

Perspectives of AIDS Vaccine Development: T Cell-based Vaccine

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ABSTRACT

Estimated number of adults and children newly infected with HIV-1 during 2001 alone is 5 million in total. An effective vaccine, in addition to education & public health approaches, has been believed to be the best option to stop the HIV-1 transmission, especially for developing countries. Among AIDS vaccine candidates, DNA vaccine is relatively safe and, in a certain extent, mimics some attributes of live attenuated vaccine, with regard to in vivo gene expression & the type of immunity induced. We recently demonstrated that DNA vaccines expressing SIVmac239 structural and regulatory genes, augmented with coadministration of IL-12 mutant induced the strongest T cell responses, resulting in low to undetectable setpoint viral loads, stable CD4⁺ T cell counts, and no evidence of clinical diseases or mortality by day 420 after challenge. This finding is the second demonstration, following the protective result of live attenuated SIV vaccine in SIVmac-rhesus monkey model, which was known to have safety problem. So, our DNA vaccines could give a significant impact on HIV-1 epidemic by slowing or stopping the spread of HIV-1, leading to eventual eradication of HIV-1 and AIDS in the population. (**Immune Network 2002;2(1):1-5**)

Key Words: AIDS vaccine, HIV-1, DNA, T cell

More than 40 million adults and children are estimated to be living with HIV-1 & AIDS in the end of the year 2001 (Fig. 1). About 80% of them are living in the poorest country of the world such as, Sub-Saharan Africa & Southeast Asia. In some parts of Sub-Saharan Africa, 50% of the young generation are positive for HIV-1. Worldwide, the number of HIV-infected individuals distributes more equally between males and females. In addition, what we are worrying about is that the HIV and AIDS epidemic is rapidly spreading other parts of the world, such as Eastern Europe and central Asia. So far, education & public health approaches have been quite effective in preventing HIV-1 transmission. However, estimated number of adults and children newly infected with HIV-1 during 2001 alone is 5 million in total. In other words, about 14,000 persons are infected each day, indicating that education and public health approaches are not sufficient for preventing HIV-1 transmission. So, other approaches are needed for stopping the spread of HIV-1. Among them, an ef-

fective vaccine has been believed to be the best option to stop the HIV-1 transmission, especially for developing countries.

Generally speaking, understanding immune correlates of protection are needed for developing an effective vaccine. Although we have not fully understood which immune responses are required for protection, there are several evidences for an effective HIV immunity, which help us to design for HIV vaccine development. For example, 1) Protection was

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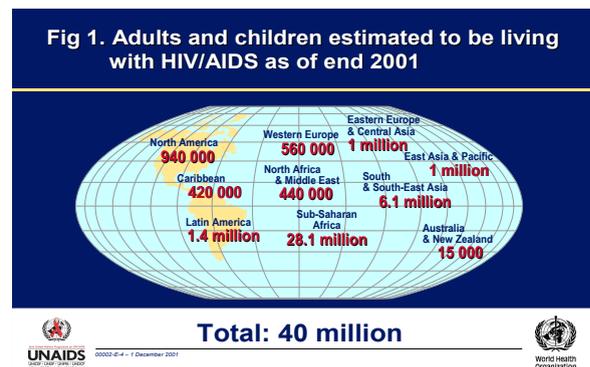


Figure 1. Adults and children estimated to be living with HIV/AIDS as of end 2001.

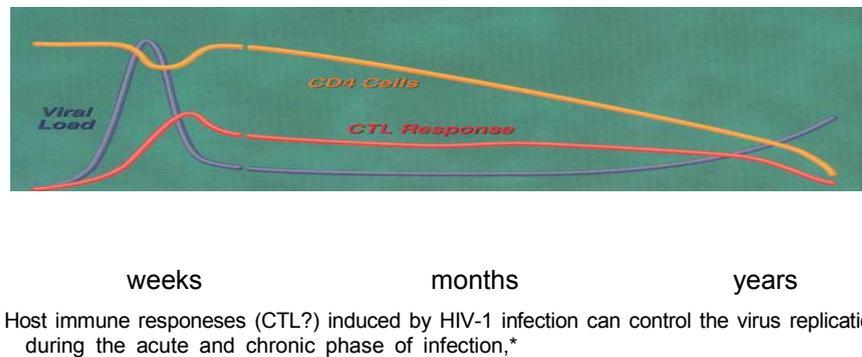


Figure 2. Consequences of HIV infection.

