R et al. Cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed, persistently seronegative subjects. J Acquir Immune Defic Syndr. 2002; 30(4):413-420. **PubMed | Google Scholar**

39. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Rev Immunol. 2006; 6(2):148-158. **PubMed** | **Google Scholar**

40. Lohman BL, Miller CJ, McChesney MB. Antiviral cytotoxic T lymphocytes in vaginal mucosa of simian immunodeficiency virus-infected rhesus macaques. J Immunol. 1995; 155(12):5855-5860. **PubMed** | **Google Scholar**

41. Lekkerkerker AN, Van Kooyk Y, Geijtenbeek TB. Mucosal-targeted AIDS vaccines: the next generation?. Trends Microbiol. 2004; 12(10):447-450. **PubMed** | **Google Scholar**

42. Iijima H, Takahashi I, Kiyono H. Mucosal immune network in the gut for the control of infectious diseases. Rev Med Virol. 2001; 11(2):117-133. **PubMed** | **Google Scholar**

43. Crotty S, Miller CJ, Lohman BL et al. Protection against simian immunodeficiency virus vaginal challenge by using Sabin poliovirus vectors. J Virol. 2001; 75(16):7435-7452. **PubMed** | **Google Scholar**

44. Pantaleo G1, Koup RA. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. Nat Med. 2004; 10(8):806-810.**PubMed** | **Google Scholar**

45. Vaccari M, Trindade CJ, Venzon D, et al. Vaccine-induced CD8+ central memory T cells in protection from simian AIDS. J Immunol. 2005; 175(6):3502-3507. **PubMed**| **Google Scholar**

46. Letvin NL, Mascola JR, Sun Y et al. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science. 2006; 312(5779):1530-3.**PubMed** | **Google Scholar**

47. Madhavi V, Kent SJ, Stratov I. HIV-specific antibody-dependent cellular cytotoxicity: a novel vaccine modality. Expert Rev Clin Immunol. 2012; 8(8):767-774. **PubMed** |**Google Scholar**

48. Wren LH, Chung AW, Isitman G et al. Specific antibody-dependent cellular cytotoxicity responses associated with slow progression of HIV infection. Immunology. 2013; 138(2):116-123. **PubMed** | **Google Scholar**

49. Chung AW, Isitman G, Navis M et al. Immune escape from HIV-specific antibody-dependent cellular cytotoxicity (ADCC) pressure. Proc Natl Acad Sci U S A. 2011; 108(18):7505-7510. **PubMed** | **Google Scholar**

50. Subbramanian RA, Xu J, Toma E et al. Comparison of human immunodeficiency virus (HIV)-specific infection-enhancing and -inhibiting antibodies in AIDS patients. J Clin Microbiol. 2002; 40(6):2141-2146. **PubMed** | **Google Scholar**

51. Peeters M, Sharp PM. Genetic diversity of HIV-1: the moving target. AIDS. 2000; 14(S3):S129-S140. **PubMed** | **Google Scholar**

52. Ndung'u T, Weiss RA. On HIV diversity. AIDS. 2012; 26(10):1255-1260. **PubMed** |**Google Scholar**

53. Peeters M, Toure-Kane C, Nkengasong JN. Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development and trials. AIDS. 2003; 17(18):2547-2560. **PubMed** | **Google Scholar**

54. Thomson MM, Perez-Alvarez L and Najera R. Molecular epidemiology of HIV-1 genetic forms and its significance for vaccine development and therapy. Lancet Infect Dis. 2002; 2(8):461-471. **PubMed** | **Google Scholar**

55. Geretti AM. HIV-1 subtypes: epidemiology and significance for HIV management. Curr Opin Infect Dis. 2006; 19(1):1-7. **PubMed** | **Google Scholar**

56. Plotkin SA. Complex Correlates of Protection After Vaccination: clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2013; 56(10):1458-1465. **Google Scholar**

57. Guthrie BL, de Bruyn G, Farquhar C. HIV-1-discordant couples in sub-Saharan Africa: explanations and implications for high rates of discordancy. Curr HIV Res. 2007; 5(4):416-29. **PubMed** | **Google Scholar**

58. Letvin NL, King NW. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Acquir Immune Defic Syndr. 1990; 3(11): 102-104. **PubMed** | **Google Scholar**

59. Akkina R. New generation humanized mice for virus research: comparative aspects and future prospects. Virology. 2013; 435(1):14-28. **PubMed** | **Google Scholar**

60. Sirugo G, Hennig BJ, Adeyemo AA et al. Genetic studies of African populations: an overview on disease susceptibility and response to vaccines and therapeutics. Hum Genet. 2008; 123(6):557-598. **PubMed** | **Google Scholar**

61. Fowke KR, Nagelkerke NJ, Kimani J, et al. Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. Lancet. 1996; 348(9038):1347-1351. **PubMed** | **Google Scholar**

