JYI

REVIEW

Understanding Challenges and Advances in HIV Vaccine Development

Brittany Frisch[1] and Rachel L. Robson, Ph.D.[2, 3]
[1]*The Morningside College*
[2]*Department of Biology, The Morningside College*
[3]*Faculty advisor*

Correspondence to: bsf003@morningside.edu

ABSTRACT

Human immunodeficiency virus (HIV) has one of the highest incidence and mortality rates of any infectious disease, with more than 33 million people infected worldwide. Specifically, HIV causes the destruction of helper T cells, ultimately resulting in the suppression of the immune system and leaving its human host susceptible to countless other pathogenic agents. The development of an effective HIV vaccine has continued for more than 20 years. But the use of preventative vaccines using traditional vaccine technologies, which have proven successful for other diseases, has thus far failed with HIV. One vaccine, AIDSVAX, was the first HIV vaccine to reach a phase III efficacy trial, but has not yet been shown to eradicate HIV. Hope now lies in the development of therapeutic vaccines using novel technologies. One such vaccine is ALVAC-HIV, which, when used in conjunction with other vaccines (AIDSVAX or Lipo-6T with IL-2 injections) has shown a great deal of promise in clinical trials suppressing viral replication and improving the immune system. Other therapeutic vaccines, such as Ad5, however, have been unsuccessful. While many believe developing an effective HIV vaccine to be impossible, efforts continue into researching its structure, transmission, immune system suppression, genetic variability, and immune system evasion. As long as research continues, hope remains that someday an effective vaccine will be developed.

INTRODUCTION

First documented in 1984, human immunodeficiency virus (HIV) has become one of the most notorious and evasive viruses to date. HIV causes the destruction of CD4+ lymphocytes, cells important to robust immune system functionality (Kwong et al., 1998). HIV is a retrovirus, which typically do not kill the cells they infect. It has been observed, however, that some helper T-cells are killed when infected with HIV (Fan et al. 1998). The mechanism by which helper T-cells are destroyed is not yet fully understood, although research has shown that it is linked to a cell's apoptotic activity (Stocker et al., 2008; Banda et al., 1992; Groux et al., 1992). Most HIV research has focused on CD4+ T-cell depletion, but it has also been observed that CD8+ T-cell counts decrease in response to HIV infection (Brenchley et al., 2004; Roederer et al., 1995). This decrease results when infected helper T-cells are destroyed, subsequently affecting the response of B-cells

and cytotoxic T-cells. Some HIV variants have also been recognized to affect cytotoxic T-cells by inactivating their T-cell receptor, disabling them from interacting with the major histocompatibility complex (MHC) class 1 molecules (Meier et al., 1995). HIV then compromises the immune system because the destruction of lymphocytes results in the inadequate production of antibodies to fight off the infection and an inability to engulf and destroy the virus. Immune system suppression ultimately results in the development of acquired immunodeficiency syndrome (AIDS) (Kwong et al., 1998).

Approximately 33 million people are infected with HIV, with more than 7,000 new infections reported every day (Dolin, 2009). Originally, HIV infection was considered an adult health problem, but due to transmission from infected mother to newborn, it has become a major killer of children under the age of five (Adetunji, 2000). This is particularly evident in developing nations, where more than 90% of the world's HIV-related deaths occur and where 25-30% of children born to infected mothers die before the age of five (Adetunji 2000). In developed countries, a great deal of funding has gone into educating, researching, and treating HIV infection. But despite these efforts, it is estimated that at least 1 million people in the United States live with HIV. That figure continues to rise, with 56,300 new infections reported each year (Centers for Disease Control and Prevention, 2010). It is also estimated that 21% (232,700 people) of those infected with HIV in the US are not even aware of their status (U.S. Department of Health and Human Services, 2010; CDC, 2010). With the prevalence of HIV infection and mortality occurring at high levels it is critical that an effective and safe vaccine be developed. Despite more than 20 years of intense research, however, this has proven to be no easy task, leaving many to question whether an effective vaccine is even possible. This paper aims to call attention to the need for an effective vaccine and to discuss how the pathogenesis and nature of HIV makes developing a vaccine especially difficult.

WHAT IS A VACCINE?