obtained with vaccine candidates in primate model (1,2). 2) In western Africa, prior infection with the less virulent HIV-2 may protect against HIV-1 infection, indicating cross-strain reactivity (3). 3) In some HIV-infected individuals, high levels of HIV-specific Th1& CTL responses were associated with a decreased viremia (4,5). 4) In Gambia and Kenya, some of the sex workers, who are exposed but not infected with HIV, have strong HIV-specific CTL responses (6,7). 5) Finally, CTL responses, during acute phase of HIV infection, appeared to control viral replication (8). The virological and immunological profiles after HIV infection indicate that the decrease of viral loads, after peak viremia, which is closely associated with appearance of HIV-specific CTL responses (Fig. 2). This level of viral load is maintained thereafter for 8~10 years throughout the chronic phase of infection. In the long run, the virus inevitably breaks through host immune defenses, resulting in an increase of viral load and a decrease of CD4+ T cell number and CTL responses. These observations indicate that host immune responses, presumably CTL responses induced by HIV-1 infection is likely to be able to control the viral replication during both acute and chronic phase of infection, although they are not strong enough to eradicate the virus completely from the host. Therefore, if we developed an HIV-1 vaccine which is able to specifically and strongly induce these kinds of immune responses such as HIV-1 specific CTL responses, we can predict that an effective HIV vaccine will be available to us.

Unfortunately, there are a number of obstacles to effective HIV vaccine development as follows.

1) HIV can be transmitted by cell-free and cell associated virus as well. 2) As a retrovirus, it is able to integrate into chromosome. So, HIV is capable of hiding quiescent memory T cells and remain latent for tens of years (9). 3) It is also sequestered into

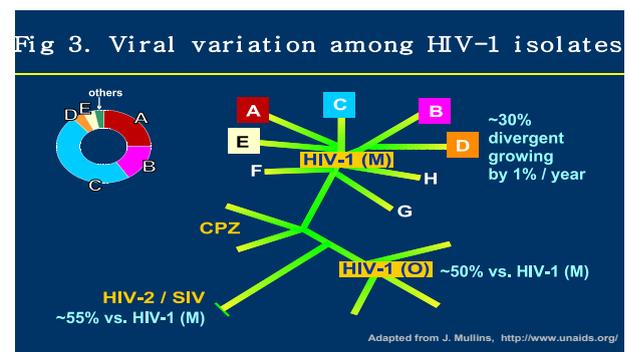


Figure 3. Viral variation among HIV-1 isolates.

immune-privileged sites: central nervous system (CNS) and lymphoid organs (10). 4) In particular, HIV infects and destroys CD4 T cells which orchestrate both Ab and T cell responses and are critical for protective immunity. 5) In addition, immune-correlates of protection are still obscure. Since no one has yet been cured from AIDS, it is not easy to identify which immune responses are necessary for protection in nature. 6) There are limitations of good animal models. 7) Another major hindrance is the high rate of mutation. The mutation rate in HIV is 65 times higher than that observed in Influenza. 8) Among HIV-1 isolates, there are tremendous genetic&viral variation even in a single host, so-called quasispecies, and geographically. Among HIV-1 isolates, there are 2 major groups, (M & O group), whose env sequences differ by about 50% (Fig. 3). SIV, a monkey AIDS virus, exhibited about 55% sequence homology with M group. So, SIV appeared to be a little bit more closely related to M group of HIV-1 than O group. Within the M group alone, there are about 10 clades, so called genetic subtypes, clade A, B, C etc. The B clade is prevalent in the America, Europe, and Korea,

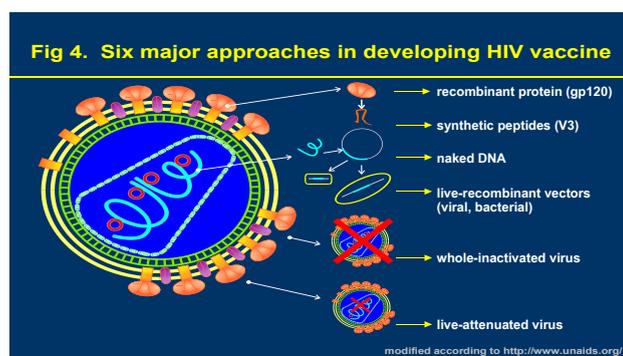


Figure 4. Six major approaches in developing HIV vaccine.

although the C clade is the most frequent virus worldwide. Ab and T cell responses against one HIV subtype may not recognize the other viruses within different subtypes. So, it will be better to consider the geographic subtypes in developing HIV vaccine.