62. Sobieszczyk ME, Lingappa JR, McElrath MJ. Host genetic polymorphisms associated with innate immune factors and HIV-1. Curr Opin HIV AIDS. 2011; 6(5):427-434.**PubMed** | **Google Scholar**

63. Taborda-Vanegas N, Zapata W, Rugeles MT. Genetic and Immunological Factors Involved in Natural Resistance to HIV-1 Infection. Open Virol J. 2011; 5:35-43.**PubMed** | **Google Scholar**

64. Sheppard HW. Inactivated- or killed-virus HIV/AIDS vaccines. Curr Drug Targets Infect Disord. 2005; 5(2):131-141. **PubMed** | **Google Scholar**

65. Lifson JD, Rossio JL, Piatak M Jr et al. Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccines with conformationally and functionally intact envelope glycoproteins. AIDS Res Hum Retroviruses. 2004; 20(7): 772-787. **PubMed** | **Google Scholar**

66. Koff WC, Johnson PR, Watkins DI, et al. HIV vaccine design: insights from live attenuated SIV vaccines. Nat Immunol. 2006; 7(1):19-23. **PubMed** | **Google Scholar**

67. Learmont JC, Geczy AF, Mills J et al. Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1: a report from the Sydney Blood Bank Cohort. N Engl J Med. 1999; 340(22):1715-1722. **PubMed** | **Google Scholar**

68. Whitney JB and Ruprecht RM. Live attenuated HIV vaccines: pitfalls and prospects. Curr Opin Infect Dis. 2004; 17(1):17-26. **PubMed** | **Google Scholar**

69. Doan LX, Li M, Chen C et al. Virus-like particles as HIV-1 vaccines. Rev Med Virol. 2005; 15(2):75-88. **PubMed** | **Google Scholar**

70. Zhang X, Wang X, Zhao D et al. Design and immunogenicity assessment of HIV-1 virus-like particles as a candidate vaccine. Sci China Life Sci. 2011; 54(11):1042-1047. **PubMed** | **Google Scholar**

71. Chege GK, Shephard EG, Meyers A, et al. HIV-1 subtype C Pr55gag virus-like particle vaccine efficiently boosts baboons primed with a matched DNA vaccine. J Gen Virol. 2008; 89(Pt 9):2214-2227. **PubMed** | **Google Scholar**

72. Donnelly JJ, Wahren B and Liu MA. DNA vaccines: progress and challenges. J Immunol. 2005; 175(2):633-639. **PubMed** | **Google Scholar**

73. MacGregor RR, Boyer JD, Ugen KE et al. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. J Infect Dis. 1998; 178(1):92-100. **PubMed** | **Google Scholar**

74. Boyer JD, Ugen KE, Wang B et al. Protection of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination. Nat Med. 1997; 3(5):526-532.**PubMed** | **Google Scholar**

75. Barnett SW, Rajasekar S, Legg H et al. Vaccination with HIV-1 gp120 DNA induces immune responses that are boosted by a recombinant gp120 protein subunit. Vaccine. 1997; 15(8):869-873. **PubMed** | **Google Scholar**

76. Estcourt MJ, McMichael AJ, Hanke T. DNA vaccines against human immunodeficiency virus type 1. Immunol Rev. 2004; 199:144-155. **PubMed | Google Scholar**

77. Hanke T, McMichael AJ, Mwau M et al. Development of a DNA-MVA/HIVA vaccine for Kenya. Vaccine. 2002; 20(15):1995-1998. **PubMed | Google Scholar**

78. Wee EG, Patel S, McMichael AJ et al. A DNA/MVA-based candidate human immunodeficiency virus vaccine for Kenya induces multi-specific T cell responses in rhesus macaques. J Gen Virol. 2002; 83(Pt 1):75-80. **PubMed | Google Scholar**

79. Monahan SJ, Salgaller ML. Viral vectors for gene transfer into antigen presenting cells. Curr Opin Mol Ther. 1999;1(5):558-564. **PubMed | Google Scholar**

80. Dudek T, Knipe DM. Replication-defective viruses as vaccines and vaccine vectors. Virology. 2006; 344(1):230-239. **PubMed | Google Scholar**

81. Truckenmiller ME, Norbury CC. Viral vectors for inducing CD8+ T cell responses. Expert Opin Biol Ther. 2004; 4(6):861-868. **PubMed | Google Scholar**

82. Wang SW, Bertley FM, Kozlowski PA et al. An SHIV DNA/MVA rectal vaccination in macaques provides systemic and mucosal virus-specific responses and protection against AIDS. AIDS Res Hum Retroviruses. 2004; 20(8):846-859. **PubMed | Google Scholar**