A vaccine is any preparation of killed or living microorganisms introduced into the body to produce an immune response to a specific disease. Vaccines designed to prevent individuals from contracting a disease are considered preventive (Idoko & Isa, 2005). The role of a preventative vaccine is to introduce a person's immune system to the virus, stimulating a response that will allow the body to "remember" and later recognize the disease (Idoko & Isa, 2005). In addition to preventative vaccines, scientists are developing therapeutic vaccines, which can be used to treat individuals who have already been infected (Idoko & Isa, 2005). The premise behind the development of therapeutic vaccines is to suppress HIV replication, thereby allowing the immune system to induce T-cell responses to the infection (Fauci, 1993; Lu et al., 2004). The ultimate goal of therapeutic vaccines is to reduce dependence on antiretroviral drugs and to control viral suppression (Idoko & Isa, 2005).

VIRUS STRUCTURE AND TRANSMISSION

HIV is a polyhedral virus with an outer double layer lipid envelope (Hutchinson, 2001). Embedded in the outer envelope membrane are spikes composed of glycoproteins (gp) 120 and 41 subunits (Hutchinson, 2001; Burton et al., 2004). The surface of each spike is made up of the outer envelope glycoprotein, gp120, which is non-covalently bound to gp41, which anchors the complex in the envelope membrane (Kwong et al., 1998). Beneath the envelope is a protein matrix that surrounds the protein capsid (Hutchinson, 2001; Johnston & Fauci,

2008). The capsid is a hollow core made of proteins where HIVs genetic material, RNA, and the enzyme reverse transcriptase are stored (Hutchinson, 2001).

HIV causes the destruction of CD4+ lymphocytes (Kwong et al., 1998). There are two kinds of lymphocytes, T-cells and B-cells, which are used to recognize and respond to substances foreign to the body (Hutchinson, 2001). Three different types of T-cells exist: helper, cytotoxic, and suppressor T-cells (Hutchinson 2001; Wexler 2010). Helper T-cells are used to alert and activate other cells of the immune system to respond to invaders (Hutchinson, 2001; Wexler, 2010). Helper T-cells recruit and amplify the response of B-cells and cytotoxic T-cells (Hutchinson 2001; Wexler 2010). Cytotoxic T-cells destroy antigen-infected cells, while suppressor T-cells suppress the immune system after the destruction of infected cells (Hutchinson, 2001; Wexler, 2010). In response to the presence of antigens, B-cells will produce and secrete antibodies (Hutchinson 2001; Wexler 2010). Receptors found on the surface of T-cells enable them to recognize different antigens. Helper T-cells carry a CD4+ glycoprotein receptor while cytotoxic and suppressor T-cells have CD8+ glycoprotein receptors (Hutchinson, 2001; Kwong et al., 1998). The receptors expressed on these different T-cells will bind with complementary antigens initiating a defensive immune response (Hutchinson, 2001).

Studies in HIV transmission have shown that mucosal tissues are the primary sites for HIV entry and infection (Belyakov et al., 1998). Once HIV enters the body, it will target specific cells that contain the cell receptor CD4+ such as helper T-cells, and some macrophages and dendritic cells (Hutchinson, 2001; McDonald et al., 2003). HIV attaches itself to cells by binding its envelope glycoprotein, gp120, to the CD4+ receptor (Hutchinson, 2001; Kwong et al., 1998; Marsh, 1984). However, the binding of gp120 to CD4+ alone does not result in the entry of HIV into a cell (Kwong et al., 1998). The presence of a co-receptor, mainly CCR5 on macrophages or CXCR4 on T-cells, must also be present on the cell surface in order for HIV to enter the cell (Moore et al., 2004; Kwong et al., 1998; Murakami et al., 1997). Once HIV is brought into the cell, its RNA genome will be replicated and expressed as is usual for retroviruses (Fan et al., 1998).

VACCINE STRATEGIES

One determinant of vaccine efficacy is its ability to elicit humeral and cellular responses (Oh et al., 2003). An effective HIV vaccine will need to be able to induce both strong cytotoxic T lymphocyte (CTL) and neutralizing antibody responses (Lemckert et al., 2004; Oh et al., 2003). In doing so, viral replication could be restricted and virus particles destroyed (Oh et al., 2003). Initially, HIV vaccine designs followed traditional vaccine technologies, using live attenuated viruses, whole killed viruses, and protein subunits (Barouch, 2008). These technologies were expected to raise antibodies and MHC class II restricted CTL responses (Yang, 2009). While these approaches have historically been successful in eliciting protective immune responses against other viruses, these strategies have thus far been slow to yield any break-through results (Yang, 2009).

