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Evolutionarily conserved T-cell epitopes on FIV for designing an HIV/AIDS vaccine

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Abstract

This review will discuss the current state of the human HIV-1 vaccine trials including the safety consideration of vaccine composition and difficulties in determining and defining protective immunity and epitopes to HIV-1. Vaccines in animal models of lentivirus infection are compared. In particular, the findings from the prototype FIV vaccine and the HIV-1 protein immunizations studies in cats are discussed, as well as the resulting research regarding a potential HIV-1 vaccine design based on evolutionarily conserved T-cell epitopes.

Keywords

HIV vaccine; feline immunodeficiency virus; T-cell epitopes

1. Introduction

The commercial dual-subtype FIV vaccine (Fel-O-Vax[®] FIV), although moderately to highly effective in protection against heterologous strains of FIV, has been underutilized clinically due to the lack of a diagnostic test that distinguishes between vaccination and natural infection. In addition, the difference in efficacy between the prototype and the commercial FIV vaccines has raised questions (Pu et al., 2010; Yamamoto et al., 2010a). Information regarding the types of immunity generated by Fel-O-Vax[®] FIV vaccine is limited although results of its clinical efficacy have been more widely studied (Yamamoto et al., 2010a). Because of the impressive efficacy of the prototype FIV vaccine, studies predominantly regarding the prototype FIV vaccine have been used for the basis of a new vaccine design. This information is useful in formulating both a second-generation FIV vaccine for cats and potentially a HIV-1 vaccine for humans. One vaccine approach is to identify viral protein regions high in evolutionarily conserved vaccine epitopes for T-cell and B-cell immunity, which can be combined into a complex of vaccine immunogens (Pu et

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al., 2010; Yamamoto et al., 2010a, 2010b). Taking one aspect of such a design, the current review describes the studies leading to a vaccine design based on evolutionarily conserved T-cell epitopes. In order to gain a full appreciation of such a vaccine design, this review also highlights the findings from recent human HIV-1 vaccine trials and trials in other lentivirus/AIDS vaccine models.

2. The state of human HIV-1 vaccine trials

Four major vaccine trials of either phase-IIb or phase-III classification afforded varying results in humans (Table 1) (Flynn et al., 2005; Pitisuttithum et al., 2006; McElrath et al., 2008; Rerks-Ngarm et al., 2009). The first phase-III trial called VAX004 initiated in 1998 utilized recombinant HIV-1 surface envelope glycoproteins (gp120) from two subtype-B HIV-1 viruses (Flynn et al., 2005). This large-scale trial was immediately followed by another phase-III trial (VAX003) with vaccine immunogens (AIDSVAX B/E) consisting of subtype-B and subtype-E gp120 glycoproteins (Pitisuttithum et al., 2003, 2006). The goal of this vaccine design was to induce robust virus neutralizing antibodies (VNAs) against clinical isolates, especially from subtype B but also from subtype E in the latter trial. These vaccines produced significant levels of VNAs to laboratory HIV-1 strains in the vaccine subtype but produced limited levels of neutralizing activities to clinical isolates even from the same subtype. The result was absence of protective efficacy. In addition to humoral immunity, these vaccines induced low-levels of cellular immunity against the HIV-1 Env antigens (Karnasuta et al., 2005; Jones et al., 2009).

