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SUMMARY

It has been almost 30 years since the identification of HIV as the causative agent of AIDS. Despite considerable efforts to halt the spread of the virus, the epidemic continues in many parts of the world. The most efficient way to stop new infections is a prophylactic vaccine that blocks transmission at the viral portal of entry. To generate an efficacious vaccine, neutralizing antibodies directed against the envelope glycoproteins (Env), the only virally expressed proteins on the surface of the virion, likely need to be elicited.

Early attempts to develop an HIV vaccine used soluble recombinant monomeric Env. This vaccine candidate generated high titers of Env binding antibodies in a large clinical trial; however, no protection against infection was observed. Since then attempts have been made to design Env immunogens that more closely mimic the native Env spike with the aim to improve the quality of vaccine-elicited humoral immune responses. Due to the lack of high resolution three dimensional structures of Env in its native conformation, these design efforts are still empirical. To guide future immunogen design efforts, it is important to understand what current Env vaccine candidates elicit in relevant pre-clinical models, both at the serological and cellular level.

The focus of this thesis has been to investigate B cell responses in non-human primates following immunization with soluble HIV-1 Env trimers. We show that Env immunization in adjuvant elicits high levels of binding antibodies as well as robust peripheral memory B cell and plasma cell populations. The kinetics and magnitude of the responses at early time points after immunization are similar to those elicited by the successful human influenza and rabies vaccines. Despite these potent responses, we only observed a modest protective effect upon mucosal SHIV challenge of vaccinated animals. When quantifying the levels of Env-specific antibodies in the mucosa we found them to be 1000-10 000 fold lower than in serum, which may explain the lack of protection in this heterologous challenge model.

From *in vitro* studies it has been proposed that interactions between Env and CD4-expressing T cells may detrimentally affect T cell function. To investigate if *in vivo* interactions between Env immunogens and CD4-expressing cells would negatively affect the elicitation of Env-specific immune responses, CD4 binding-competent and CD4 binding-defective trimers were constructed and inoculated into non-human primates. No difference was observed between the groups in regards to Env-specific antibody titers, memory B cell or T cell levels and functionality, indicating that Env binding to CD4 *in vivo* was not detrimental to vaccine elicited responses. Furthermore, using the CD4 binding-competent and CD4 binding-defective Env immunogens, we assessed if *in vivo* CD4 binding affected the outcome of an intravenous SHIV challenge. Antibodies directed against the conserved co-receptor binding site of Env were only observed following immunization with the CD4-binding competent trimers. However, the presence or absence of this class of antibodies did not influence the level of protection against SHIV infection. These results indicate that Env-CD4 *in vivo* interactions, and specifically co-receptor binding site-directed antibodies, did not contribute to the control of viremia observed in this challenge model.

LIST OF PUBLICATIONS

I. **Sundling, C**. Forsell, M. N. E. O'dell, S. Feng, Y. Rao, S. S. Loré, K. Mascola, J. R. Wyatt, R. T. Douagi, I. Karlsson Hedestam, G. B. *Soluble HIV-1 Env trimers in adjuvant elicit potent and diverse functional B cell responses in primates*. In revision for Journal of Experimental Medicine.

II. Douagi, I. Forsell, M. N. E. **Sundling, C**. O'dell, S. Feng, Y. Dosenovic, P. Li, Y. Seder, R. Loré, K. Mascola, J. R. Wyatt, R. T. Karlsson Hedestam, G. B. *Influence of novel CD4 binding-defective HIV-1 Envelope glycoprotein immunogens on neutralizing antibody and T-cell responses in nonhuman primates*. Journal of Virology. 2010. 84:1683-1695.

III. **Sundling, C**. O'dell, S. Douagi, I. Forsell, M. N. E. Loré, K. Mascola, J. R. Wyatt, R. T. Karlsson Hedestam, G. B. *Immunization with wildtype or CD4 binding-defective HIV-1 Env trimers comparably reduces viremia following heterologous SHIV challenge*. Manuscript

ADDITIONAL PUBLICATIONS NOT INCLUDED IN THIS THESIS

I. Douagi, I. Gujer, C. **Sundling, C**. Adams, W. C. Smed-Sörensen, A. Seder, R. A. Karlsson Hedestam, G. B. Loré, K. *Human B cell responses to TLR ligands are differentially modulated by myeloid and plasmacytoid dendritic cells*. Journal of Immunology. 2009. 182:1991-2001.

II. Mörner, A. Douagi, I. Forsell, M. N. **Sundling, C**. Dosenovic, P. O'dell, S. Dey, B. Kwong, P. D. Voss, G. Thorstensson, R. Mascola, J. R. Wyatt, R. T. Karlsson Hedestam G. B. *Human immunodeficiency virus type 1 Env trimer immunization of macaques and impact of priming with viral vector or stabilized core protein*. Journal of Virology. 2009. 83:541-551.

III. **Sundling, C**. Schön, K. Mörner, A. Forsell, M. N. Wyatt, R. T. Thorstensson, R. Karlsson Hedestam, G. B. Lycke, N. Y. *CTA1-DD adjuvant promotes strong immunity against human immunodeficiency virus type 1 envelope glycoproteins following mucosal immunization*. Journal of General Virology. 2008. 89:2954-2964.

IV. Forsell, M. N. McInerney, G. M. Dosenovic, P. Hidmark, Å. S. **Eriksson, C**. Liljeström, P. Grundner, C. Karlsson Hedestam, G. B. *Increased human immunodeficiency virus type 1 Env expression and antibody induction using an enhanced alphavirus vector*. Journal of General Virology. 2007. 88:2774-2779.

POPULAR SCIENCE PUBLICATIONS

I. **Eriksson, C**. Forsell, M. N. E. Karlsson Hedestam, G. B. *Hur HIV-1 värjer sig från neutraliserande antikroppar – implikationer för vaccinutveckling.* Incitament. 2007. 7:535-539.

CONTENTS

ABBREVIATIONS

Ab	Antibody
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
APOBEC3G	Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G
CDRH	Complementary determining region heavy chain
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
DNA	Deoxyribonucleic acid
ELISpot	Enzyme-linked immunosorbent spot
Env	Envelope
Gag	Group-specific antigen
GALT	Gut-associated lymphoid tissue
ART	Anti-retroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
MHC	Major histocompatibility complex
Nef	Negative factor
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
Pol	Polymerase gene
PRR	Pattern recognition receptor
RNA	Ribonucleic acid
RT	Reverse transcriptase
SHIV	Simian/Human immunodeficiency virus
SIV	Simian immunodeficiency virus
TLR	Toll-like receptor
TRIM5α	Tripartite motif protein 5α
Vif	Viral infectivity factor

INTRODUCTION

Human Immunodeficiency Virus type 1 (HIV-1)

History

In the beginning of the 1980s there were reports of opportunistic infections from *Pneumocystis Carinii* and *Candida albicans* as well as aggressive forms of the normally rare Karposi's sarcoma in homosexual males in the USA [2]. These symptoms lead researchers to believe that the affected individuals were immune deficient and upon investigating lymphocyte levels they were found to have low levels of CD4+ T cells. The symptoms of immune deficiency gave the disease the name Acquired Immune Deficiency Syndrome (AIDS). In 1983, two groups independently isolated a new retrovirus from patients with AIDS and proposed it to be the causative agent of the disease [3-4] . The virus showed resemblance to Human T cell leukemia virus I and II (HTLV-I,II) and was called HTLV-III by the American group [5-8], while the French group called it lymphadenopathy-associated virus (LAV) [4]. In 1986 the virus was re-named Human Immunodeficiency Virus type I (HIV-1) [9].

Epidemiology

There are two major forms of HIV that infect humans; HIV-1 and HIV-2. HIV-1 is responsible for the majority of infections worldwide. HIV-2 was found later [10] and is mainly restricted to infections in some regions of western and central Africa [11] and is not as pathogenic [12]. HIV-1 and HIV-2 are further subdivided into clades. Each clade can vary as much as 20-50% in the *Env* sequence. HIV-2 contain clades A-H while the HIV-1 clades are designated; M (Major), N (Non-M, Non-O, New), O (Outlier). The vast majority of HIV-1 infections stem from clade M [11, 13]. Clade M is further subdivided into subtypes (A-D, F-H, J and K) of which subtype B is most prominent in the Western world while subtype C is common in Africa, the Middle east and Asia [13]. HIV-1 infections of subtype C are now responsible for more than 50% of HIV-1 infections word wide. The different clades are thought to have arisen through transmission from non-human primates into the human population in the early 1900s [14-15]. HIV-1 has been shown to stem from Chimpanzees (*Pan troglodytes troglodytes*) [16-17] while HIV-2 stems from Sooty mangabeys (*Cercocebus atys*) [13].

HIV-1 structure and replication

HIV-1 is a +ve stranded RNA virus of approximately 9.2 kbp and belongs to the Lentivirus genus of the *Retroviridae* family. Viruses in the *Retroviridae* are enveloped by a lipid membrane derived from the infected host cell upon budding. All viruses in the *Retroviridae* family encode the three major structural genes; *gag*, *pol*, and *env* (Figure 1). The *gag* polyprotein codes for the matrix, capsid, nucleocapsid and p6 proteins. *pol* encodes protease, reverse transcriptase and integrase, while *env* codes for the envelope glycoproteins gp41 and gp120. In HIV-1, an additional three accessory proteins are encoded; *vif, vpr, vpu,* and three regulatory proteins *tat, rev, and nef* (reviewed in [18-19]).

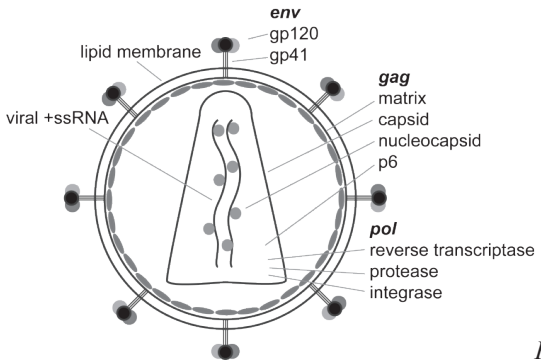

Figure 1. The structure of HIV-1.

HIV-1 binding to the host cell occurs in a two-step process. It is initiated through binding of gp120 to the primary host cell receptor, CD4 [20]. This induces a conformational change in Env, which exposes or forms the co-receptor binding site (CoRbs) [21-23]. The CoRbs then interacts with CCR5 or CXCR4 depending on the tropism of the virus [24]. This leads to further conformational changes and membrane fusion, with subsequent release of the virus capsid into the cell cytoplasm. Upon entering the cytoplasm the nucleocapsid uncoats and the viral RNA is released. The RNA is reverse transcribed into double stranded DNA, called provirus, by the error prone reverse transcriptase. The provirus interacts with the HIV-1 integrase and additional viral and cellular components to form the pre-integration complex [25], which is transported into the nucleus where the provirus is integrated with the host cell genome [18, 26]. After integration the virus can become latent and survive for the lifetime of the infected cell, making eradication of infection very difficult [27-29].