The first promising vaccine to be developed was AIDSVAX. AIDSVAX, a monomeric version of the HIV trimeric gp120 envelope glycoprotein, was aimed at inducing envelope-specific antibody immune responses (Barouch, 2008). Thus far, AIDSVAX is the only HIV vaccine to reach a phase III efficacy trial. However, results of two independent studies have been disappointing. The first study, known as VAX004, was conducted in the United States, Puerto Rico, Canada, and the Netherlands (Singh et al., 2005). Volunteers were randomized to either the

vaccine, (AIDSVAX B/B), or a placebo (Flynn et al., 2005). The vaccine contained two forms of gp120 from the HIV subgroup B (Singh et al., 2005). The vaccine, AIDSVAX B/B, was chosen because it reflected the common virus subgroups found in those areas (Singh et al., 2005). Results of the study showed that the production of neutralizing antibodies and CD4+-blocking antibody responses were apparent, but the vaccine was not effective in preventing HIV infection or modifying disease progression (Flynn et al., 2005).

Disappointing results were reinforced by conclusion of the second study, which was conducted in Thailand. The vaccine was changed to AIDSVAX B/E to reflect common virus subgroups as before (Pitisuttithum et al., 2006). Again, the results of the study showed that the vaccine did not prevent HIV infection nor did it delay disease progression (Pitisuttithum et al., 2006).

Given the slow progress in developing effective vaccines using traditional approaches, novel vaccine technologies—which include plasmid DNA vaccines and live recombinant vectors—are also being investigated (Barouch, 2008). Live recombinant vectors are vaccines made of a non-HIV virus, which is engineered to carry genes that encode recombinant proteins, like gp120, of the HIV virus. The use of such vaccines focuses on eliciting MHC class I restricted CTL responses (Yang, 2009).

The most notable novel vaccine strategy to be clinically tested is ALVAC-HIV. ALVAC-HIV is a recombinant canarypox vector vaccine genetically engineered to express HIV gp120 (Brander & Walker 1999). Despite initially disappointing results, AIDSVAX is being researched in clinical trials along with ALVAC-HIV. Studies that evaluated the tolerance of the combined vaccine regimen have showed promising results (Nitayaphan et al., 2004). A study conducted in Thailand using the AIDSVAX B/E and ALVAC-HIV vaccines, showed that the use of both vaccines was well tolerated and immunogenic (Nitayaphan et al., 2004). The study's findings allowed researchers to advance to a phase III trial, which evaluated the efficacy of using four priming injections of ALVAC-HIV and two booster injections of AIDSVAX B/E (Rerks-Ngarm et al., 2009). This study was designed to evaluate the prevention of HIV infection and the effects of early vaccination on viral load after infection (Rerks-Ngarm et al. 2009). Results of the study were promising. Within the "to treat" group, which at enrollment was composed of HIV-positive subjects, evidence to suggested that the vaccine regimen had the ability to prevent virus infection (Rerks-Ngarm et al., 2009).

ALVAC-HIV has also been investigated in combination with other vaccine. One such study examined a therapeutic regimen combining ALVAC-HIV and a Lipo-6T vaccine followed by subcutaneous IL-2 injections as a booster for antiviral therapy (Le'vy et al., 2005). The use of boosters in antiviral therapy is critical since antiviral drugs can sometimes induce unbearable adverse effects just as painful and malicious as HIV itself. Effects of the antiviral drugs can sometimes be so agonizing that patients will either temporarily stop usage, known as "drug holidays," or completely discontinue their use. The results of the ALVAC-HIV/Lipo-6T booster study demonstrated that the therapeutic regimen induced and sustained CD4+ T lymphocyte immune responses in chronically infected patients (Le'vy et al., 2005). While additional trials will need to be conducted, this particular study provided evidence for the concept that a therapeutic vaccine given prior to antiviral drug holidays may contribute to the containment of viral replication (Le'vy et al., 2005).

Another novel vaccine that has been developed is Ad5, a live recombinant adenoviral vector vaccine (Cheng et al., 2007). The efficacy of adenovirus vector vaccines has shown promise in animal models and early clinical evaluations testing their immunogenicity, but their ability to elicit CTL responses in humans has thus far failed (Singh et al., 2005; Cheng et al., 2007). Ad5 was tested in two efficacy trials, but neither trial was carried out to completion. Results from the first trial, the STEP study, revealed that the vaccine did not prevent HIV infection nor did it lower early viral load in vaccinated volunteers (Buchbinder et al., 2008). These results led to the immediate termination of the STEP study as well as the second efficacy trial, known as Phambili (Johnston & Fauci, 2008).