83. Berglund P, Quesada-Rolander M, Putkonen P et al. Outcome of immunization of cynomolgus monkeys with recombinant Semliki Forest virus encoding human immunodeficiency virus type 1 envelope protein and challenge with a high dose of SHIV-4 virus. AIDS Res Hum Retroviruses. 1997; 13(17):1487-1495. **PubMed |Google Scholar**

84. Murphy CG, Lucas WT, Means RE et al. Vaccine protection against simian immunodeficiency virus by recombinant strains of herpes simplex virus. J Virol. 2000; 74(17):7745-7754. **PubMed | Google Scholar**

85. Matano T, Kano M, Nakamura H et al. Rapid appearance of secondary immune responses and protection from acute CD4 depletion after a highly pathogenic immunodeficiency virus challenge in macaques vaccinated with a DNA prime/Sendai virus vector boost regimen. J Virol. 2001; 75(23):11891-11896. **PubMed | Google Scholar**

86. Arnold G, Velasco P, Wrin T, et al. Recombinant human rhinovirus displaying the HIV-1 gp41 ELDKWA epitope can elicit broad neutralization of HIV-1 primary isolates. AIDS Vaccine 2005 Conference, Montreal, Canada, 2005. **Google Scholar**

87. Roland KL, Tinge SA, Killeen KP et al. Recent advances in the development of live, attenuated bacterial vectors. Curr Opin Mol Ther. 2005; 7(1):62-72. **PubMed |Google Scholar**

88. Chujoh Y, Matsuo K, Yoshizaki H et al. Cross-clade neutralizing antibody production against human immunodeficiency virus type 1 clade E and B' strains by recombinant Mycobacterium bovis BCG-based candidate vaccine. Vaccine. 2001; 20(5-6):797-804.**PubMed | Google Scholar**

89. Chapman R, Chege G, Shephard E et al. Recombinant Mycobacterium bovis BCG as an HIV vaccine vector. Curr HIV Res. 2010; 8(4):282-298. **PubMed | Google Scholar**

90. Paterson Y, Johnson RS. Progress towards the use of Listeria monocytogenes as a live bacterial vaccine vector for the delivery of HIV antigens. Expert Rev Vaccines. 2004; 3(4 Suppl):S119-S134. **PubMed | Google Scholar**

91. Lindberg AA, Pál T. Strategies for development of potential candidate Shigella vaccines. Vaccine. 1993; 11(2):168-179. **PubMed | Google Scholar**

92. Xu F, Hong M and Ulmer JB. Immunogenicity of an HIV-1 gag DNA vaccine carried by attenuated Shigella. Vaccine. 2003; 21(7-8):644-648. **PubMed | Google Scholar**

93. Chin'ombe N, Ruhanya V. Recombinant Salmonella bacteria vectoring HIV/AIDS Vaccines. The Open Virology Journal. 2013; 6:121-126. **PubMed | Google Scholar**

94. Chin'ombe N, Bourn WR, Williamson AL et al. Oral vaccination with a recombinant Salmonella vaccine vector provokes systemic HIV-1 subtype C Gag-specific CD4+ Th1 and Th2 cell immune responses in mice. Virology Journal 2009; 6:87. **Google Scholar**

95. Chin'ombe N. Recombinant Salmonella enterica Serovar Typhimurium as a Vaccine Vector for HIV-1 Gag. Viruses. 2013; 5(9):2062-2078. **PubMed | Google Scholar**

96. Paris RM, Kim JH, Robb ML et al. Prime-boost immunization with poxvirus or adenovirus vectors as a strategy to develop a protective vaccine for HIV-1. Expert Rev Vaccines. 2010; 9(9):1055-1069. **PubMed | Google Scholar**

97. Newman MJ. Heterologous prime-boost vaccination strategies for HIV-1: augmenting cellular immune responses. Curr Opin Investig Drugs. 2002; 3(3):374-378. **PubMed| Google Scholar**

98. Yu S, Feng X, Shu T et al. Potent specific immune responses induced by prime-boost-boost strategies based on DNA, adenovirus, and Sendai virus vectors expressing gag gene of Chinese HIV-1 subtype B. Vaccine. 2008; 26(48):6124-6131. **PubMed |Google Scholar**

99. Koup RA, Roederer M, Lamoreaux L, Fischer J, Novik L, Nason MC, Larkin BD, Enama ME, Ledgerwood JE, Bailer RT, Mascola JR, Nabel GJ, Graham BS; VRC 009 Study Team; VRC 010 Study Team. Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. PLoS One. 2010; 5(2):e9015. **PubMed | Google Scholar**

100. Jaoko W, Karita E, Kayitenkore K et al. Safety and immunogenicity study of Multiclade HIV-1 adenoviral vector vaccine alone or as boost following a multiclade HIV-1 DNA vaccine in Africa. PLoS One. 2010; 5(9):e12873. **PubMed | Google Scholar**