Infection and immunity

HIV-1 infection

The most common form of HIV-1 transmission is via sexual contact. There are several factors influencing the transmission, such as viral load in the infected partner, presence of other sexually transmitted diseases, lesions in the mucosa, male circumcision, type of sexual act etc. [30-32]. Commonly productive infection is established in the gut from as few as one singe virus [33-34]. Upon entering the gut-associated lymphoid tissue (GALT) the virus infects and replicates to high levels in resting $CCR5^+$ memory CD4 T cells [35-39], which are the main GALT resident T cells [35-37]. At peak viremia, as much as 80% of the GALT memory T cells can be infected or killed. A large proportion of the human memory T cells reside in the GALT and even after initiation of antiretroviral therapy and suppression of viremia the reconstitution of the memory T cell compartment is poor [40-41]. The first adaptive T cell mediated response can be detected around the time of peak viremia [42]. Up until this time the virus replicates in the absence of an adaptive immune responses, but escape mutants will then rapidly be selected for as viremia decreases to set-point levels [43]. The magnitude of the viral load set-point is a good predictive value of the disease progression to AIDS [44]. After the decline to set-point the infected person is often clinically asymptomatic, so called clinical latency. This latency can last for several years, however, gradually the $CD4^+$ T cell count

drops and upon reaching below 200 cells per µl blood, the infected person usually reaches the clinical status of AIDS and becomes sensitive to opportunistic pathogens [45] (Figure 2).

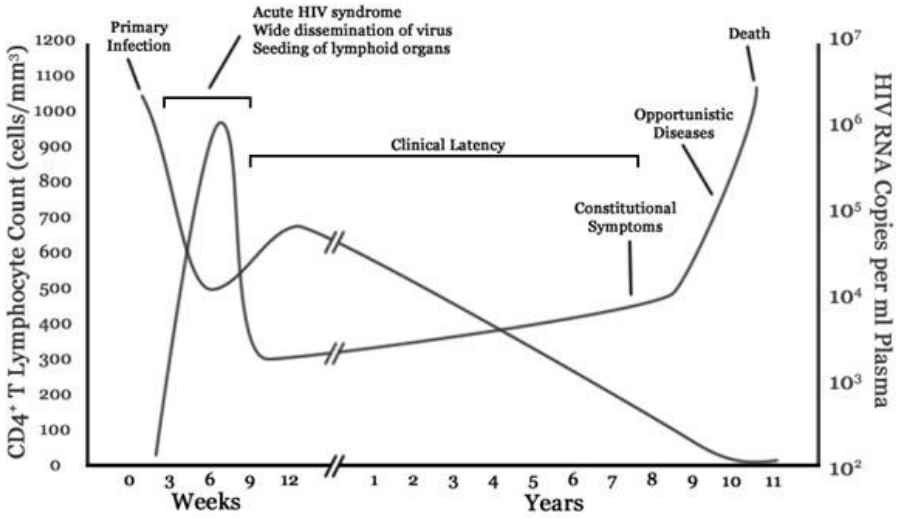

Figure 2. HIV-1 replication and $CD4^+$ T cell counts during infection.

Innate immune responses

The innate response to pathogens is characterized by fast but rather non-specific actions. The response is initiated through recognition of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) on host cells. Several different types of PRRs exist, which enable the innate immunity to shape the adaptive response according to the type of pathogen responsible for the infection [46-50]. PAMPs are structures that cannot be changed easily by the pathogen, e.g. peptidoglycan, LPS, non-methylated DNA, and single-stranded RNA etc. The early innate response to HIV-1 infection is very strong [51] and has been suggested to be able to protect from, or limit infection in some cases [52]. Strong NK cell activation has been shown to precede T cell activation and be important for initial control of virus replication and shaping of the adaptive immune response [53-55].

Adaptive immune responses

T cell responses

The first adaptive immune response detected toward HIV-1 is mediated by $CD8^+$ T cells and is observed close to the time of peak viremia [42, 56]. The T cell response then peaks 1-2 weeks later, concurrent with control of replication and viral set-point. Between the time of peak viremia and viral set-point is a phase of intense immune pressure, where a large pool of infected cells is present. Due to the pressure exerted by the cytotoxic T lymphocytes (CTLs) the virus rapidly mutates to produce escape variants [43, 57-63]. Much of the initial T cell response to HIV-1 is directed toward *env* and *nef* [61], but changes later in infection toward more conserved epitopes in *gag* and *pol* [61, 64]. Early responses are also directed toward few epitopes, while later responses show an increased broadness, especially toward more

conserved residues [61, 65-66]. Some individuals are associated with a better control of HIV-1 replication. This has been associated to the expression of certain human leukocyte antigen (HLA) genes, which interact with conserved epitopes of HIV. Studies have shown that mutations in these epitopes results in viral loss of fitness [67-68].

B cell responses

The initial B cell response to HIV-1 is observed, in the form of immune complexes, as early as eight days after detectable viremia. These antibodies (Abs) are directed toward the gp41 ectodomain of the surface envelope glycoproteins (Env). It takes an additional two weeks before the appearance of Abs directed toward gp120 [69]. Autologous neutralizing Abs to HIV-1 are not detectable until months after infection [70-73] but upon appearance exert a significant selective pressure on the circulating virus [74] resulting in strain-specific Abs with limited breadth [75-76]. Abs that neutralizes a broader range of virus isolates are usually not detected until after several years of chronic infection [77-80]. As many as 25% of infected people have been shown to produce broadly neutralizing antibodies (bNabs) after more than two years of infection [77, 79]. Among these about 1% can be classified as elite neutralizers that exhibit broad neutralization to different clades of HIV-1 isolates [81].

The autologous neutralizing Abs are often directed toward the immunogenic variable regions (V1-V5) of Env [82-84] while the bNabs are directed toward conserved areas, which cannot easily mutate due to extensive fitness cost or production of non-viable viral particles [85]. As V3 is important for co-receptor binding and infection, some Abs directed to this region can exhibit some breadth of neutralization, at least within the same clade [86-90]. Env utilizes several different mechanisms for subversion of the immune response [91], e.g. sequence variation and insertion of glycans [71, 92-95], conformational masking [96-97], nonfunctional Env [98] and direct interaction with other immune cells [99-102].

Although rare, several bNabs have been isolated from infected patients. These Abs target conserved areas of gp41 [103-104], the CD4-binding site [105], high-mannose glycans [106] and quaternary epitopes of the trimeric spike [107]. An additional conserved area within Env is the immunogenic CoRbs and upon infection large amounts of Abs are elicited toward this surface [73, 108]. However, few of these Abs show neutralization of primary viruses, likely due to sterical restriction [109-110]. A common feature of bNabs isolated from chronically infected individuals is the highly mutated, elongated CDRH3 loops [111]. This has been associated with increased risk of auto-reactivity [112-113]; however, this remains a controversial area [114].

Animal models

HIV-1 can only productively infect humans and chimpanzees. The reasons behind this are the host restriction factors APOBEC3 and TRIM5α, which limit infection if not countered by HIV-encoded proteins (reviewed in [15, 115]). Early in the field of HIV-1 research, chimpanzees were used for pathogenesis and vaccine studies [116-118]. However, due to ethical considerations, cost and the fact that they seldom develop AIDS after infection, the field has turned the focus toward other models [119-120]. The most prominent model for pathogenesis studies is now SIV infection in Asian macaques, which well recapitulates HIV-1

infection in humans, although disease progression to AIDS tends to be faster (reviewed in [121-122]). SIV challenge models are not well suited for the evaluation of antibody based vaccines due to the lack of cross-reactivity between the HIV-1 and SIV envelope glycoproteins. However, the SIV model is useful for evaluation of T cell based vaccines [123-130]. Depending on which SIV isolate and which route of infection is used for challenge, it is possible to mimic conditions resembling natural infection in humans [131]. For evaluation of antibody based vaccines chimeras between SIV and HIV, called SHIVs, have been created. The SHIVs contain the *env*, *tat, vpu* and *rev* genes from HIV-1 and the remaining genes, important for counter of the host cell restriction factors from SIV (Figure 3).

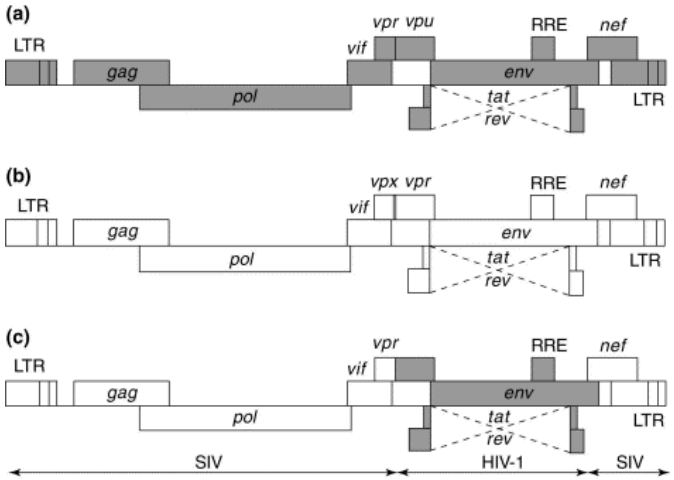

Figure 3.

Genetic organization of (a) HIV, (b) SIV, and (c) SHIV. Adapted from [1].

Initially SHIVs utilizing the CXCR4 co-receptor were constructed [132-138]. These viruses were after passage often very pathogenic and replicated well in macaques depleting peripheral but not gut $CD4^+$ T cells [139]. However upon vaccination they were relatively easy to protect against [128, 140-142], raising questions about whether CXCR4-using SHIVs are an appropriate model virus for HIV-1 challenge studies [143]. CCR5 co-receptor using SHIVs were subsequently developed [144-147]. These are considered to mimic natural HIV-1 infection better and stocks have been generated that are more or less resistant to neutralizing Abs [139, 148].

As non-human primate experiments are expensive and the supply of animals is limited efforts are underway to develop humanized mouse models capable of recapitulating HIV-1 infection [149].

Vaccine approaches

The rationale behind a vaccine is to stimulate the recipient to induce an adaptive immune response that can protect against an invading pathogen or attenuate disease without having encountered the pathogen before. The correlate of protection for most successful vaccines existing today is neutralizing Abs [150]. For HIV, proof-of-principle studies using the SHIV

model, indicate that if sufficient amounts of neutralizing Abs are present at the portal of virus entry the recipient will be protected from infection [151-161].

Clinical HIV-1 vaccine trials

To date four late phase clinical efficacy trials have been conducted. The first two trials were conducted by the company Vaxgen and based on recombinant monomeric gp120 Env given with alum adjuvant in seven consecutive doses in North America and Holland (clade B) [162] or Thailand (clade B/E) [163]. The primary vaccine targets were men who have sex with men (MSM) and women with high risk of HIV-1 exposure or injection drug users. More than 7500 participants joined the two studies and the incidence was 7% and 8.4% respectively in the placebo groups. No effect on acquisition [162-163] or other immune parameters [164] was observed between the placebo and vaccine arms in regards to infection although almost all vaccine recipients developed strong, but short-lived, Env-specific antibody responses.

After the failure of the strictly protein based vaccine, focus turned toward T cells as the main targets for a vaccine. Promising data showed that adenovirus vectors could protect monkeys from SHIV infection [128] and it was known that CTL responses were important for suppression of viremia in natural HIV-1 infection [165-167]. A clinical study was initiated by Merck with a replication-defective adenovirus type 5 vector expressing the HIV-1 genes *gag, pol*, and *nef* of clade B [168]. The vaccine was given three times to 3000 participants. At a planned interim analysis it was found that the vaccine had no effect upon acquisition of infection or viral loads and furthermore that participants presenting high levels of adenovirus type 5-directed Abs upon study initiation and were un-circumcised exhibited an increased risk of HIV-1 infection in comparison to the placebo group.