There are six major approaches in developing HIV vaccine (Fig. 4). For example, 1) recombinant protein vaccine using gp120 protein, 2) synthetic peptide vaccine using V3 loop, 3) naked DNA vaccine, 4) live recombinant vectored vaccine, 5) whole inactivated virus vaccine, 6) live attenuated virus vaccine. Among them, more than 25 different HIV vaccine candidates using protein vaccine, peptide vaccine, DNA vaccine, and vectored vaccine, have currently entered human clinical trials.

Generally speaking, a suitable animal model is needed for a vaccine development. HIV-1 is only able to infect chimpanzee and human, and causes AIDS-like diseases in human, but not in chimpanzee. In contrast, monkey AIDS virus, such as SIV and SHIV, are able to chronically infect macaques and cause virus-induced diseases. So, these two monkey AIDS viruses have been widely used for evaluating the efficacy of AIDS vaccine candidates. SHIV is a hybrid virus composed of a HIV envelope and SIV nucleocapsid plus replication machinery. Recently, there are great success in protection against SHIV as a challenging virus with the following vaccine candidates. For example, IL2-augmented DNA vaccine induced potent secondary CTL responses, resulting in low to undetectable setpoint viral loads & no-evidence of clinical diseases or mortality by day 140 after challenge (11). Multiprotein DNA priming and MVA boosting vaccine can raise a memory immune responses capable of controlling a highly virulent mucosal immunodeficiency virus challenge administered 7 months after the booster (12), replication-incompetent adenovirus vaccine elicits effective anti-immunodeficiency-virus immunity which exhibited the significant attenuation of the virus infection (13).

However, many leading scientists warned that this newfound optimism may be based on misleading science, because SHIV produces a different immune response from SIV. In addition, SHIV produces a different disease that is like flu: high fever, acute fatigue, and nausea. Dr. Ron Desrosiers at Harvard Medical School suggested that every potential vaccine that is working against SHIV should be retested against SIVmac before entering clinical trials (14). In 1990, gp120 vaccine, developed by Genentech, was reported to be protective against HIV-1 challenge in chimpanzee (15), but not protective in humans, indicating that using a suitable animal model is critical for HIV vaccine development. In comparison with SHIV, SIVmac is more similar to HIV-1 in terms of 1) similar peak in plasma antigenemia, cellular VLs & level of plasma RNA, 2) Similar immune responses following infection, 3) Clinical diseases associated drop in CD4⁺ T cells. In this regard, it has been recommended that HIV-1 vaccine candidates be evaluated for their efficacy in SIVmac rhesus monkey model. However, it has been known that, in this animal model, it is extremely difficult to achieve protection against i.v. challenge with SIVmac like HIV-1-Human. So far, live attenuated SIV vaccine with a deletion of regulatory gene such as *nef* or *vif*, etc is the only one that clearly shows protection against i.v. challenge with SIVmac (16). However, these kinds of live attenuated, multiply deleted SIV were reported to cause AIDS in infant and adult macaques (17) suggesting that live attenuated vaccine appeared to have potential safety problem. In this point, a potential AIDS vaccine should be safe as well as effective. Among AIDS vaccine candidates, DNA vaccine is relatively safe and, in a certain extent, mimics some attributes of live attenuated vaccine, with regard to *in vivo* gene expression & the type of immunity induced. Based on these facts, it has been suggested that DNA vaccine may be one of the best AIDS vaccine candidates

DNA vaccine is just a bacterial plasmid DNA which is able to produce antigen under eukaryotic promoter. Another important feature of plasmid DNA is the presence of unmethylated CpG motifs that have Th1-inducing activity by stimulating macrophages and DCs to produce a variety of cytokines including IL12, functioning as mitogenic effect (18). When the DNA vaccine is i.m. or i.d. delivered into host, the injected DNA is transfected into target cells such as myocytes, keratinocytes, and BM-derived APCs, and then the DNA-encoded antigen is produced by host transcriptional and translational machinery (Fig. 5). In this regard, the immunized host functions as a vaccine factory. The DNA-encoded antigen is processed and then complexed with MHC class I and II