After the failed gp120 trials, the vaccine studies focused more on a vaccine that induces robust cellular immunity against HIV-1, such as CTL activity against HIV-infected cells. The next notable HIV-1 vaccine trial was a phase-IIb STEP trial consisting of adenovirus serotype-5 vectored HIV-1 *gag/pol/nef* (Ad5-HIV-1 *gag/pol/nef*) vaccine (McElrath et al., 2008). This vaccine was aimed at inducing potent cellular immunity against HIV-1 as it contained no *env* sequence for generating VNAs (Buchbinder et al., 2008). This approach showed moderate efficacy in preclinical trials with Ad5-SIV *gag/pol/nef*-vaccinated rhesus macaques against challenges with simian-human immunodeficiency virus (SHIV, SIV with HIV *env* sequence) (Watkins et al., 2008). The preliminary results in humans showed more infection in the vaccinated group than in the placebo-immunized group, and the trial was terminated before completion of the full phase-IIb trial (Barouch and Korber, 2010). The enhancement of infection was attributed to a variety of factors including non-specific cell activation, pre-existing antibodies to the vector, and decreased risk factor (more circumcised subjects) in the placebo group (Buchbinder et al., 2008). A lesson learned from this failed trial was the significance of using appropriate challenge systems in preclinical trials. Since this trial was initiated based on the positive results from preclinical trials using the SHIV/macaque system, many primate AIDS researchers complained that the SHIV challenge system was too weak. This is supported by the absence of significant efficacy against pathogenic SIV challenges (Watkins et al., 2008; Barouch and Korber, 2010). As to the use of Ad5 vector, a major controversy still exists over the significance of the pre-existing immunity to the vector in enhancing infection in the vaccinated subjects (Hutnick et al., 2009; O'Brien et al., 2009; Tatsis et al., 2009). A number of phase-I and -II trials using adenovirus vector system were either withdrawn or terminated, while some, especially those

evaluating a combination strategy with DNA vaccine with adenovirus vector, are still ongoing (IAVI Reports Trials Database; <http://www.iavireport.org/trials-db/Pages/default.aspx>).

A recent phase-III trial called RV144 evaluated the efficacy of the prime-boost system, using a canarypox virus vectored HIV-1 *gag/pol/env* priming (ALVAC-HIV [vCP1521]) along with boosts of recombinant gp120 subunit vaccine (AIDSVAX B/E). This trial showed a modest overall vaccine efficacy of 31.2% but little efficacy (3.7%) for the high risk group (Rerks-Ngarm et al., 2009). The goal of such vaccine design is to generate both humoral and cellular immunity against HIV-1 for prophylaxis (Pitisuttithum et al., 2003; Nitayaphan et al., 2004; Karnasuta et al., 2005). According to the current list of ongoing trials, more investigators are utilizing vaccine combinations that simulate an attenuated HIV-1 vaccine without incorporating an infectious HIV-1 genome such as the combination of a DNA vaccine and viral vector vaccine (IAVI Reports Trials Database). Importantly, there must be a thorough evaluation during preclinical trials to minimize large-scale failures, devastating the hopes for developing an effective HIV-1 vaccine.

3. Deciphering the vaccine immunity from the immunity of HIV-1 infected subjects

Many HIV-1 researchers are studying the immunity required for controlling HIV-1 infection by comparing the anti-viral immunity generated by HIV-1 infected subjects with rapid progression to those with long-term non-progression to AIDS. The two most popular subject groups of such studies are HIV-1 positive long-term survivors (LTS) and elite controllers (Bello et al., 2009; Blankson et al., 2010). LTS are those subjects who, without antiretroviral drug therapy (ART), control viral load to low levels and remain clinically asymptomatic but may have some CD4⁺ T-cell loss. Elite controllers belong to a small subset of LTS with undetectable levels of HIV-1 and normal CD4⁺ and CD8⁺ T-cell counts. The major goal for such studies is to identify the immunity generated for the control of HIV-1 infection in hope that such immunity can be induced by vaccination to confer prophylaxis.

Many of the results from the above studies have been compiled in the Los Alamos National Laboratory (LANL) Epitope Table (<http://www.hiv.lanl.gov/content/immunology/tables/tables.html>). This database catalogs epitopes of viral proteins detected by CD8⁺ CTLs, CD4⁺ Th cells, and B cells (antibodies). The LANL database also correlates the CD8⁺ and CD4⁺ T-cell epitopes with the HLA class-I and II alleles, respectively. A cursory evaluation of the database indicates that the CD8⁺ CTL epitopes are concentrated on p24, gp120, Nef, RT, and p17 (Table 2). Although still limited in the number of studies, CD4⁺ Th epitopes are concentrated on p24 and gp120, but found sporadically on other HIV-1 proteins. Perhaps, the most remarkable observation from this analysis is the vast number of CD8⁺ CTL epitopes as compared to CD4⁺ Th epitopes and B-cell epitopes (B-cell data not shown). In spite of abundant CTL epitopes, an effective HIV-1 vaccine still remains elusive.