The fourth late phase clinical trial, called RV144, was based on a prime-boost regiment with the canary-pox vector (ALVAC) encoding a modified clade B/E Env and clade B *gag/protease* together with clade B/E gp120 protein monomers or clade E oligomeric gp160 as boosts [169]. The vaccine was given to more than 16 000 participants in Thailand and upon completion; vaccine efficacy was in the modified intention-to-treat analysis calculated to 31%, with the largest effect in low to medium risk groups [170]. Although no clear correlations were observed between immune responses and HIV-1 acquisition, the results are intriguing.

Recombinant subunit vaccines

For a prophylactic HIV-1 vaccine, recombinant protein based vaccines are pursued for several reasons, not the least because of safety concerns with whole particle vaccines [171]. Although often very potent [172-175], all live attenuated vaccines carry the potential of reverting to a pathogenic form, potentially harming or killing the vaccine recipient [176-178]. This has been observed, with a high frequency, for attenuation attempts of SIV [179]. Encouraging for recombinant protein vaccines is the development of the successful hepatitis B and human papilloma virus vaccines [180-182]. With a recombinant subunit vaccine it is possible to direct the immune response to desired targets, representing neutralization sensitive areas of the invading pathogen. However, often it is necessary to include immune stimulatory

components, in the form of adjuvants, as proteins by themselves are relatively inert to the immune system [183].

As the only virally encoded proteins on the surface of HIV, the trimeric gp41-gp120 heterodimeric envelope glycoproteins (Env) [184-185] are of main interest for antibody-based HIV-1 vaccines. To rationally design Env trimer mimetics capable of inducing bNabs it is important to know the three-dimensional structure of Env [85, 186]. Currently several sub-domains of Env have been crystallized [187-189]. However, the structure of the important non-liganded trimeric form present on infectious circulating virus is still lacking. Cryotomography has given a low-resolution structure of the functional trimer although not at the level of detail necessary for immunogen design [190]. Instead bNabs (see section B cell responses) to conserved determinants of Env can be used as probes to evaluate the antigenicity of the mimetics generated [191-194].

The first generation Env mimetics were based on monomeric gp120 and were subsequently used in the first clinical trials (see section about clinical vaccine trials). Although such immunogens elicited high levels of gp120 specific Abs these exhibited limited breadth and no protection from infection was observed [164]. The next generation mimetics were various forms of soluble, stabilized oligomeric Envs [195-201]. These have been shown to be superior to monomeric gp120 Env in eliciting neutralization breath [202-206] as measured by *in vitro* infection blocking assays [207-209]. However no broad NAb activity has been induced by vaccination so far [210-211]. Much of the reactivity elicited by the oligomeric Env mimetics is directed toward the variable loops, similarly to what is seen in autologous neutralizing Abs upon infection [95, 205, 212-214]. To focus the antibody response toward more neutralization-sensitive areas, or areas where known bNabs bind, several different approaches have been tested [92, 215-219], so far with limited success. However, there are some indications that stabilization of specific epitopes generate enhanced antibody responses to these determinants [220], suggesting that such engineering efforts are promising for the future.

Concurrent with the development of recombinant soluble protein immunogens is the evaluation of virus-like particles (VLPs) as HIV-1 vaccines. VLPs are considered safe and induce strong and diverse immune responses including both the humoral and cellular arm of the immune system [221-222]. However, although strong immune responses are elicited there is still a need for improved immunogen design to focus the response on conserved determinants as current VLPs do not protect non-human primates from SHIV challenge [223-224].

Viral vector and DNA vaccines

Genetic vaccines are based on the delivery of genes encoding antigens of interest into the host. These genes are then produced by the host cells and presented to the immune system for induction of primarily T cell responses. There are two major techniques to deliver the genetic material. One is via the use of recombinant viral vectors and the other is via injection of naked DNA. Recombinant viral vectors are based on non-pathogenic viruses, unrelated to the pathogen of interest, and function as *in vivo* gene expression systems. There are several

classes of viruses that can function as viral vector vaccines, either replication-competent or replication-incompetent [225-227]. Upon vaccination the vector triggers the recipient to induce insert-specific immune responses without the need for adjuvants [228]. A large proportion of the antiviral response is mediated through T cells [229-230] and when vectors are used as the only vaccine component the goal is mainly a T cell mediated vaccine effect [126, 231]. However, vectors that have been designed to express high levels of HIV-1 Env protein do induce significant B cell mediated responses [232-233], although upon comparison with protein only immunizations the levels are significantly lower [234]. An approach to elevate the antibody titers is to use heterologous prime-boost regiments, where the recipient is first immunized with viral vectors and then boosted with recombinant protein. This approach elicits antibody levels as high as with protein alone, but retains the anti-viral phenotype of the immune response induced by the viral vector [234-235]. One of the caveats with viral vectors is that the vaccine recipient will also mount an immune response toward the vector. This immunity can then attenuate further injections with decreased immune responses to intended antigens as result.

DNA vaccines are based on DNA plasmids encoding the vaccine gene of interest under influence of a strong promoter. Immunization with DNA will lead to transfection of both APCs and tissue resident cells. Via cross-presentation the gene products can access both MHC I and II and mount primarily T cell responses, but also weak B cell responses [236-238]. In general the responses elicited by DNA vaccines alone are weak, most likely due to low transfection rate of host cells. However as a priming component in a prime-boost regiment with recombinant protein or viral vectors, DNA has been shown to enhance the vaccine-induced response prior to a protein boost [239-243]. Efforts are also invested into increasing the transfection of cells after immunization. Methods that are evaluated are e.g. *in vivo* electroporation, shooting DNA coated gold particles or shooting DNA dissolved in liquid into the skin. These techniques have been shown to increase the transfection and thereby immune responses [244-246]. Two of the strengths with DNA vaccines are the ease of manufacturing and stability of the vaccine, features that could be important to make a successful vaccine against HIV-1 available worldwide [247-248].

AIMS

The overall aim of this thesis was to characterize HIV-1 Env-specific B cell responses following protein subunit immunization of non-human primates. The specific aims of the individual papers were:

Paper I. To evaluate the B cell response in the periphery and bone marrow following immunization with soluble trimeric Env and to assess the protective effect of the response upon mucosal SHIV challenge.

Paper II. To investigate the *in vivo* influence of Env-CD4 binding for cellular and humoral immune responses elicited by soluble trimeric Env immunogens.

Paper III. To evaluate the protective effect of CD4 binding-competent and CD4 binding-defective Env trimers upon intravenous SHIV challenge.

RESULTS AND DISCUSSION

The methods used in this thesis are described in detail in the included papers.

Paper I

Soluble HIV-1 Env trimers in adjuvant elicit potent and diverse functional B cell responses in primates

For a successful prophylactic HIV-1 vaccine the elicitation of bNAbs is thought to be critical. Efforts are underway to characterize the antibody repertoire in HIV-1-infected individuals exhibiting broad serological neutralizing activity [77-79, 81, 249] and monoclonal Abs are isolated for epitope mapping and subsequent immunogen design [107, 250-251]. However, equally important is the understanding of how the immune response develops to the antigens during immunization. It is currently not known why it takes so long for bNabs to develop in the HIV-1 infected individuals who possess such activity. The examination of selected monoclonal Abs from these individuals shows that they exhibit a high degree of somatic hypermutation suggesting that extensive affinity maturation is necessary [105, 107]. However, little is known about what drives the selection process of B cells recognizing different epitopes on complex antigens in the germinal centers and the establishment of memory and long lived plasma cell pools. We also lack knowledge regarding the effect of different immunization regiments on the establishment of the cells that populate these immune compartments.

In Paper I we immunized rhesus macaques with current state-of-the-art Env trimers [198] to comprehensively analyze the elicited B cell responses. To analyze the memory B cell compartment we developed an *in vitro* stimulation protocol to differentiate memory B cells into antibody secreting cells (ASCs), which could be measured via B cell ELISpot analysis. We also used a recently described assay for evaluation of Env region-specific B cell responses [252] to determine how these evolve during the course of the immunizations. To further evaluate the Env trimer immunogens used here we benchmarked the capacity of the trimer-elicited responses to neutralize a diverse set of viruses in direct comparison to the responses elicited by the gp120 monomers used in the human clinical VAX04 study. We then evaluated the protective effect of trimer-elicited Abs on repeated rectal challenge with a heterologous SHIV.

Upon immunization with Env trimers in Abisco-100 and CpG adjuvant, peak antibody levels were reached after the second immunization. Following subsequent immunizations the antibody levels could not be elevated further although we detected an increase in the overall antibody avidity, suggesting maturation of the Abs (Figure 1).

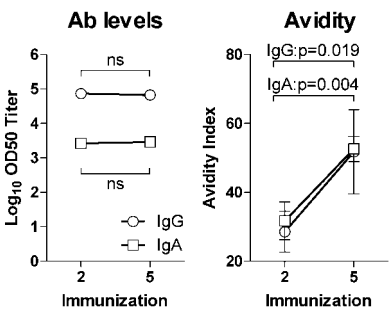

Figure 1. Antibody titers (left) and avidity index (right) two weeks after immunizations 2 and 5. Shown is mean ±SEM (n=6 per group). Statistics was measured with a paired t-test.

Peak levels of Env-specific peripheral plasma cells (PC), memory B cells, and long lived PCs in the bone marrow were also reached after the second immunization. Similarly to the antibody levels, the magnitude of these compartments did not increase by subsequent immunizations (Figure 2).

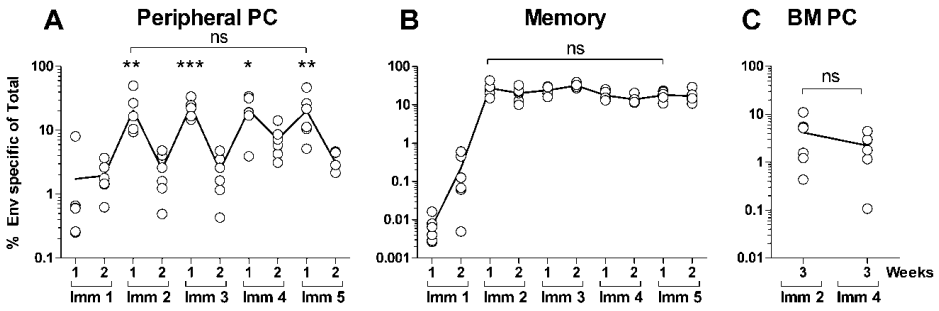

Figure 2. Env-specific peripheral plasma cell (A), memory B cell (B), and bone marrow plasma cell (C) levels following indicated weeks after immunization. Statistics was done with a paired t-test.

Peripheral plasma cells peaked seven days after immunization with ~20% of IgG producing cells being specific for Env, an observation that is consistent with influenza and rabies vaccines in humans [253-254]. As peripheral levels of antigen-specific plasma and memory B cells are similar to what is observed for other successful human vaccines and high titers of Env-specific Abs are elicited this indicates that HIV-1 Env trimers are not intrinsically weak as antigens for induction of humoral responses, when administered in adjuvant. However, these observations are directly following immunization and cannot predict sustainability (or duration) of the Env-specific response.