Fig 5. Concept of DNA immunization

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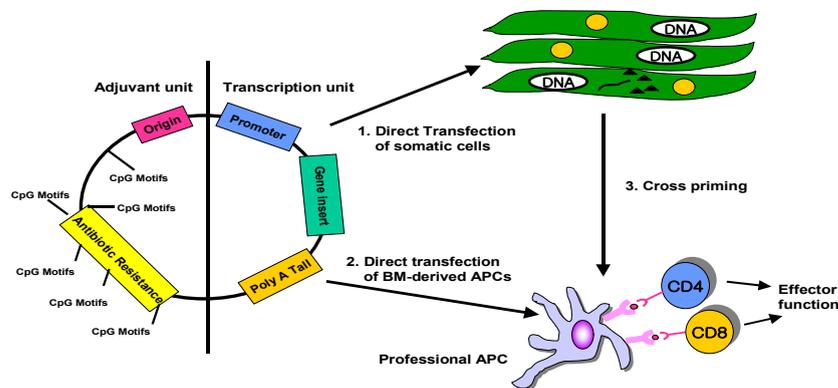


Figure 5. Concept of DNA immunization.

molecules, leading to the activation of CD4 and CD8 T cells. Also, DNA-encoded antigen is able to directly bind to specific B cells to secrete antibody. So, DNA vaccine is able to induce both humoral and cellular immunity. In particular, qualitatively, DNA vaccine is quite effective in inducing TH1 & CTL responses, compared with other immune responses (19). DNA vaccine is relatively safe, because of its noninfectious nature&high purity. Also, it is faster to develop than other vaccines, because expression plasmid itself is used as a vaccine. In a sense, it is inexpensive to manufacture, because identical production&formulation protocols for different vaccines could reduce the operating costs. Plasmid DNA itself is stable molecule at room temperature. So, there will be no need for a cold chain in distribution which also reduce the cost and potential side effects. Finally, it is easy for DNA vaccines to formulate into cocktail or multivalent vaccines, which will be very effective for controlling highly variable virus, like HIV.

In general, ideal vaccine is to induce sterilizing immunity which is achieved by neutralizing antibody. Since neutralizing Ab was known to be not working well against hypervariable virus, like HIV-1, more realistic HIV vaccine is to achieve secondary endpoints, that is, protection against disease rather than infection, in other words, either initial infection, but controlled or establishment of chronic infection with low viral load. In fact, most currently licensed&commercialized vaccines, for examples, polio and influenza vaccines, do not induce sterilizing immunity, but prevent diseases. We have recently developed AIDS DNA vaccines containing SIV structural genes and regulatory genes plus IL-12 mutant. IL-12 mutant was demonstrated to induce long-term TH1 and CTL responses in DNA vaccination (20). Our DNA vac-

cine do not develop sterilizing immunity, but are capable of decreasing viral loads & preventing SIV-induced diseases, which meets the secondary endpoints of potential HIV vaccine. In particular, our DNA vaccines were demonstrated to be protective against i.v. infection with SIVmac in rhesus monkey which was known to be extremely difficult to achieve protection. This finding is the second demonstration, following the protective result of live attenuated SIV vaccine which was known to have safety problem. So, our DNA vaccine, which is protective under experimental condition that the infecting dose used (10 MID_{50}) may be 500 ~ 50,000 times higher than those of a human sexual contact and i.v. drug injection, may offer a chance of complete protection from new HIV-1 infection. Even without complete eradication of HIV-1, our DNA vaccine may benefit the infected individual with low viral loads by slowing or preventing AIDS-like diseases. Furthermore, since it was demonstrated that those who have low viral loads are less infectious, our DNA vaccines could give a significant impact on HIV-1 epidemic by slowing or stopping the spread of HIV-1, leading to eventual eradication of HIV-1 and AIDS in the population.