DNA-based vaccines are also of significant interest because of their ability to elicit strong cellular responses (MacGregor et al., 1998). The first DNA based vaccine to be tested in humans was APL 400-003, which contained genes that encode the *env* and *rev* proteins of HIV (MacGregor et al., 1998). Participants of the study did not experience any local or systemic reactions to the vaccine and it was shown that the vaccine increased gp120 antibody concentrations in individual patients (MacGregor et al., 1998). Some increases in CTL activity and proliferation were also identified. While the results of the study suggested that DNA-based vaccines could be significantly immunogenic and safe, more trials must be conducted in order to better understand the potential benefits and/or downsides to this approach.

OBSTACLES IN EFFECTIVE VACCINE DEVELOPMENT

Unique biological characteristics of HIV make effective vaccine development exceptionally difficult. HIV is quite genetically diverse, despite its small genome. Its genome is composed of nine genes, but only two, *gag* and *env,* are reliable genes for vaccine development, since they are less affected by the high mutation rate of HIV (Hutchinson, 2001).

In general, any gene has the potential to be mutated. Due to the mutation rates of reverse transcriptase, a vast number of HIV strains and subgroups have arisen (Barouch, 2008). Each strain can be classified into three groups: M (major), O (outlier), and N (non-M/non-O), from which they can be further classified into subgroups—known as clades—and recombinant forms (Barouch, 2008; Hutchinson, 2001; Spira et al., 2003). Accounting for over 90% of HIV infections are M-group strains (Spira et al., 2003). Within the M group are nine major subgroups that include A-D, F-H, J, and K (Spira et al., 2003). Clades are classified by their envelope (*env*) sequences that make up the envelope proteins, gp120 and gp41, which can differ by 10% to >25% (McMichael & Hanke, 2003; Spira et al., 2003).

Despite the relatively diverse assortment of clades, the vast majority (98%) of vaccines used in preclinical trials are derived from strains from clade B, the predominant form of virus in the US and Europe (Olin et al., 2006). The majority of HIV infections, however, occur in Africa and Asia, where an array of different clades can be found (Spira et al., 2003). Sub- Saharan Africa alone is estimated to be home to >70% of the world's HIV infected adults (Cao et al., 2003). Unfortunately, due to a lack of clinical and laboratory infrastructure in these regions, the development of new vaccines rarely occurs in countries with the highest incidences of HIV infection (Cao et al., 2003). Some have the question as to whether existing and newly developed vaccines based on clade B strains will be effective in eliciting cross-clade responses (Cao et al., 2003).

Complexity is further compounded when considering the ability of different HIV

subtypes to co-infect and coexist in different body fluids and organs within a host (Hutchinson, 2001; Jobes et al., 2006; Pandit & Sinha, 2010; Ball et al., 1994). The ability of more than one HIV strain to co-infect and exist within a single person has important implications in understanding HIV transmission, particularly regarding the development of an effective vaccine (Jobes et al., 2006). Most of these cases observe infection with two different sub-types. However, some have reported infection of variants from the same subtype (Jobes et al., 2006). Incidences of recombination—although less common—have also been observed (Jobes et al., 2006). A case of dual infection and recombination was documented from a participant in the phase III efficacy trial VAX004 (Jobes et al., 2006). This volunteer appeared to be infected with two strains of HIV subtype B, but was later found to have been infected by a recombinant form of clades A and B strains B (Jobes et al., 2006). The number of possible dual and recombinant forms that HIV can take suggests that the virus may possess a greater ability to defeat the immune system and defend against vaccines than previously understood, further complicating vaccine development.

Another obstacle to overcome is the extraordinary ability of HIV to shield conserved epitopes and evade neutralizing antibody responses (Lemckert et al., 2004). Neutralizing antibodies is important because they bind to proteins (e.g. gp120 and gp41) on the surface of HIV, inhibiting its ability to enter and infect cells (Lemckert et al., 2004; Koff, 2010). HIV is able to evade neutralizing antibodies for three general reasons. First, the gp120 and gp41 glycoprotein complexes have loop domains that are highly variable (Lemckert et al. 2004; Koup, 2002). Second, the envelope is heavily glycosylated, which protects the susceptible antibody regions of the gp120 and gp41 glycoprotein complex (Lemckert et al., 2004; Koup, 2002). Finally, gp120 is very flexible which makes it more difficult to bind to than a rigid protein (Koup, 2002).