4. Animal models for identifying an effective HIV-1 vaccine design

Both SIV-macaque and equine infectious anemia virus (EIAV)-equine models utilize attenuated live vaccine to confer more consistent protection in the respective host than other vaccine designs so far tested (Koff et al., 2006; Shen et al., 2006). Although the attenuated live vaccine is not being currently considered as an approach for HIV-1 vaccine due to major safety concerns (Ruprecht, 1999), studies using this design can help identify the immunity needed for vaccine protection. A major concern stems from the growing number of recombinant HIV-1 viruses circulating in the world when two viruses infect and recombine in one host (Leitner et al., 2005). The increasing prevalence of recombinants may be an indication of increasing co-infections resulting from insufficient levels of immunity generated by the first infection to block the superinfection of the second virus.

Another version of a vaccine that contains a majority of the viral proteins is the inactivated whole-virus (IWV) vaccines. This approach, much like the attenuated vaccine, has been used extensively in human and veterinary vaccines (Uhl et al., 2002; USDA, 2010). Early studies in 1990s using the IWV approach in SIV-macaque models afforded mixed results against varying challenge doses and sources of challenge strains (Gardner, 1994; Siegel et al., 1995). Such an approach is not currently advocated for HIV-1 vaccine due to the potential for insufficient inactivation of the vaccine virus causing accidental infections. However, for cats, a commercial dual-subtype IWV vaccine for FIV (Fel-O-Vax® FIV) has been released in the U.S. and selected countries without any cases of infection by the vaccine virus (Yamamoto et al., 2007, Yamamoto et al., 2010a).

5. Lessons learned from FIV vaccine immunity for developing an effective HIV-1 vaccine

The commercial dual-subtype FIV vaccine consisting of chemically inactivated whole-viruses (IWVs) from subtypes A and D are derived from strains which cause infection in laboratory cats without clinical symptoms for a prolonged period of time (Yamamoto et al., 2007). The subtype-A strain FIV_{Pet} of the vaccine virus has been extensively studied (Elyar et al., 1997; Uhl et al., 2002; Pistello et al., 2010). Infection with this virus or vaccination with IWV of this virus induced high levels of virus neutralizing antibodies (VNAs) to autologous or homologous virus and to strains with envelope (Env) sequences closely related to the FIV_{Pet}, generally from the same subtype A (Pu et al., 2004, 2010). This observation was expected knowing that Env sequences contain the epitopes for VNAs (Yamamoto et al., 2010a). However, infection or vaccination with subtype-D strain FIV_{Shi} (or IWV_{Shi}) induced minimal levels of VNAs to homologous virus, but when IWV_{Shi} was used together with IWV_{Pet}, the prototype dual-subtype vaccine conferred protection against heterologous viral strains from the same subtype as well as those from differing subtypes and the recombinant subtypes (Pu et al., 2004, 2010). Moreover, adoptive-transfer of T cells from dual-subtype IWV-vaccinated cats conferred protection against homologous virus in MHC-matched cats (Pu et al., 2006; Uhl et al., 2008). This observation was confirmed by another pilot adoptive-transfer study showing protection even against heterologous subtype-B virus (Pu et al., 2006). Although more studies are needed, these results suggest that an

AIDS lentivirus vaccine must induce a broad spectrum of VNAs and T-cell immunity to be effective against circulating global subtypes and recombinants.