References

1. Boyer JD, Ugen KE, Wang B, Agadjanyan M, Gilbert L, Bagarazzi ML, Chattergoon M, Frost P, Javadian A, Williams WV, Refaeli Y, Ciccarelli RB, McCallus D, Coney L, Weiner DB: Protection of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination. *Nat Med* 3;526-532, 1997
2. Rose NF, Marx PA, Luckay A, Nixon DF, Moretto WJ, Donahoe SM, Montefiori D, Roberts A, Buonocore L, Rose JK: An effective AIDS vac-

- cine based on live attenuated vesicular stomatitis virus recombinants. *Cell* 106;539-549, 2001
3. Travers K, Mboup S, Marlink R, Gueye-Nidaye A, Siby T, Thior I, Traore I, Dieng-Sarr A, Sankale JL, Mullins C, Ndoye I, Hsieh CC, Essex M, Kanki P: Natural protection against HIV-1 infection provided by HIV-2. *Science* 268; 1612-1615, 1995
 4. Clerici M, Shearer GM: A TH1-TH2 switch is a critical step in the etiology of HIV infection. *Immunol Today* 14;107-111, 1993
 5. Ogg, GS, Jin, X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, Segal JP, Cao Y, Rowland-Jones SL, Cerundolo V, Hurley A, Markowitz M, Ho DD, Nixon DF, McMichael AJ: Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 279;2103-2106, 1998
 6. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, Whitby D, Sabally S, Galimore A, Corrah T, et al: HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat Med* 1;59-64, 1995
 7. Kaul R, Plummer FA, Kimani J, Dong T, Kiama P, Rostron T, Njagi E, MacDonald KS, Bwayo JJ, McMichael AJ, Rowland-Jones SL: HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. *J Immunol* 164;1602-1611, 2000
 8. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB: Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 68;6103-6110, 1994
 9. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278; 1295-1300, 1997
 10. Chiodi F: Tissue lesions in AIDS patients involve both the lymphoid and the nervous system. *AIDS* 9 Suppl A;S41-8, 1995
 11. Barouch DH, Santra S, Schmitz JE, Kuroda MJ, Fu TM, Wagner W, Bilska M, Craiu A, Zheng XX, Krivulka GR, Beaudry K, Lifton MA, Nickerson CE, Trigona WL, Punt K, Freed DC, Guan L, Dubey S, Casimiro D, Simon A, Davies ME, Chastain M, Strom TB, Gelman RS, Montefiori DC, Lewis MG, Emini EA, Shiver JW, Letvin NL: Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science* 290; 486-492, 2000
 12. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, Miller J, McClure HM, McNicholl JM, Moss B, Robinson HL: Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 292;69-74, 2001.
 13. Shiver JW, Fu TM, Chen L, Casimiro DR, Davies ME, Evans RK, Zhang ZQ, Simon AJ, Trigona WL, Dubey SA, Huang L, Harris VA, Long RS, Liang X, Handt L, Schleif WA, Zhu L, Freed DC, Persaud NV, Guan L, Punt KS, Tang A, Chen M, Wilson KA, Collins KB, Heidecker GJ, Fernandez VR, Perry HC, Joyce JG, Grimm KM, Cook JC, Keller PM, Kresock DS, Mach H, Troutman RD, Isopi LA, Williams DM, Xu Z, Bohannon KE, Volkin DB, Montefiori DC, Miura A, Krivulka GR, Lifton MA, Kuroda MJ, Schmitz JE, Letvin NL, Caulfield MJ, Bett AJ, Youil R, Kaslow DC, Emini EA: Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415;331-335, 2002
 14. *Newsday*, *Newsday*, Inc. Sep. 5, 2001
 15. Berman PW, Gregory TJ, Riddle L, Nakamura GR, Champe MA, Porter JP, Wurm FM, Hershberg RD, Cobb EK, Eichberg JW: Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. *Nature* 345;622-625, 1990
 16. Daniel MD, Kirchhoff F, Czajak SC, Sehgal PK, Desrosiers RC: Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science* 258;1938-1941, 1992
 17. Baba TW, Liska V, Khimani AH, Ray NB, Dailey PJ, Penninck D, Bronson R, Greene MF, McClure HM, Martin LN, Ruprecht RM: Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques *Nat Med* 5;194-203, 1999
 18. Jakob T, Walker PS, Krieg AM, Udey MC, Vogel JC: Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. *J Immunol* 161;3042-3049, 1998
 19. Gurunathan S, Klinman DM, Seder RA: DNA vaccines: immunology, application, and optimization. *Annu Rev Immunol* 18;927-974, 2000
 20. Ha SJ, Chang J, Song MK, Suh YS, Jin HT, Lee CH, Nam GH, Choi G, Choi KY, Kim WB, Sung YC: Engineering N-glycosylation mutations in IL-12 enhances sustained cytotoxic T lymphocyte responses for DNA immunization. *Nat Biotechnol* in press