In early attempts to use monomeric Env as an HIV-1 vaccine candidate (VAX04) no protective effect was observed [163]. However, recently a prime-boost regiment using a viral vector prime with an Env protein boost (RV144) suggested a reduced risk of acquisition of HIV-1 infection in the immunized group [170]. Although the protective effect was transient and the mechanisms behind the protection have not been identified this merits further investigation of the role of Env protein in human HIV-1 vaccines. As studies in rabbits have shown that the soluble Env trimers are superior to monomers in terms of eliciting Abs exhibiting some neutralization breadth and potency [202, 204-205], we benchmarked the Env trimer-elicited Ab responses in non-human primates to gp120 monomer responses elicited in twenty randomly selected human subjects from the VAX04 trial. To do this we evaluated the neutralizing capacity in plasma or serum against several tier I and II viruses from clade B and selected viruses from clade A and C using standardized methods [207] (Table I).

Table I. Neutralization potential from immunization with monomeric or trimeric Env .

			VAX04[b]	NHP[c]
	Virus	Clade	monomers	trimers
Tier I	MN	B	100[a]	100
	HxB2	B	15	100
	SF162	B	5	100
	BaL0.1	B	0	37.5
Tier II	YU2	B	0	75
	89.6	B	0	27
	6536	B	0	38
	ADA	B	95	55
Tier I	DJ263	A	0	75
Tier I	MW965	C	95	100

[a]values indicate percent responders (ID_{50}>10) vs non-responders (ID_{50}<10)

[b]n=20 randomly selected human donors from the VAX04 study.

[c]n=16 macaques pooled from the present study, [234], and [255].

The plasma obtained from Env trimer-immunized macaques expressed a broader neutralization profile with activities against all the viral isolates tested. The monomer-induced human responses, on the other hand, showed more narrow neutralizing activity, although when reactivity was detected it was generally potent. These responses indicate that the Env trimers used here elicit superior neutralizing Abs in this highly relevant non-human primate model compared to the monomers used in the human clinical trials and as such the trimers could be an interesting component in a future HIV-1 vaccine. However, it is also important to address the potential role of the different adjuvants for their capacity to influence the response. In this regard, Alum was used in the human study while AS01B or Abisco-100 together with CpG was used in the non-human primates, which limits direct comparisons of the data.

Following immunization with Env a large proportion of the vaccine elicited antibody response is directed toward the variable regions [205, 212] and can, similarly to early autologous neutralizing Abs, protect against homologous strains of virus [256-257]. However, so far only partial protection against heterologous strains has been observed (Paper III and [223]). To evaluate the protective capacity of the Env-specific Abs elicited in this study we first determined the neutralizing antibody titers *in vitro* against the CCR5 tropic SHIV-SF162P4. We observed a moderate ID_{50} neutralization titer of ~200 against both the challenge virus and a cloned pseudovirus generated from the SHIV stock. When performing repeated low/medium dose rectal SHIV-SF162P4 challenge a trend toward delayed kinetics of infection was observed in Env-immunized animals compared to control animals, but this was not statistically significant. To investigate the basis for the limited protection, we determined the levels of Env-specific Abs at the virus portal of entry. We quantified the levels of Env-specific IgG and IgA in rectal and vaginal washes and analyzed these levels in relation to the circulating Abs in blood in each animal (Figure 3).

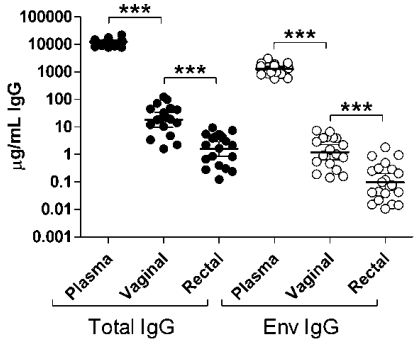

Figure 3. Total IgG and Env-specific IgG antibody responses in plasma and in mucosal washes (n=21-24 depending on successful sampling). Statistics was evaluated with ANOVA on log transformed values.

Although there was a correlation in titers for each animal, indicating equilibrium between the anatomical compartments, the levels of Abs in the vaginal wash were 1000-fold lower and in the rectal wash 10 000-fold lower than measured in circulation. This raises the possibility that the neutralizing antibody titers, although high in blood, were too low at the mucosal surfaces for efficient protection. These results highlight the importance of inducing Abs possessing specificities with enhanced neutralizing activity so that lower effective titers are needed. It also illustrates the need for adjuvants that either increase systemic neutralizing antibody titers or direct the Abs toward mucosal surfaces.

Paper II

Influence of novel CD4 binding-defective HIV-1 envelope glycoprotein immunogens on neutralizing antibody and T-cell responses in non-human primates

HIV-1 Env is the only virally encoded protein on the virion surface. This makes it the only viral target available for a neutralizing antibody-based vaccine against HIV-1. Upon interaction with the primary receptor, CD4, Env undergoes a conformational change that leads to the exposure of the conserved co-receptor binding site (CoRbs). The CoRbs interacts with either CCR5 or CXCR4 on host cells, which leads to further conformational changes and subsequent fusion of the virus and host cell membranes and infection. In chronically infected hosts, there are several forms of Env in addition to the functional Env spikes present on infectious virions. For example, free gp120 is released into the blood and cell surface-bound full-length Env is expressed on infected cells. Previous *in vitro* work has suggested that Env interactions with CD4 may lead to downstream signaling events in T cells, which could influence the pathogenic outcome of the infection [100-101, 258-260]. However, the consequences of Env-CD4 *in vivo* interactions during subunit Env immunization were not previously addressed.

To address the effect of Env-CD4 binding in a vaccine setting we designed two trimer mutants, referred to as 368 and 423/425/431. Both mutants were deficient in CD4 binding, but by distinct mechanisms (See Paper II). We evaluated the antigenicity and immunogenicity of the trimer variants in rhesus macaques possessing CD4 molecules with high affinity for Env. To determine if the antigens were equally immunogenic we measured the antigen-specific binding antibody titers. We also investigated the functionality and responsiveness of T cells

and memory B cells. As the mutants and Wt Envs were equally immunogenic and we did not detect any detrimental effect on Env-specific T cell responses we could conclude that immunization with trimeric CD4 binding-competent Env, at the concentrations used in our studies, will not in a detrimental manner affect the vaccine-induced T cell and B cell response. However, only peak responses after immunization were measured and future studies are therefore needed to determine if Env-CD4 *in vivo* interactions would affect the long term response to immunization.

Furthermore, a central aim in these studies was to investigate the neutralizing capacity of the Env-specific Abs against a selected panel of pseudoviruses. A marked difference in the elicited Abs was the reactivity toward the CoRbs. Earlier studies by our group had shown that the presence of high affinity CD4 was necessary for the induction of Abs toward the CoRbs upon Env immunization [261]. These results were confirmed in Paper II as we only observed CoRbs-directed Abs in the Wt trimer-immunized animals and not in the CD4 binding-defective trimer immunized animals (Figure 1). These data was also confirmed by solid-phase absorptions.

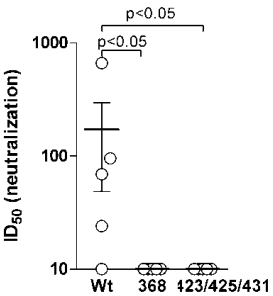

Figure 1. Co-receptor binding site directed antibody levels as determined with the HIV-2 neutralization assay [108] (n=5 per group). Statistics was evaluated with the Kruskal-Wallis test.

The 423/431/425 trimer mutant was designed to retain the capacity to bind a panel of CD4 binding site (CD4bs)-directed Abs and to interact with CD4 via the gp120 outer domains, but it was unable to undergo the conformational change necessary to fix CD4 in a stable complex; with this the overall affinity to CD4 was very low. In contrast, the 368 trimer variant harbored a substitution mutation in the center of the CD4bs, which rendered this molecule completely CD4 binding-deficient. As we found a marked difference between the groups in terms of their neutralization of the clade B, tier I virus HxBc2 (Figure 2), with no neutralization in the 368 group, our results indicated that the HxBc2 virus is primarily sensitive to Abs directed against the CD4bs. This was confirmed using solid-phase absorptions where reactivity toward the CD4bs was only found in the Wt and 423/425/431 trimer groups.

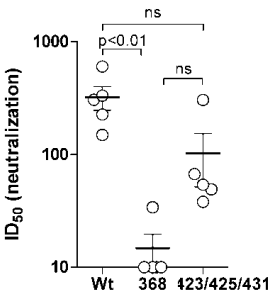

Figure 2. Pseudovirus neutralization of HxBc2. (n=5 per group). Statistics was evaluated with the Kruskal-Wallis test.

CoRbs-directed Abs are elicited abundantly in HIV-1 infected individuals [73, 108]. However, they appear before the detection of autologous neutralization and have been shown to be non-neutralizing in *in vitro* assays, likely due to the CoRbs being sterically restricted after CD4 binding [109, 262]. As such the CoRbs may act as a decoy for the immune system, leading to induction of non-neutralizing Abs instead of other, possibly more effective specificities. The 423/425/431 Env trimers are therefore of interest as a future immunogen as

they contain an intact and unmasked CD4 binding site and they do not elicit CoRbs-directed Abs due to their inability to strongly bind CD4.

Paper III

Immunization with wildtype CD4 binding-defective HIV-1 Env trimers comparably reduces viremia following heterologous SHIV challenge

In Paper II, we show that Wt and two CD4 binding-defective Env trimer immunogens elicit similar levels of antigen-specific B and T cell responses. However, due to the inability of the mutant Envs to strongly bind CD4 they cannot expose the CoRbs for induction of Abs toward this surface. The role of CoRbs Abs in control of viremia and protection is controversial. The CoRbs is a conserved and immunogenic surface although Abs directed toward this site are non-neutralizing in *in vitro* assays and this class of Abs are detected in HIV-1 infected individuals before the appearance of autologous neutralization [108, 262-263]. However, CoRbs-directed Abs have been found in patient sera exhibiting broadly neutralizing reactivities [78, 251, 264] and have been suggested to play a role in protection of rhesus macaques upon SHIV challenge [265].

As immunization with Wt or CD4 binding-defective Env trimers resulted in the clear presence or absence of CoRbs-directed Abs, we extended these studies to address the role of CoRbs-directed Abs in protection of rhesus macaques against intravenous challenge with SHIV-SF162P4. SHIV-SF162P4 is related to SHIV-SF162P3, the challenge virus used in the study were CoRbs-directed Abs were suggested to contribute to protection [265]. The Wt Env trimers have been shown to induce high amounts of V3-directed Abs [234] mediating neutralization of the parental SF162 virus. To determine if there was a difference in elicitation of V3-directed Abs in the animals immunized with the three trimer variants we quantified the levels of V3-binding Abs in all animals and no difference was observed.

When challenging the macaques we observed a statistically significant reduction in viremia in all immunized groups of animals compared to the unimmunized group (Figure 1). Monkey F77 in the 423/425/431 group was completely protected. However, we could not detect any difference between the groups when comparing cumulative or peak viral loads, suggesting that the Env-CD4 interaction or the CoRbs-directed Abs elicited in the Wt-immunized animals did not affect the outcome of the SHIV-SF162P4 challenge.