5.1. Mapping T-cell epitopes on the FIV proteins

Only a few studies have performed epitope mapping and are limited to a subset of FIV proteins, and mapping for the whole virus is currently unavailable (Dean et al., 2004; Uhl et al., 2008). Consequently, the T-cell epitope mapping of HIV-1 posted in LANL database (<http://hiv-web.lanl.gov/content/immunology/maps/maps.html>) was used as a template to identify the viral proteins with a large number of CTL epitopes. Based on LANL database, p24, Nef, RT, and gp120 had the most CTL epitopes (Table 2). Since FIV has no *nef* gene and FIV gp100 does not have high homology to HIV-1 gp120 (Table 2), the PBMC from dual-subtype IWV-vaccinated cats were evaluated for responses to overlapping peptides (15mer peptides with an overlap of 8-11 aa's) derived from FIV RT and p24 proteins by IFN γ ELISpot analysis followed by CD4 $^{+}$ and CD8 $^{+}$ T-cell carboxyfluorescein succinimidyl ester (CFSE) proliferation. This approach identified many peptide pools from FIV p24 that induced IFN γ and slightly fewer from FIV RT (Pu and Yamamoto, pers. com.). In addition, many peptide pools of both p24 and RT induced proliferation of CD4 $^{+}$ T cells. Fewer peptide pools induced proliferation of CD8 $^{+}$ T cells (Pu and Yamamoto, pers. com.). To determine if such proliferation responses correlated with IL-2 production, overlapping peptides were used with IL-2 ELISpot analysis. The majority of peptide pools that induced IL-2 responses also induced proliferation of CD4 $^{+}$ T cells (Fig. 1).

Since CD4 $^{+}$ T-cell responses producing IFN- γ and IL-2 to HIV-1 Gag (polyprotein of MA-CA-NC, Table 2) were associated with non-progression to AIDS (Boaz et al., 2002), IFN γ and IL-2 responses of PBMC from the vaccinated cats were compared. In p24 and in RT peptide analyses, few of the peptide pools induced both IFN γ and IL-2 responses (Fig. 1, RT data not shown). An interesting observation was that the correlation of IFN γ and IL-2 responses to RT peptide pools was more consistent than those for p24 peptide pools. Intracellular cytokine/cytotoxin staining (ICS) analyses for IFN γ , perforins, granzymes, TNF- α , and IL-2 are currently being performed with RT and p24 peptide pools to identify the CD4 $^{+}$ and CD8 $^{+}$ T-cell identity of the producers of these cytokines and to determine if cytolytic mediators are also produced. CD4 $^{+}$ T cells expressing cytolytic mediator(s) to FIV peptide pools will indicate FIV-specific CD4 $^{+}$ CTLs, while CD8 $^{+}$ T cells expressing cytolytic mediators and/or IFN γ will indicate FIV-specific CD8 $^{+}$ CTLs.

5.2. Immunization with recombinant HIV and FIV p24 and RT proteins against low-dose FIV challenges

Although the vaccine approach based on recombinant FIV protein(s) lacked any significant protection, this approach was used with two modifications to enhance the readout (e.g., efficacy) of such studies. The challenge dose was lowered to only 5-8 times the natural transmission dose (i.e., 5-10 mean cat infectious dose, CID $_{50}$), and the adjuvant used was supplemented with IL-12. IL-12 has been reported to enhance cellular immunity while retaining adequate humoral immunity (Kim et al., 1999; Coleman et al., 2005; Chong et al., 2007). The FIV p24 immunizations conferred minimal to no protection against low-dose challenge, while HIV-1 p24 immunizations conferred statistically significant protection to

cats challenged with a low-dose FIV inoculation (Coleman et al., 2005). These results were further corroborated by later trials (Coleman et al., 2009). Moreover, the FIV p24 and HIV-1 p24 vaccinations did not induce similar IFN γ ELISpot and proliferation responses to FIV p24 peptide pools (data not shown), even though the vaccine formulation and the immunization schedule were identical. These differences in the immune responses may provide the key to why HIV-1 p24 vaccine was more effective at conferring protection than the FIV p24 vaccine. The next step was to immunize cats with recombinant FIV RT proteins alone or together with FIV p24. Preliminary results from FIV RT immunizations show a more consistent protection against low-dose challenge with heterologous FIV, while addition of FIV p24 to RT caused only a slight enhancement in protection (Coleman et al., 2009).