FUTURE DIRECTIONS

Since 1987 more than 10,000 individuals worldwide have received preventative HIV vaccine immunizations in clinical trials, with roughly 7,000 in the United States (Ackers et al., 2003). Despite substantial efforts to advance research in vaccine development, a truly functional and interventional therapy has yet to be established. But while research continues, HIV prevalence and mortality continue to grow. Understanding and researching HIV structure, transmission, and its mode of suppressing the immune system by destroying helper T-cells will be pivotal in developing and utilizing an effective vaccine.

As noted above, there are many hurdles and unsolved problems to overcome before a truly effective preventative or therapeutic vaccine can be produced. Overall, there are two main challenges that need to be addressed before an effective vaccine can be developed. First, vaccines designed for more than one subgroup must be investigated. Second, an effective mechanism or mechanisms need to be developed that can elicit strong CTL responses to HIV. If any one of these challenges can be met, vaccine development would take a significant step forward.

Despite disappointing clinical trials, there is hope for an effective vaccine. While a preventative vaccine appears to be less promising, given the genetic variability and mutation rate of the virus, considerable potential remains in developing a therapeutic vaccine regimen. Research has identified monoclonal antibodies against HIVs proteins such gp120 and gp41 that have been shown to

elicit strong neutralizing antibody responses in different clade isolates, as well as vaccine regimens involving the suppression of HIV replication. While therapeutic vaccines are unable to prevent infection, the use of a truly effective therapeutic vaccine would be able to control and suppress HIV replication, thereby presenting infected individuals with tolerable symptoms and hopefully improved longevity. At present, education, public policy, and antiretroviral approaches have not prevented the rampant spread of HIV. For this reason, development of an effective vaccine may be our best hope. As to when and whether an effective HIV vaccine would be available is uncertain, but a commitment to furthering our understanding about HIV, how it is transmitted, and how affects the immune system will be required if further advances are to be achieved.

REFERENCES

1. Singh, M. et al. (2005) HIV vaccine development. Frontiers in Bioscience 10, 2064-2081.

2. Lemckert, A. et al. (2004) Challenges in the search for an HIV vaccine. European Journal of Epidemiology 19, 513-516.

3. Barouch, D. (2008) Challenges in the development of an HIV-1 vaccine. Nature 455, 613-619.

4. Idoko, J.A. and S.O. Isa (2005) HIV vaccine development. Annals of Ibadan Postgraduate Medicine 3, 19-25.

5. Hutchinson, J. (2001) The biology and evolution of HIV. Annual Review of Anthropology 30, 85-108.

6. Letvin, N. (2005) Progress toward an HIV vaccine. Annual Review of Medicine 56, 213-223.

7. Koff, W. (2010) Accelerating HIV vaccine development. Nature 464, 161-162.

8. McMichael, A. and T. Hanke (2003) HIV vaccines 1983-2003. Nature Medicine 9, 874-880.

9. Brander, C. and B. Walker (1999) T lymphocyte responses in HIV-1 infection: implications for vaccine development. Current Opinion in Immunology 11, 451-459.

10. Dolin, R. (2009) HIV vaccine trial results: an opening for further research. New England Journal of Medicine 361, 2279-2280.

11. Moore, J. et al. (2004) The CCR5 and CXCR4 coreceptors: central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Research and Human Retroviruses 20, 111-126.

12. Spira, S. et al. (2003) Impact of clade diversity on HIV-1 virulence, antiretroviral drug sensitivity and drug resistance. Journal of Antimicrobial Chemotherapy 51, 229-240.

13. Burton, D. et al. (2004) HIV vaccine design and the neutralizing antibody problem. Nature Immunology 5, 233-236.

14. Johnston, M. and A. Fauci (2008) An HIV vaccine: challenges and prospects. New England Journal of Medicine 359, 888-890.

15. Koup, R. (2002) HIV vaccine research: problems and progress. International AIDS Society USA 10, 9-13.

16. McDonald, D. et al. (2003) Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science 300, 1295-1297.

17. Kwong, P. et al. (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393, 648-659.

18. Fauci, A. (1993) Multifactorial nature of human immunodeficiency virus disease:

implications for therapy. Science 262, 1011-1018.

19. Brenchley, J. et al. (2004) CD4 T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. Journal of Experimental Medicine 200, 749-759.