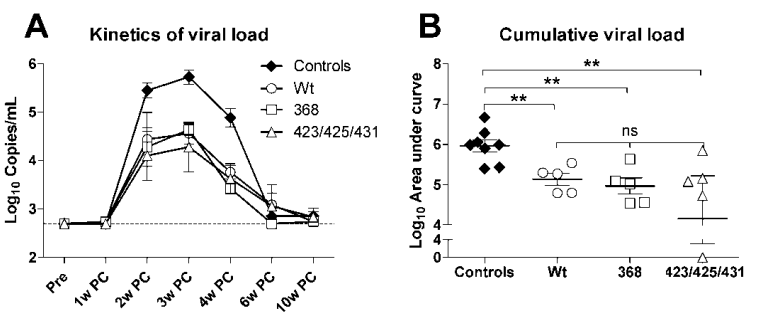

Figure 1. Viral load kinetics following challenge with SHIV-SF162P4 (A). Reduction in cumulative viral load in immunized groups compared to controls (B) (n=5-8) Shown is mean ±SEM of log transformed values. Statistics was evaluated with the Mann-Whitney test.

CONCLUSIONS AND FUTURE DIRECTIONS

In Paper I we thoroughly analyzed the B cell responses in non-human primates following immunization with purified soluble HIV-1 Env trimers in adjuvant. We could determine that peripheral plasma and memory B cell kinetics and levels were similar to what has been observed for the influenza and rabies vaccines in humans. This together with high titers of antigen-specific Abs indicates that Env is not an intrinsically poor antigen when administered in adjuvant. Furthermore we performed a benchmark neutralization comparison between sera elicited from the current state-of-the-art trimers in non-human primates compared to gp120 monomers used in the VAX04 clinical trial and show that the trimer-induced responses was superior over that elicited by the gp120 monomers. We also determined the protective role of the Env trimer-elicited Abs in a heterologous SHIV rectal challenge model and only a modest effect was observed. This was most likely due to the insufficient titers of neutralizing Env-specific Abs at the mucosal surfaces.

It has previously been shown *in vitro* that interactions between Env and CD4 can suppress T cell activity. In Paper II we evaluated the *in vivo* effect of Env-CD4 interactions following protein immunizations. We generated two trimeric Env mutants that were deficient in CD4 binding by distinct mechanisms and compared the B cell and T cell responses to those elicited by Wt Env trimers following immunization of non-human primates. There was no significant difference in the magnitude of Env-specific Abs, or functionality of memory B cells or T cells, indicating that there was no detrimental *in vivo* effect of Env-CD4 interaction from protein immunizations. However, this study only investigated acute responses following immunization and it will be important to follow up with longitudinal studies using CD4 binding-competent and deficient Env immunogens.

In Paper III we evaluated the effect of Env-CD4 interaction on protection against intravenous SHIV challenge. A clear difference in the induction of Abs directed toward the conserved co-receptor binding site (CoRbs) on Env was observed between animals immunized with CD4 binding-competent Env trimers and CD4 binding-defective trimers. CoRbs-directed Abs were previously suggested to contribute to protection of macaques challenged with SHIV. We observed a significant reduction of viremia in all animals after challenge with no difference between the groups, indicating that the presence or absence of CoRbs-directed Abs at the titers elicited in our study did not affect the protective responses against intravenous challenge with SHIV-SF162P4.

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REFERENCES

1. Nath, B.M., K.E. Schumann, and J.D. Boyer, *The chimpanzee and other non-human-primate models in HIV-1 vaccine research.* Trends Microbiol, 2000. **8**(9): p. 426-31.
2. Gottlieb, M.S., et al., *Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency.* N Engl J Med, 1981. **305**(24): p. 1425-31.
3. Gallo, R.C., et al., *Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 865-7.
4. Barre-Sinoussi, F., et al., *Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 868-71.
5. Gallo, R.C., et al., *Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS.* Science, 1984. **224**(4648): p. 500-3.
6. Popovic, M., et al., *Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS.* Science, 1984. **224**(4648): p. 497-500.
7. Sarngadharan, M.G., et al., *Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS.* Science, 1984. **224**(4648): p. 506-8.
8. Schupbach, J., et al., *Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS.* Science, 1984. **224**(4648): p. 503-5.
9. Coffin, J., et al., *Human immunodeficiency viruses.* Science, 1986. **232**(4751): p. 697.
10. Clavel, F., et al., *Isolation of a new human retrovirus from West African patients with AIDS.* Science, 1986. **233**(4761): p. 343-6.
11. Requejo, H.I., *Worldwide molecular epidemiology of HIV.* Rev Saude Publica, 2006. **40**(2): p. 331-45.
12. Duvall, M.G., et al., *Maintenance of HIV-specific CD4+ T cell help distinguishes HIV-2 from HIV-1 infection.* J Immunol, 2006. **176**(11): p. 6973-81.
13. Kandathil, A.J., et al., *Molecular epidemiology of HIV.* Indian J Med Res, 2005. **121**(4): p. 333-44.
14. Korber, B., et al., *Timing the ancestor of the HIV-1 pandemic strains.* Science, 2000. **288**(5472): p. 1789-96.
15. Heeney, J.L., A.G. Dalgleish, and R.A. Weiss, *Origins of HIV and the evolution of resistance to AIDS.* Science, 2006. **313**(5786): p. 462-6.
16. Keele, B.F., et al., *Chimpanzee reservoirs of pandemic and nonpandemic HIV-1.* Science, 2006. **313**(5786): p. 523-6.
17. Gao, F., et al., *Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes.* Nature, 1999. **397**(6718): p. 436-41.
18. Frankel, A.D. and J.A. Young, *HIV-1: fifteen proteins and an RNA.* Annu Rev Biochem, 1998. **67**: p. 1-25.
19. Vaishnav, Y.N. and F. Wong-Staal, *The biochemistry of AIDS.* Annu Rev Biochem, 1991. **60**: p. 577-630.
20. Dalgleish, A.G., et al., *The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus.* Nature, 1984. **312**(5996): p. 763-7.
21. Deng, H., et al., *Identification of a major co-receptor for primary isolates of HIV-1.* Nature, 1996. **381**(6584): p. 661-6.
22. Dragic, T., et al., *HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5.* Nature, 1996. **381**(6584): p. 667-73.

23. Wu, L., et al., *CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5.* Nature, 1996. **384**(6605): p. 179-83.
24. Mosier, D.E., *How HIV changes its tropism: evolution and adaptation?* Curr Opin HIV AIDS, 2009. **4**(2): p. 125-30.
25. Hare, S., et al., *Retroviral intasome assembly and inhibition of DNA strand transfer.* Nature, 2010. **464**(7286): p. 232-6.
26. Tang, H., K.L. Kuhen, and F. Wong-Staal, *Lentivirus replication and regulation.* Annu Rev Genet, 1999. **33**: p. 133-70.
27. Chomont, N., et al., *HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation.* Nat Med, 2009. **15**(8): p. 893-900.
28. North, T.W., et al., *Viral Sanctuaries during Highly Active Antiretroviral Therapy in a Nonhuman Primate Model for Aids.* J Virol, 2009.
29. Dinoso, J.B., et al., *Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy.* Proc Natl Acad Sci U S A, 2009. **106**(23): p. 9403-8.
30. Boily, M.C., et al., *Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies.* Lancet Infect Dis, 2009. **9**(2): p. 118-29.
31. Gupta, R., T. Warren, and A. Wald, *Genital herpes.* Lancet, 2007. **370**(9605): p. 2127-37.
32. Russell, D.B., *Herpes and HIV infection--has the time come to act?* Sex Health, 2006. **3**(2): p. 67-71.
33. Salazar-Gonzalez, J.F., et al., *Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing.* J Virol, 2008. **82**(8): p. 3952-70.
34. Keele, B.F., et al., *Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection.* Proc Natl Acad Sci U S A, 2008. **105**(21): p. 7552-7.
35. Mattapallil, J.J., et al., *Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection.* Nature, 2005. **434**(7037): p. 1093-7.
36. Brenchley, J.M., et al., *CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract.* J Exp Med, 2004. **200**(6): p. 749-59.
37. Veazey, R.S., P.A. Marx, and A.A. Lackner, *Vaginal CD4+ T cells express high levels of CCR5 and are rapidly depleted in simian immunodeficiency virus infection.* J Infect Dis, 2003. **187**(5): p. 769-76.
38. Veazey, R.S., et al., *Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection.* Science, 1998. **280**(5362): p. 427-31.
39. Li, Q., et al., *Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells.* Nature, 2005. **434**(7037): p. 1148-52.
40. Guadalupe, M., et al., *Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy.* J Virol, 2003. **77**(21): p. 11708-17.
41. Mehandru, S., et al., *Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection.* PLoS Med, 2006. **3**(12): p. e484.
42. Koup, R.A., et al., *Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome.* J Virol, 1994. **68**(7): p. 4650-5.

43. Bernardin, F., et al., *Human immunodeficiency virus mutations during the first month of infection are preferentially found in known cytotoxic T-lymphocyte epitopes.* J Virol, 2005. **79**(17): p. 11523-8.
44. Mellors, J.W., et al., *Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.* Science, 1996. **272**(5265): p. 1167-70.
45. Johnson, R.P., R.F. Siliciano, and M.J. McElrath, *Cellular immune responses to HIV-1.* AIDS, 1998. **12 Suppl A**: p. S113-20.
46. Koyama, S., et al., *Innate immune response to viral infection.* Cytokine, 2008. **43**(3): p. 336-41.
47. Janeway, C.A., Jr. and R. Medzhitov, *Innate immune recognition.* Annu Rev Immunol, 2002. **20**: p. 197-216.
48. Akira, S., S. Uematsu, and O. Takeuchi, *Pathogen recognition and innate immunity.* Cell, 2006. **124**(4): p. 783-801.
49. Medzhitov, R., *Recognition of microorganisms and activation of the immune response.* Nature, 2007. **449**(7164): p. 819-26.
50. Beutler, B.A., *TLRs and innate immunity.* Blood, 2009. **113**(7): p. 1399-407.
51. McMichael, A.J., et al., *The immune response during acute HIV-1 infection: clues for vaccine development.* Nat Rev Immunol, 2010. **10**(1): p. 11-23.
52. Letvin, N.L., et al., *No evidence for consistent virus-specific immunity in simian immunodeficiency virus-exposed, uninfected rhesus monkeys.* J Virol, 2007. **81**(22): p. 12368-74.
53. Alter, G., et al., *Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes.* J Exp Med, 2007. **204**(12): p. 3027-36.
54. Alter, G., et al., *Evolution of innate and adaptive effector cell functions during acute HIV-1 infection.* J Infect Dis, 2007. **195**(10): p. 1452-60.
55. Alter, G. and M. Altfeld, *NK cell function in HIV-1 infection.* Curr Mol Med, 2006. **6**(6): p. 621-9.
56. Wilson, J.D., et al., *Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection.* AIDS, 2000. **14**(3): p. 225-33.
57. Karlsson, A.C., et al., *Sequential broadening of CTL responses in early HIV-1 infection is associated with viral escape.* PLoS One, 2007. **2**(2): p. e225.
58. Allen, T.M., et al., *Selective escape from CD8+ T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution.* J Virol, 2005. **79**(21): p. 13239-49.
59. Salazar-Gonzalez, J.F., et al., *Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection.* J Exp Med, 2009. **206**(6): p. 1273-89.
60. Phillips, R.E., et al., *Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition.* Nature, 1991. **354**(6353): p. 453-9.
61. Goonetilleke, N., et al., *The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection.* J Exp Med, 2009. **206**(6): p. 1253-72.
62. Duda, A., et al., *HLA-associated clinical progression correlates with epitope reversion rates in early human immunodeficiency virus infection.* J Virol, 2009. **83**(3): p. 1228-39.
63. Kawashima, Y., et al., *Adaptation of HIV-1 to human leukocyte antigen class I.* Nature, 2009. **458**(7238): p. 641-5.