6. Mapping for evolutionarily conserved T-cell epitopes on HIV-1 and FIV

The cross-protection against FIV in the HIV-1 p24-immunized cats was the most unexpected and interesting observation (Coleman et al., 2005). This observation prompted the following study to determine whether HIV-1 infected LTS and HIV-1 p24-immunized cats can recognize similar peptide pools, and potentially the same peptides or epitopes. PBMC from greater than 30% of both HIV-1⁺ LTS and HIV-1 p24-immunized cats responded significantly to three common peptide pools (Yamamoto et al., 2010b). A careful analysis of two of these peptide pools shows that LTS and cats recognize a substantial number of the same peptides, including the established HIV-specific KK10 CTL epitope (KRWIILGLNK) important in non-progression to AIDS (Yamamoto et al., 2010b). Overall, these results demonstrate the presence of evolutionarily conserved epitopes on HIV-1 p24. Based on this observation, the PBMC from the same HIV-1⁺ subjects were tested for IFN γ responses and CFSE proliferation to FIV p24. The PBMC from 28% of the HIV-1⁺ subjects had IFN γ responses to FIV p24 peptide pool (Fp14) but not to the sequence counterpart peptide pool of HIV-1 p24 (Hp15) (Fig. 2). Furthermore, at least 22% of the subjects had IFN γ responses and CD3⁺CD4⁺ and CD3⁺CD8⁺ T-cell proliferations to another FIV p24 peptide pool (Fp9) (Yamamoto et al., 2010b). Thus, the evolutionarily conserved epitopes reside on both FIV and HIV-1 p24 proteins. Preliminary FIV RT analysis shows the PBMC from a substantial number of HIV-1⁺ subjects recognizing a number of FIV RT peptide pools (e.g., FRT3). Similar to the p24 findings, the FIV RT peptide pool (FRT3) induces a higher response than the HIV-1 sequence-counterpart peptide pool (HRT3) (Fig. 2).

Based on our blast search analysis using the LANL HIV-sequence database, the individual FIV p24 peptides in the Fp14 pool had sequence homologies to other regions of the HIV-1 proteins (Tat, Rev, and Vpr) more than the individual HIV-1 peptides in the counterpart Hp15 pool (data not shown). Thus, the high responses to the Fp14 may be due to the Fp14 stimulation causing the activation of memory cells to both the Hp15 epitopes and the homologous epitopes in other HIV-1 proteins. In the case of FRT3 and HRT3 with high sequence homology (79%) and identity (71%), few aa differences in the peptide that binds to the MHC's peptide binding region may be sufficient enough to cause the differences in the responses. Ongoing studies should determine the reasons for these unexpected observations.

6.1. Vaccine designs for the evolutionary conserved T-cell epitopes against FIV and HIV-1

These striking observations demonstrate that evolutionarily conserved epitopes on FIV are more reactive to PBMC from HIV-1⁺ subjects than expected. This suggests that such epitopes may be only homologous in sequence to the counterpart HIV-1 epitopes but are still recognized by subjects and may be important in an effective HIV-1 vaccine. Designing the vaccine will require the combination of conserved epitopes able to generate anti-HIV-1 CTL responses, Th responses, as well as neutralizing antibodies. The selected epitopes may be used in prime-boost combinations, which are effective at generating anti-HIV responses in humans, in a regimen maximizing the durability of the immunity generated. Formulation of such a multi-epitope vaccine could include supplemental viral proteins in one of the many proven delivery systems. Preliminary testing of the vaccine in cats gives the opportunity to make a new feline vaccine or improve the existing FIV vaccine by enhancing its immunity against conserved regions. The conserved T-cell epitope approach remains important in the search for an effective HIV-1 vaccine for humans and the FIV model is one of the best tools available.

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Fig. 1.