20. Ackers, M.L. et al. (2003) Human immunodeficiency virus (HIV) seropositivity among uninfected HIV vaccine recipients. Journal of Infectious Diseases 187, 879-886.

21. Belyakov, I. et al. (1998) Mucosal immunization with HIV-1 peptide vaccine induces mucosal and systemic cytotoxic T lymphocytes and protective immunity in mice against intrarectal recombinant HIV-vaccinia challenge. PNAS 95, 1709-1714.

22. Rerks-Ngarm, S. et al. (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. New England Journal of Medicine 361, 2209-2220.

23. Oh, S. et al. (2003) Coadministration of HIV vaccine vectors with vaccinia viruses expressing IL-15 but not IL-12 induces long-lasting cellular immunity. PNAS 100, 3392-3397.

24. Jobes, D. et al. (2006) Longitudinal population analysis of dual infection with recombination in two strains of HIV type 1 subtype B in an individual from a phase 3 HIV vaccine efficacy trial. AIDS Research and Human Retroviruses 22, 968-978.

25. Lu, W. et al. (2004) Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. Nature Medicine 10, 1359-1365.

26. U.S. Department of Health and Human Services (2010) MMWR morbidity and mortality weekly report. Weekly 59, 737-741.

27. Olin, J. et al. (2006) Community preparedness for HIV vaccine trials in the Democratic Republic of Congo. Culture, Health & Sexuality 8, 529-544.

28. Meier, U.C. et al. (1995) Cytotoxic T lymphocyte lysis inhibited by viable HIV mutants. Science 270, 1360-1362.

29. MacGregor, R.R. et al. (1998) First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. Journal of infectious diseases 178, 92-100.

30. Cao, H. et al. (2003) Immunogenicity of a recombinant human immunodeficiency virus (HIV)-canarypox vaccine in HIV-seronegative Ugandan Volunteers: results of the HIV network for prevention trials 007 vaccine study. Journal of Infectious Diseases 187, 887-895.

31. Pitisuttithum, P. et al. (2006) Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. Journal of Infectious Diseases 194, 1661-1671.

32. Nitayaphan, S. et al. (2004) Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. Journal of Infectious Diseases 190, 702-706.

33. Roederer, M. et al. (1995) CD8 naïve T cell counts decrease progressively in HIV-infected adults. American Society for Clinical Investigation 95, 2061-2066.

34. Murakami, T. et al. (1997) A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. Journal of Experimental Medicine 186, 1389-1393.

35. Buchbinder, S. et al. (2008) Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the step study): a double-blind, randomized, placebo-controlled,

test-of-concept trial. Lancet 29, 1881-1893.

36. Flynn, N.M. et al. (2005) The rgp 120 HIV vaccine study group: placebo controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. Journal of Infectious Diseases 191, 654-665.

37. Le'vy, Y. et al. (2005) Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. AIDS 19, 279-286.

38. Stocker, H. et al. (2008) Destruction of primary CD4 T cells by cell-cell interaction in human immunodeficiency virus type 1 infection in vitro. Journal of General Virology 81, 1907-1911.

39. Banda, N. et al. (1992) Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. Journal of Experimental Medicine 176, 1099-1106.

40. Groux, H. et al. (1992) Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. Journal of Experimental Medicine 175, 331-340.

41. Adetunji, J. (2000) Trends in under-5 mortality rates and HIV/AIDS epidemic. Bulletin of the World Health Organization 78, 1200-1206.

42. Yang, O. (2009) Candidate vaccine sequences to represent intra- and inter-clade HIV-1 variation. PLoS ONE 4.

43. Cheng, C. et al. (2007) Mechanism of Ad5 vaccine immunity and toxicity: fiber shaft targeting of dendritic cells. PLoS Pathogens 3.

44. Fan, H. et al. (1998) Aids: science and society. Jones and Barlett Publishers 62-67.

45. Wexler, B. (2010) AIDS/HIV. Information Plus 5-8.

46. Pandit, A. and S. Sinha (2010) Using genomic signatures for HIV-1 subtyping. BioMed Central 1, 1-8.

47. Ball, J. et al. (1994) Genomic variation of human immunodeficiency virus type 1 (HIV-1): molecular analysis of HIV-1 in sequential blood samples and various organs obtained at autopsy. Journal of General Virology 75, 867-879.

48. Marsh, M. (1984) The entry of enveloped viruses into cells by endocytosis. Biochem. J. 218, 1-8.

49. Center for Disease Control (2010) HIV in the United States.