64. Wang, Y.E., et al., *Protective HLA class I alleles that restrict acute-phase CD8+ T-cell responses are associated with viral escape mutations located in highly conserved regions of human immunodeficiency virus type 1.* J Virol, 2009. **83**(4): p. 1845-55.
65. Martinez-Picado, J., et al., *Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1.* J Virol, 2006. **80**(7): p. 3617-23.
66. Addo, M.M., et al., *Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load.* J Virol, 2003. **77**(3): p. 2081-92.
67. Altfeld, M., et al., *HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1.* PLoS Med, 2006. **3**(10): p. e403.
68. Carrington, M. and S.J. O'Brien, *The influence of HLA genotype on AIDS.* Annu Rev Med, 2003. **54**: p. 535-51.
69. Tomaras, G.D., et al., *Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia.* J Virol, 2008. **82**(24): p. 12449-63.
70. Aasa-Chapman, M.M., et al., *Development of the antibody response in acute HIV-1 infection.* AIDS, 2004. **18**(3): p. 371-81.
71. Wei, X., et al., *Antibody neutralization and escape by HIV-1.* Nature, 2003. **422**(6929): p. 307-12.
72. Richman, D.D., et al., *Rapid evolution of the neutralizing antibody response to HIV type 1 infection.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4144-9.
73. Gray, E.S., et al., *Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection.* J Virol, 2007. **81**(12): p. 6187-96.
74. Frost, S.D., et al., *Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection.* Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18514-9.
75. Cheng-Mayer, C., et al., *Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization.* Proc Natl Acad Sci U S A, 1988. **85**(8): p. 2815-9.
76. Profy, A.T., et al., *Epitopes recognized by the neutralizing antibodies of an HIV-1-infected individual.* J Immunol, 1990. **144**(12): p. 4641-7.
77. Sather, D.N., et al., *Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection.* J Virol, 2009. **83**(2): p. 757-69.
78. Li, Y., et al., *Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals.* J Virol, 2009. **83**(2): p. 1045-59.
79. Doria-Rose, N.A., et al., *Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies.* J Virol, 2009. **83**(1): p. 188-99.
80. Binley, J.M., et al., *Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C.* J Virol, 2008. **82**(23): p. 11651-68.
81. Simek, M.D., et al., *Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-*

throughput neutralization assay together with an analytical selection algorithm. J Virol, 2009. **83**(14): p. 7337-48.
82. Nara, P.L. and R. Garrity, *Deceptive imprinting: a cosmopolitan strategy for complicating vaccination.* Vaccine, 1998. **16**(19): p. 1780-7.
83. Tobin, G.J., et al., *Deceptive imprinting and immune refocusing in vaccine design.* Vaccine, 2008. **26**(49): p. 6189-99.
84. Laird, M.E., et al., *Importance of the V1/V2 loop region of simian-human immunodeficiency virus envelope glycoprotein gp120 in determining the strain specificity of the neutralizing antibody response.* J Virol, 2008. **82**(22): p. 11054-65.
85. Stamatatos, L., et al., *Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine?* Nat Med, 2009. **15**(8): p. 866-70.
86. Gorny, M.K., et al., *Human monoclonal antibodies specific for conformation-sensitive epitopes of V3 neutralize human immunodeficiency virus type 1 primary isolates from various clades.* J Virol, 2002. **76**(18): p. 9035-45.
87. Corti, D., et al., *Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals.* PLoS One, 2010. **5**(1): p. e8805.
88. Nandi, A., et al., *Epitopes for broad and potent neutralizing antibody responses during chronic infection with human immunodeficiency virus type 1.* Virology, 2010. **396**(2): p. 339-48.
89. Zolla-Pazner, S., et al., *Cross-clade neutralizing antibodies against HIV-1 induced in rabbits by focusing the immune response on a neutralizing epitope.* Virology, 2009. **392**(1): p. 82-93.
90. Haynes, B.F., et al., *Analysis of HIV-1 subtype B third variable region peptide motifs for induction of neutralizing antibodies against HIV-1 primary isolates.* Virology, 2006. **345**(1): p. 44-55.
91. Burton, D.R., R.L. Stanfield, and I.A. Wilson, *Antibody vs. HIV in a clash of evolutionary titans.* Proc Natl Acad Sci U S A, 2005. **102**(42): p. 14943-8.
92. Koch, M., et al., *Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition.* Virology, 2003. **313**(2): p. 387-400.
93. Sagar, M., et al., *Diversity in HIV-1 envelope V1-V3 sequences early in infection reflects sequence diversity throughout the HIV-1 genome but does not predict the extent of sequence diversity during chronic infection.* AIDS Res Hum Retroviruses, 2006. **22**(5): p. 430-7.
94. Sagar, M., et al., *Human immunodeficiency virus type 1 V1-V2 envelope loop sequences expand and add glycosylation sites over the course of infection, and these modifications affect antibody neutralization sensitivity.* J Virol, 2006. **80**(19): p. 9586-98.
95. Rong, R., et al., *Escape from autologous neutralizing antibodies in acute/early subtype C HIV-1 infection requires multiple pathways.* PLoS Pathog, 2009. **5**(9): p. e1000594.
96. Kwong, P.D., et al., *HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites.* Nature, 2002. **420**(6916): p. 678-82.
97. Chen, L., et al., *Structural basis of immune evasion at the site of CD4 attachment on HIV-1 gp120.* Science, 2009. **326**(5956): p. 1123-7.
98. Moore, P.L., et al., *Nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1.* J Virol, 2006. **80**(5): p. 2515-28.

99. Stevceva, L., et al., *Immune responses to HIV Gp120 that facilitate viral escape.* Curr HIV Res, 2007. **5**(1): p. 47-54.
100. Holm, G.H. and D. Gabuzda, *Distinct mechanisms of CD4+ and CD8+ T-cell activation and bystander apoptosis induced by human immunodeficiency virus type 1 virions.* J Virol, 2005. **79**(10): p. 6299-311.
101. Vlahakis, S.R., et al., *Chemokine-receptor activation by env determines the mechanism of death in HIV-infected and uninfected T lymphocytes.* J Clin Invest, 2001. **107**(2): p. 207-15.
102. Hu, H., et al., *HIV envelope suppresses CD4+ T cell activation independent of T regulatory cells.* J Immunol, 2008. **180**(8): p. 5593-600.
103. Muster, T., et al., *A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1.* J Virol, 1993. **67**(11): p. 6642-7.
104. Stiegler, G., et al., *A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1.* AIDS Res Hum Retroviruses, 2001. **17**(18): p. 1757-65.
105. Burton, D.R., et al., *Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody.* Science, 1994. **266**(5187): p. 1024-7.
106. Trkola, A., et al., *Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1.* J Virol, 1996. **70**(2): p. 1100-8.
107. Walker, L.M., et al., *Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target.* Science, 2009. **326**(5950): p. 285-9.
108. Decker, J.M., et al., *Antigenic conservation and immunogenicity of the HIV coreceptor binding site.* J Exp Med, 2005. **201**(9): p. 1407-19.
109. Labrijn, A.F., et al., *Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1.* J Virol, 2003. **77**(19): p. 10557-65.
110. Xiang, S.H., et al., *Characterization of CD4-induced epitopes on the HIV type 1 gp120 envelope glycoprotein recognized by neutralizing human monoclonal antibodies.* AIDS Res Hum Retroviruses, 2002. **18**(16): p. 1207-17.
111. Saphire, E.O., et al., *Crystal structure of a neutralizing human IGG against HIV-1: a template for vaccine design.* Science, 2001. **293**(5532): p. 1155-9.
112. Haynes, B.F., et al., *Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies.* Science, 2005. **308**(5730): p. 1906-8.
113. Verkoczy, L., et al., *Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance.* Proc Natl Acad Sci U S A, 2010. **107**(1): p. 181-6.
114. Scherer, E.M., et al., *Difficulties in eliciting broadly neutralizing anti-HIV antibodies are not explained by cardiolipin autoreactivity.* AIDS, 2007. **21**(16): p. 2131-9.
115. Huthoff, H. and G.J. Towers, *Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha.* Trends Microbiol, 2008. **16**(12): p. 612-9.
116. Boyer, J.D., et al., *Protection of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination.* Nat Med, 1997. **3**(5): p. 526-32.
117. Girard, M., et al., *Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus.* Proc Natl Acad Sci U S A, 1991. **88**(2): p. 542-6.
118. Fultz, P.N., et al., *Vaccine protection of chimpanzees against challenge with HIV-1-infected peripheral blood mononuclear cells.* Science, 1992. **256**(5064): p. 1687-90.
119. Novembre, F.J., et al., *Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1.* J Virol, 1997. **71**(5): p. 4086-91.

120. Prince, A.M. and L. Andrus, *AIDS vaccine trials in chimpanzees.* Science, 1998. **282**(5397): p. 2195-6.
121. Baroncelli, S., et al., *Macaca mulatta, fascicularis and nemestrina in AIDS vaccine development.* Expert Rev Vaccines, 2008. **7**(9): p. 1419-34.
122. Geretti, A.M., *Simian immunodeficiency virus as a model of human HIV disease.* Rev Med Virol, 1999. **9**(1): p. 57-67.
123. Letvin, N.L., et al., *Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys.* Science, 2006. **312**(5779): p. 1530-3.
124. Martins, M.A., et al., *T-cell correlates of vaccine efficacy after a heterologous SIV challenge.* J Virol, 2010.
125. Wang, H.B., et al., *Partial protection against SIV challenge by vaccination of adenovirus and MVA vectors in rhesus monkeys.* Gene Ther, 2010. **17**(1): p. 4-13.
126. Liu, J., et al., *Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys.* Nature, 2009. **457**(7225): p. 87-91.
127. Reynolds, M.R., et al., *Macaques vaccinated with live-attenuated SIV control replication of heterologous virus.* J Exp Med, 2008. **205**(11): p. 2537-50.
128. Shiver, J.W., et al., *Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity.* Nature, 2002. **415**(6869): p. 331-5.
129. Matano, T., et al., *Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial.* J Exp Med, 2004. **199**(12): p. 1709-18.
130. Mattapallil, J.J., et al., *Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge.* J Exp Med, 2006. **203**(6): p. 1533-41.
131. Keele, B.F., et al., *Low-dose rectal inoculation of rhesus macaques by SIVsmE660 or SIVmac251 recapitulates human mucosal infection by HIV-1.* J Exp Med, 2009. **206**(5): p. 1117-34.
132. Crawford, J.M., et al., *Characterization of primary isolate-like variants of simian-human immunodeficiency virus.* J Virol, 1999. **73**(12): p. 10199-207.
133. Etemad-Moghadam, B., et al., *Characterization of simian-human immunodeficiency virus envelope glycoprotein epitopes recognized by neutralizing antibodies from infected monkeys.* J Virol, 1998. **72**(10): p. 8437-45.
134. Reimann, K.A., et al., *A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys.* J Virol, 1996. **70**(10): p. 6922-8.
135. Shibata, R., et al., *Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing.* J Infect Dis, 1997. **176**(2): p. 362-73.
136. Karlsson, G.B., et al., *Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys.* J Virol, 1997. **71**(6): p. 4218-25.
137. Joag, S.V., et al., *Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4+ T cells and AIDS in pig-tailed macaques.* J Virol, 1996. **70**(5): p. 3189-97.
138. Li, J.T., et al., *Persistent infection of macaques with simian-human immunodeficiency viruses.* J Virol, 1995. **69**(11): p. 7061-7.
139. Harouse, J.M., et al., *Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs.* Science, 1999. **284**(5415): p. 816-9.
140. Amara, R.R., et al., *Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine.* Science, 2001. **292**(5514): p. 69-74.