Carboxyfluorescein succinimidyl ester (CFSE) proliferation, IL-2, and IFN γ responses to FIV p24 overlapping peptide pools of prototype IWV-vaccinated cats. Six specific-pathogen-free cats vaccinated for 8-11 times over four years with prototype dual-subtype IWV FIV vaccine are shown. The PBMC of these cats were tested for CD3⁺CD8⁺ (A1) and CD3⁺CD4⁺ (A2) T-cell proliferation, exogenous IL-2 production (B), and IFN γ responses (C). All results for ELISpot were performed in duplicate and adjusted to spot forming units (SFU) per 10⁶ cells as previously described (Abbott et al., 2010). The results for CFSE proliferation were performed as described by others (Lichterfeld et al., 2004). The number below the peptide pool, FIV p24, FIV IWV, or T-cell mitogen (ConA) is the total number of responders (above the threshold, dotted horizontal line) with a responder frequency of $\geq 50\%$

for ELISpot results and 66% for proliferation. The thresholds for ELISpot analyses and proliferation were set at 300 SFU (B & C) and 30% CFSE^{low} (A1 & A2), respectively. These results were evaluated based on both total responses and responder frequency, which evaluated responses of the individual cat at thresholds of 50 SFU for ELISpot and 5% CFSE^{low} for proliferation (data not shown). The numbers in dark color represent those peptide-pool responses common between the results in panels A1 and A2, and between those in panels B and C; while the numbers in light color are the responses observed predominantly in the corresponding panel only. Note that the PBMC from the vaccinated cats responded to FIV p24 peptide pool Fp14 in all four of the immune parameters (vertical boxed/dashed line), while Fp12 induced responses in all immune responses except for the responder frequency of the CD3⁺CD8⁺ T-cell response (shorter boxed/dashed line).

Fig. 2.

IFN γ ELISpot responses of PBMC from HIV-1 infected subjects and vaccinated cats. The data is presented as photographs of actual IFN γ ELISpot results. The top four rows are results from PBMC of HIV-infected subjects with no stimulation (column 5) or after stimulation with the FIV-p24 peptide-pool Fp14 (column 1), counterpart HIV-p24 peptide-pool Hp15 (column 2; sequence counterpart of Fp14), FIV-RT peptide-pool FRT3 (column 3), counterpart HIV-1 peptide-pool HRT3 (column 4, counterpart of FRT3), or T-cell mitogen (positive control, ConA) (column 6). A representative of a duplicate well is shown. The description of the subject or the cat is shown on the left along with the multiplication factor (#X) to obtain a SFU result for 10^6 cells. The results are from a long-term survivor (LTS19 infected for 12 years), an HIV⁺ long-time infected subject (HL20 infected for 23

years), and two HIV⁺ short-time infected subjects (HS17 and HS23 infected for 8 and 7 years, respectively). The SFU number of each result is shown immediately below the corresponding ELISpot well. The last two rows are the results from PBMC of an IWV-vaccinated cat (row 5) and an HIV-1 p24-immunized cat (row 6). Peptides not tested are shown as (ND). The PBMC from LTS19, HL20, HS17, HS23 and cat BDA have moderate to large responses to FIV-p24 peptide-pool Fp14, while their responses to HIV-p24 counterpart Hp15 are moderate to none. Subject HS23 has a robust response to FIV-RT peptide-pool FRT3 but not to the counterpart HRT3. Subject LTS19 and cat BDA have moderate to high responses to FRT3. Hence, the most notable observation was more robust responses of HIV-infected subjects to FIV peptide pools than to the counterpart HIV peptide pools.

Table 1

Major phase-IIb and phase-III HIV-1 vaccine trials.

Vaccine Trial (Developer) [Reference]	Trial Classification Sample Size (Vaccine:Placebo)	Trial Period	Trial Location & Study Population (pop.)	Vaccine type or immunogen(s)	Trial Outcome Infected Events Vaccine:Placebo	Antibody Immunity (VNAs, virus neutralizing Ab)	Cellular Immunity (ADCC, antibody-dependent cellular cytotoxicity)	Vector-Specific Immune Responses Produced
VaxGen 004 (VaxGen) [Flynn et al., 2005]	Phase III 5403 (2:1)	1998-2003	North America High Risk Men sex w/men (MSM)	gp120 envelopes from subtype-B MN and GNE8 strains, called AIDSVAX B/B. (alum adjuvant)	No efficacy 368 infected 241:127	gp120 binding Ab. Type-specific VNAs to lab strains & vaccine types. [Gilbert et al., 2005, 2010]	CD8 ⁺ & CD4 ⁺ T-cell proliferations and CD8 ⁺ T-cell cytokine responses to gp120. [Jones et al., 2009]	No
VaxGen 003 (VaxGen) [Pitisuttithum et al., 2003]	Phase III 2527 (1:1)	1999-2003	Thailand High Risk IV Drug User	gp120 envelopes from subtypes B (MN) & E (CRF01_AE A244) called AIDSVAX B/E. (alum adjuvant)	No efficacy 211 infected 106:105	gp120 binding Ab. Type-specific VNA to vaccine types. [Pitisuttithum et al., 2004, 2006]	T-cell proliferation [Pitisuttithum et al., 2003]	No
Step HVTN 502 (Merck) [Buchbinder et al., 2008]	Phase IIb Plan 3000 (1:1)	2004-2007	Americas High Risk MSM + Women	Ad5- <i>gag/pol/nef</i> subtype B strains: CAM-1 <i>gag</i> , JRF1 <i>pol</i> (<i>RT & IN</i>), IIB <i>nef</i> (Ad5 vector)	Enhancement of infection 82 infected 49:33	NA	CD4 ⁺ T-cell & CD8 ⁺ CTL responses to Gag, Pol, and Nef. [McElrath et al., 2008]	Yes Ab & T cells to Ad5. [Buchbinder et al., 2008]
RV144 prime-boost trial (prime – Sanofi boost – VaxGen) [Rerks-Narm et al., 2009]	Phase (IIb)-III Cancelled at 1836	2004-2009	Thailand	Prime - ALVAC-HIV vCP1521 subtype-B LAI strain <i>gag-pr-gp41</i> & CRF01_AE <i>gp120</i> (canarypox vector)	Overall efficacy 31.2%	gp120 binding Ab. Ab. Type-specific VNAs. [Nitayaphan et al., 2004; Rerks-Narm et al., 2009]	Low CTL responses to Gag, PR, gp120, & gp160. [Nitayaphan et al., 2004; Rerks-Narm et al., 2009]	Yes
	16,402 (1:1)		Low-High Risk General pop.	Boost - AIDSVAX B/E	High risk group 3.7%	Some ADCC Ab. [Karnasuta et al., 2005]	Some ADCC (NK cell?) responses. [Karnasuta et al., 2005]	Ab & T cells to ALVAC. [Weiner, et al., 2010]

Table 2CD8⁺ CTL and CD4⁺ Th epitopes on HIV-1 proteins.

Protein	Acronym	HIV-1 Size	Number of HIV-1 Epitopes ^a			HIV-1 & FIV Homology	FIV Size
			CD8 ⁺ CTL	CD4 ⁺ Th			
Capsid	Gag CA	p24	252	102		63.1%	p24
Negative factor (accessory)	Nef	p27	182	25		None	None
Reverse Transcriptase	Pol RT	p66	141	21		72.0%	p61
Surface Envelope	Env SU	gp120	135	92		42.5%	gp100
Matrix	Gag MA	p17	105	36		50.4%	p17
Transmembrane Envelope	Env TM	gp41	83	23		42.4%	gp39
Integrase	Pol IN	p32	54	11		64.7%	p31
Nucleocapsid	Gag NC	p7	40	19		54.2%	p5
Protease	Pol PR	p11	39	1		48.4%	p13
Total:			1031	330			

^a HIV-1 epitopes were determined from the lists in LANL's Epitope Tables of the HIV Molecular Immunology Database <http://www.hiv.lanl.gov/content/immunology/tables/tables.html>.