141. Barouch, D.H., et al., *Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination.* Science, 2000. **290**(5491): p. 486-92.
142. Nehete, P.N., et al., *Protection against chronic infection and AIDS by an HIV envelope peptide-cocktail vaccine in a pathogenic SHIV-rhesus model.* Vaccine, 2001. **20**(5-6): p. 813-25.
143. Feinberg, M.B. and J.P. Moore, *AIDS vaccine models: challenging challenge viruses.* Nat Med, 2002. **8**(3): p. 207-10.
144. Luciw, P.A., et al., *Persistent infection of rhesus macaques with T-cell-line-tropic and macrophage-tropic clones of simian/human immunodeficiency viruses (SHIV).* Proc Natl Acad Sci U S A, 1995. **92**(16): p. 7490-4.
145. Song, R.J., et al., *Molecularly cloned SHIV-1157ipd3N4: a highly replication-competent, mucosally transmissible R5 simian-human immunodeficiency virus encoding HIV clade C Env.* J Virol, 2006. **80**(17): p. 8729-38.
146. Nishimura, Y., et al., *Generation of the Pathogenic R5-Tropic Shivad8 by Serial Passaging in Rhesus Macaques.* J Virol, 2010.
147. Pal, R., et al., *Characterization of a simian human immunodeficiency virus encoding the envelope gene from the CCR5-tropic HIV-1 Ba-L.* J Acquir Immune Defic Syndr, 2003. **33**(3): p. 300-7.
148. Tan, R.C., et al., *In vivo adaptation of SHIV(SF162): chimeric virus expressing a NSI, CCR5-specific envelope protein.* J Med Primatol, 1999. **28**(4-5): p. 164-8.
149. Ince, W.L., et al., *Evolution of the HIV-1 env gene in the Rag2-/- gammaC-/- humanized mouse model.* J Virol, 2010. **84**(6): p. 2740-52.
150. Plotkin, S.A., *Vaccines: correlates of vaccine-induced immunity.* Clin Infect Dis, 2008. **47**(3): p. 401-9.
151. Shibata, R., et al., *Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys.* Nat Med, 1999. **5**(2): p. 204-10.
152. Mascola, J.R., et al., *Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies.* J Virol, 1999. **73**(5): p. 4009-18.
153. Mascola, J.R., 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): p. 207-10.
154. Parren, P.W., et al., *Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro.* J Virol, 2001. **75**(17): p. 8340-7.
155. Ferrantelli, F., et al., *Complete protection of neonatal rhesus macaques against oral exposure to pathogenic simian-human immunodeficiency virus by human anti-HIV monoclonal antibodies.* J Infect Dis, 2004. **189**(12): p. 2167-73.
156. Hessell, A.J., et al., *Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers.* PLoS Pathog, 2009. **5**(5): p. e1000433.
157. Hessell, A.J., et al., *Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L.* J Virol, 2010. **84**(3): p. 1302-13.
158. Hessell, A.J., et al., *Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques.* Nat Med, 2009. **15**(8): p. 951-4.

159. Veazey, R.S., et al., *Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120.* Nat Med, 2003. **9**(3): p. 343-6.
160. Baba, T.W., et al., *Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection.* Nat Med, 2000. **6**(2): p. 200-6.
161. Nishimura, Y., et al., *Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies.* J Virol, 2002. **76**(5): p. 2123-30.
162. Flynn, N.M., et al., *Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection.* J Infect Dis, 2005. **191**(5): p. 654-65.
163. Pitisuttithum, P., et al., *Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand.* J Infect Dis, 2006. **194**(12): p. 1661-71.
164. Connor, R.I., et al., *Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines.* J Virol, 1998. **72**(2): p. 1552-76.
165. Yang, O.O., et al., *Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms.* J Virol, 1997. **71**(4): p. 3120-8.
166. Kaslow, R.A., et al., *Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection.* Nat Med, 1996. **2**(4): p. 405-11.
167. Carrington, M., et al., *HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage.* Science, 1999. **283**(5408): p. 1748-52.
168. Priddy, F.H., et al., *Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults.* Clin Infect Dis, 2008. **46**(11): p. 1769-81.
169. Thongcharoen, P., et al., *A phase 1/2 comparative vaccine trial of the safety and immunogenicity of a CRF01_AE (subtype E) candidate vaccine: ALVAC-HIV (vCP1521) prime with oligomeric gp160 (92TH023/LAI-DID) or bivalent gp120 (CM235/SF2) boost.* J Acquir Immune Defic Syndr, 2007. **46**(1): p. 48-55.
170. Rerks-Ngarm, S., et al., *Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand.* N Engl J Med, 2009. **361**(23): p. 2209-20.
171. Amanna, I. and M.K. Slifka, *Public fear of vaccination: separating fact from fiction.* Viral Immunol, 2005. **18**(2): p. 307-15.
172. Amanna, I.J., N.E. Carlson, and M.K. Slifka, *Duration of humoral immunity to common viral and vaccine antigens.* N Engl J Med, 2007. **357**(19): p. 1903-15.
173. Letvin, N.L., *Correlates of immune protection and the development of a human immunodeficiency virus vaccine.* Immunity, 2007. **27**(3): p. 366-9.
174. Koff, W.C., et al., *HIV vaccine design: insights from live attenuated SIV vaccines.* Nat Immunol, 2006. **7**(1): p. 19-23.
175. Johnson, R.P., *Mechanisms of protection against simian immunodeficiency virus infection.* Vaccine, 2002. **20**(15): p. 1985-7.
176. Kretzschmar, M., et al., *Frequency of adverse events after vaccination with different vaccinia strains.* PLoS Med, 2006. **3**(8): p. e272.
177. Lindsey, N.P., et al., *Adverse event reports following yellow fever vaccination.* Vaccine, 2008. **26**(48): p. 6077-82.
178. Alexander, L.N., et al., *Vaccine policy changes and epidemiology of poliomyelitis in the United States.* JAMA, 2004. **292**(14): p. 1696-701.

179. Whitney, J.B. and R.M. Ruprecht, *Live attenuated HIV vaccines: pitfalls and prospects.* Curr Opin Infect Dis, 2004. **17**(1): p. 17-26.
180. Harper, D.M., et al., *Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial.* Lancet, 2004. **364**(9447): p. 1757-65.
181. Joura, E.A., et al., *Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials.* Lancet, 2007. **369**(9574): p. 1693-702.
182. McAleer, W.J., et al., *Human hepatitis B vaccine from recombinant yeast.* Nature, 1984. **307**(5947): p. 178-80.
183. Lore, K. and G.B. Karlsson Hedestam, *Novel adjuvants for B cell immune responses.* Curr Opin HIV AIDS, 2009. **4**(5): p. 441-6.
184. Earl, P.L., R.W. Doms, and B. Moss, *Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein.* Proc Natl Acad Sci U S A, 1990. **87**(2): p. 648-52.
185. Thomas, D.J., et al., *gp160, the envelope glycoprotein of human immunodeficiency virus type 1, is a dimer of 125-kilodalton subunits stabilized through interactions between their gp41 domains.* J Virol, 1991. **65**(7): p. 3797-803.
186. Wyatt, R., et al., *The antigenic structure of the HIV gp120 envelope glycoprotein.* Nature, 1998. **393**(6686): p. 705-11.
187. Kwong, P.D., et al., *Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody.* Nature, 1998. **393**(6686): p. 648-59.
188. Huang, C.C., et al., *Structure of a V3-containing HIV-1 gp120 core.* Science, 2005. **310**(5750): p. 1025-8.
189. Ofek, G., et al., *Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope.* J Virol, 2004. **78**(19): p. 10724-37.
190. Liu, J., et al., *Molecular architecture of native HIV-1 gp120 trimers.* Nature, 2008. **455**(7209): p. 109-13.
191. Hu, S.L. and L. Stamatatos, *Prospects of HIV Env modification as an approach to HIV vaccine design.* Curr HIV Res, 2007. **5**(6): p. 507-13.
192. Pantophlet, R. and D.R. Burton, *GP120: target for neutralizing HIV-1 antibodies.* Annu Rev Immunol, 2006. **24**: p. 739-69.
193. Phogat, S., R.T. Wyatt, and G.B. Karlsson Hedestam, *Inhibition of HIV-1 entry by antibodies: potential viral and cellular targets.* J Intern Med, 2007. **262**(1): p. 26-43.
194. Phogat, S. and R. Wyatt, *Rational modifications of HIV-1 envelope glycoproteins for immunogen design.* Curr Pharm Des, 2007. **13**(2): p. 213-27.
195. Forsell, M.N., W.R. Schief, and R.T. Wyatt, *Immunogenicity of HIV-1 envelope glycoprotein oligomers.* Curr Opin HIV AIDS, 2009. **4**(5): p. 380-7.
196. Pancera, M., et al., *Soluble mimetics of human immunodeficiency virus type 1 viral spikes produced by replacement of the native trimerization domain with a heterologous trimerization motif: characterization and ligand binding analysis.* J Virol, 2005. **79**(15): p. 9954-69.
197. Dey, B., et al., *Characterization of human immunodeficiency virus type 1 monomeric and trimeric gp120 glycoproteins stabilized in the CD4-bound state: antigenicity, biophysics, and immunogenicity.* J Virol, 2007. **81**(11): p. 5579-93.

198. Yang, X., et al., *Highly stable trimers formed by human immunodeficiency virus type 1 envelope glycoproteins fused with the trimeric motif of T4 bacteriophage fibritin.* J Virol, 2002. **76**(9): p. 4634-42.
199. Binley, J.M., et al., *A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure.* J Virol, 2000. **74**(2): p. 627-43.
200. Sanders, R.W., et al., *Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1.* J Virol, 2002. **76**(17): p. 8875-89.
201. Nkolola, J.P., et al., *Breadth of Neutralizing Antibodies Elicited by Stable, Homogeneous Clade A and Clade C HIV-1 gp140 Envelope Trimers in Guinea Pigs.* J Virol, 2010.
202. Kim, M., et al., *Comparison of HIV Type 1 ADA gp120 monomers versus gp140 trimers as immunogens for the induction of neutralizing antibodies.* AIDS Res Hum Retroviruses, 2005. **21**(1): p. 58-67.
203. Yang, X., et al., *Characterization of the outer domain of the gp120 glycoprotein from human immunodeficiency virus type 1.* J Virol, 2004. **78**(23): p. 12975-86.
204. Earl, P.L., et al., *Immunogenicity and protective efficacy of oligomeric human immunodeficiency virus type 1 gp140.* J Virol, 2001. **75**(2): p. 645-53.
205. Li, Y., et al., *Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimeric and monomeric envelope glycoproteins in selected adjuvants.* J Virol, 2006. **80**(3): p. 1414-26.
206. Beddows, S., et al., *A comparative immunogenicity study in rabbits of disulfide-stabilized, proteolytically cleaved, soluble trimeric human immunodeficiency virus type 1 gp140, trimeric cleavage-defective gp140 and monomeric gp120.* Virology, 2007. **360**(2): p. 329-40.
207. Li, M., et al., *Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies.* J Virol, 2005. **79**(16): p. 10108-25.
208. Mascola, J.R., et al., *Human immunodeficiency virus type 1 neutralization measured by flow cytometric quantitation of single-round infection of primary human T cells.* J Virol, 2002. **76**(10): p. 4810-21.
209. Seaman, M.S., et al., *Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies.* J Virol, 2010. **84**(3): p. 1439-52.
210. Burton, D.R., et al., *HIV vaccine design and the neutralizing antibody problem.* Nat Immunol, 2004. **5**(3): p. 233-6.
211. Mascola, J.R. and D.C. Montefiori, *The role of antibodies in HIV vaccines.* Annu Rev Immunol, 2010. **28**: p. 413-44.
212. Derby, N.R., et al., *Antibody responses elicited in macaques immunized with human immunodeficiency virus type 1 (HIV-1) SF162-derived gp140 envelope immunogens: comparison with those elicited during homologous simian/human immunodeficiency virus SHIVSF162P4 and heterologous HIV-1 infection.* J Virol, 2006. **80**(17): p. 8745-62.
213. Ching, L.K., et al., *The first hypervariable region of the gp120 Env glycoprotein defines the neutralizing susceptibility of heterologous human immunodeficiency virus type 1 isolates to neutralizing antibodies elicited by the SF162gp140 immunogen.* J Virol, 2008. **82**(2): p. 949-56.

214. Pinter, A., *Roles of HIV-1 Env variable regions in viral neutralization and vaccine development.* Curr HIV Res, 2007. **5**(6): p. 542-53.
215. Humbert, M., et al., *Inducing cross-clade neutralizing antibodies against HIV-1 by immunofocusing.* PLoS One, 2008. **3**(12): p. e3937.
216. Wu, L., et al., *Cross-clade recognition and neutralization by the V3 region from clade C human immunodeficiency virus-1 envelope.* Vaccine, 2006. **24**(23): p. 4995-5002.
217. Wu, L., et al., *Enhanced exposure of the CD4-binding site to neutralizing antibodies by structural design of a membrane-anchored human immunodeficiency virus type 1 gp120 domain.* J Virol, 2009. **83**(10): p. 5077-86.
218. Bontjer, I., et al., *Optimization of human immunodeficiency virus type 1 envelope glycoproteins with V1/V2 deleted, using virus evolution.* J Virol, 2009. **83**(1): p. 368-83.
219. Barnett, S.W., et al., *The ability of an oligomeric human immunodeficiency virus type 1 (HIV-1) envelope antigen to elicit neutralizing antibodies against primary HIV-1 isolates is improved following partial deletion of the second hypervariable region.* J Virol, 2001. **75**(12): p. 5526-40.
220. Dey, B., et al., *Structure-based stabilization of HIV-1 gp120 enhances humoral immune responses to the induced co-receptor binding site.* PLoS Pathog, 2009. **5**(5): p. e1000445.
221. Young, K.R., et al., *Virus-like particles: designing an effective AIDS vaccine.* Methods, 2006. **40**(1): p. 98-117.
222. McBurney, S.P., K.R. Young, and T.M. Ross, *Membrane embedded HIV-1 envelope on the surface of a virus-like particle elicits broader immune responses than soluble envelopes.* Virology, 2007. **358**(2): p. 334-46.
223. Zhao, J., et al., *Preclinical studies of human immunodeficiency virus/AIDS vaccines: inverse correlation between avidity of anti-Env antibodies and peak postchallenge viremia.* J Virol, 2009. **83**(9): p. 4102-11.
224. Lai, L., et al., *GM-CSF DNA: an adjuvant for higher avidity IgG, rectal IgA, and increased protection against the acute phase of a SHIV-89.6P challenge by a DNA/MVA immunodeficiency virus vaccine.* Virology, 2007. **369**(1): p. 153-67.
225. Schnell, M.J., *Viral vectors as potential HIV-1 vaccines.* FEMS Microbiol Lett, 2001. **200**(2): p. 123-9.
226. Polo, J.M. and T.W. Dubensky, Jr., *Virus-based vectors for human vaccine applications.* Drug Discov Today, 2002. **7**(13): p. 719-27.
227. Liniger, M., A. Zuniga, and H.Y. Naim, *Use of viral vectors for the development of vaccines.* Expert Rev Vaccines, 2007. **6**(2): p. 255-66.
228. Barefoot, B., et al., *Comparison of multiple vaccine vectors in a single heterologous prime-boost trial.* Vaccine, 2008. **26**(48): p. 6108-18.
229. Sundback, M., et al., *Efficient expansion of HIV-1-specific T cell responses by homologous immunization with recombinant Semliki Forest virus particles.* Virology, 2005. **341**(2): p. 190-202.
230. Honda, M., et al., *Different vaccine vectors delivering the same antigen elicit CD8+ T cell responses with distinct clonotype and epitope specificity.* J Immunol, 2009. **183**(4): p. 2425-34.
231. Robinson, H.L., *New hope for an AIDS vaccine.* Nat Rev Immunol, 2002. **2**(4): p. 239-50.
232. Forsell, M.N., et al., *Biochemical and immunogenic characterization of soluble human immunodeficiency virus type 1 envelope glycoprotein trimers expressed by semliki forest virus.* J Virol, 2005. **79**(17): p. 10902-14.

233. Forsell, M.N., et al., *Increased human immunodeficiency virus type 1 Env expression and antibody induction using an enhanced alphavirus vector.* J Gen Virol, 2007. **88**(Pt 10): p. 2774-9.
234. Morner, A., et al., *Human immunodeficiency virus type 1 env trimer immunization of macaques and impact of priming with viral vector or stabilized core protein.* J Virol, 2009. **83**(2): p. 540-51.
235. Patterson, L.J., et al., *Replicating adenovirus HIV/SIV recombinant priming alone or in combination with a gp140 protein boost results in significant control of viremia following a SHIV89.6P challenge in Mamu-A*01 negative rhesus macaques.* Virology, 2008. **374**(2): p. 322-37.
236. Arrode-Bruses, G., et al., *Characterization of T-cell responses in macaques immunized with a single dose of HIV DNA vaccine.* J Virol, 2010. **84**(3): p. 1243-53.
237. Estcourt, M.J., A.J. McMichael, and T. Hanke, *DNA vaccines against human immunodeficiency virus type 1.* Immunol Rev, 2004. **199**: p. 144-55.
238. Giri, M., K.E. Ugen, and D.B. Weiner, *DNA vaccines against human immunodeficiency virus type 1 in the past decade.* Clin Microbiol Rev, 2004. **17**(2): p. 370-89.
239. Lu, S., *Combination DNA plus protein HIV vaccines.* Springer Semin Immunopathol, 2006. **28**(3): p. 255-65.
240. Amara, R.R. and H.L. Robinson, *A new generation of HIV vaccines.* Trends Mol Med, 2002. **8**(10): p. 489-95.
241. Yu, S., 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): p. 6124-31.
242. Dale, C.J., et al., *Prime-boost strategies in DNA vaccines.* Methods Mol Med, 2006. **127**: p. 171-97.
243. Lu, S., *Immunogenicity of DNA vaccines in humans: it takes two to tango.* Hum Vaccin, 2008. **4**(6): p. 449-52.
244. Fynan, E.F., et al., *DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations.* Proc Natl Acad Sci U S A, 1993. **90**(24): p. 11478-82.
245. Mumper, R.J. and Z. Cui, *Genetic immunization by jet injection of targeted pDNA-coated nanoparticles.* Methods, 2003. **31**(3): p. 255-62.
246. Widera, G., et al., *Increased DNA vaccine delivery and immunogenicity by electroporation in vivo.* J Immunol, 2000. **164**(9): p. 4635-40.
247. Suhrbier, A., *Multi-epitope DNA vaccines.* Immunol Cell Biol, 1997. **75**(4): p. 402-8.
248. McMichael, A., M. Mwau, and T. Hanke, *HIV T cell vaccines, the importance of clades.* Vaccine, 2002. **20**(15): p. 1918-21.
249. Li, Y., et al., *Broad HIV-1 neutralization mediated by CD4-binding site antibodies.* Nat Med, 2007. **13**(9): p. 1032-4.
250. Pietzsch, J., et al., *Anti-gp41 antibodies cloned from HIV-infected patients with broadly neutralizing serologic activity.* J Virol, 2010.
251. Scheid, J.F., et al., *Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals.* Nature, 2009. **458**(7238): p. 636-40.
252. Dosenovic, P., et al., *Selective expansion of HIV-1 envelope glycoprotein-specific B cell subsets recognizing distinct structural elements following immunization.* J Immunol, 2009. **183**(5): p. 3373-82.
253. Blanchard-Rohner, G., et al., *Appearance of peripheral blood plasma cells and memory B cells in a primary and secondary immune response in humans.* Blood, 2009. **114**(24): p. 4998-5002.

254. Wrammert, J., et al., *Rapid cloning of high-affinity human monoclonal antibodies against influenza virus*. Nature, 2008. **453**(7195): p. 667-71.
255. Douagi, I., et al., *Influence of Novel CD4 binding-defective HIV-1 Envelope Glycoprotein Immunogens on Neutralizing Antibody and T Cell Responses in Non-human Primates.* J Virol, 2009.
256. Barnett, S.W., et al., *Protection of macaques against vaginal SHIV challenge by systemic or mucosal and systemic vaccinations with HIV-envelope.* AIDS, 2008. **22**(3): p. 339-48.
257. Bogers, W.M., et al., *Systemic neutralizing antibodies induced by long interval mucosally primed systemically boosted immunization correlate with protection from mucosal SHIV challenge.* Virology, 2008. **382**(2): p. 217-25.
258. Fernando, K., et al., *Vaccine-delivered HIV envelope inhibits CD4(+) T-cell activation, a mechanism for poor HIV vaccine responses.* Blood, 2007. **109**(6): p. 2538-44.
259. Schwartz, O., et al., *Impairment of T cell receptor-dependent stimulation in CD4+ lymphocytes after contact with membrane-bound HIV-1 envelope glycoprotein.* Virology, 1994. **198**(1): p. 360-5.
260. Weinhold, K.J., et al., *HIV-1 GP120-mediated immune suppression and lymphocyte destruction in the absence of viral infection.* J Immunol, 1989. **142**(9): p. 3091-7.
261. Forsell, M.N., et al., *B cell recognition of the conserved HIV-1 co-receptor binding site is altered by endogenous primate CD4.* PLoS Pathog, 2008. **4**(10): p. e1000171.
262. Chen, W., et al., *Human domain antibodies to conserved sterically restricted regions on gp120 as exceptionally potent cross-reactive HIV-1 neutralizers.* Proc Natl Acad Sci U S A, 2008. **105**(44): p. 17121-6.
263. Robinson, J.E., et al., *High frequencies of antibody responses to CD4 induced epitopes in HIV infected patients started on HAART during acute infection.* Hum Antibodies, 2005. **14**(3-4): p. 115-21.
264. Gray, E.S., et al., *Antibody specificities associated with neutralization breadth in plasma from human immunodeficiency virus type 1 subtype C-infected blood donors.* J Virol, 2009. **83**(17): p. 8925-37.
265. DeVico, A., et al., *Antibodies to CD4-induced sites in HIV gp120 correlate with the control of SHIV challenge in macaques vaccinated with subunit immunogens.* Proc Natl Acad Sci U S A, 2007. **104**(44): p. 17477-82.