Part One
Immunology and Vaccination Strategies for AIDS and TB

AIDS and Tuberculosis: A Deadly Liaison. Edited by Stefan H.E. Kaufmann and Bruce D. Walker

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HIV Immunology and Prospects for Vaccines

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1.1
Introduction

As the HIV epidemic approaches its fourth decade, the world remains without a vaccine for a disease that has claimed more than 25 million lives, and currently infects over 33 million persons. The vast majority of these infections are in resource-scarce settings, and in most places the humanitarian crisis is enhanced because of overlap with the expanding global tuberculosis (TB) epidemic. The introduction of highly active antiretroviral therapy (HAART) in 1995–1996 resulted in a dramatic decrease in the mortality and morbidity of HIV infection in developed countries fortunate enough to have access to these life-extending medicines [1], and more recent expanded global access has resulted in more than three million persons receiving treatment in 2008. However, this still leaves an enormous gap in those who have advanced disease and are in desperate need of therapy, and in addition there are likely to be nearly 2.5 million new infections in 2009 (UNAIDS, http://www.unaids.org).

There is no doubt that the development of a safe and effective HIV-1 vaccine will be the best solution for the ultimate control of the worldwide AIDS pandemic [2], and this will likely also impact the TB epidemic. However, all attempts to achieve this have failed so far, reinforcing the fact that an AIDS vaccine is unlikely to be available in the near future [3]. As the TB and HIV epidemics intersect across the globe, the need for a vaccine to prevent the immunodeficiency induced by HIV that is accelerating expansion of the TB epidemic is even more acute [4]. In this chapter we will discuss the current challenges to the development of an effective AIDS vaccine, and address the progress made and persisting gaps in our quest for an effective method to prevent new infections.

1.2
Challenges for HIV Vaccine Design

The history of successful immunization dates back to the time of Jenner, whose success with a smallpox vaccine in 1796 was achieved with little understanding of the

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actual mechanisms of protection that were being induced. By mimicking infection with smallpox by inducing a benign cowpox infection, Jenner laid the foundation for modern vaccinology. Most vaccines currently in use, if not all, do not actually prevent infection, but rather attenuate disease caused by the pathogen. In fact, most mimic something that happens naturally – namely that some fraction of people who become infected clear their infections [5].

The situation with HIV is quite different as HIV is an infection in which, to our knowledge, spontaneous clearance never occurs. The natural history of HIV infection is one of progressive viremia, in which the targets of the virus are cells of the immune system itself, particularly CD4+ T-lymphocytes. Following infection, there is a gradual decline in CD4+ cell number and an increase in viral load, typically resulting in AIDS within 8–10 years, which is defined by a CD4+ cell count of less than 200 or specific AIDS-defining illnesses. HIV is actually an infection of the immune system, with CD4+ T-lymphocytes being a key target of the virus, which enters these cells through its coreceptors CCR5 (or occasionally other chemokine coreceptors such as CXCR4) and CD4.

There are five main properties of HIV that render the development of an HIV vaccine an unprecedented challenge.

1. **Massive infection of immune cells:** HIV uses its envelope protein to gain access to cells bearing its coreceptors, CD4 and the chemokine receptor CCR5 or CXCR4. The major target of the infection are CD4+ T-cells, and because activated cells are preferentially infected by HIV, the infection preferentially appears to deplete HIV-specific CD4+ cells. The infection of CD4+ cells is massive at the acute stage of infection, when up to 60% of CD4+ T-cells in the gut-associated lymphoid tissue (GALT) are depleted [6].

2. **Integration into the host chromosome:** HIV is a retrovirus, and following viral entry the viral reverse transcriptase initiates the production of a double-stranded proviral DNA that can remain as free circular DNA and undergo processes of transcription and translation to make new virion particles. Alternatively, it can use the viral integrase protein to create a nick in the host chromosome, and integrate. Once integration occurs – which all indications suggest happens very early after acute infection [7] – the virus can remain in an immunologically latent state. This is possible because the lack of transcription and translation of viral proteins means that the normal immune mechanisms, which rely on the detection of foreign viral protein within cells to induce immune attack, do not occur.

3. **Viral diversity:** HIV is a retrovirus, and viral replication is dependent on an error-prone viral reverse transcriptase that has a poor proofreading function. As a result, with each replication cycle there is likely to be at least one nucleotide mis-incorporation. At least some of this diversity is driven by immune selection pressure, which has been shown to be progressively deleting some key epitopes of the virus at a population level [8]. Globally there are three main groups of HIV – M, N, and O – with group M (the largest) being further divided into nine distinct clades and additional circulating recombinant forms. Viruses within a clade may

differ by up to 20% in the highly variable Env protein, which is the target for neutralizing antibodies, and by up to 38% between clades. Even within a single individual HIV mutates such that individuals carry unique strains. Developing a vaccine to target all of these viruses simultaneously is an enormous task.

4. **Envelope glycosylation:** The HIV envelope is heavily glycosylated, and also very flexible, in that it allows for a high degree of random mutations to be stably incorporated. This combination of Env variability, together with heavy glycosylation that renders key epitopes poorly exposed to antibody-mediated immune attack, has been a major challenge for any vaccine to provide broad cross-neutralizing protective antibody responses (for a review, see Ref. [5]). Indeed, at the current time this is such a challenge that many in the field have focused not on a preventive HIV vaccine – which would require the induction of broadly cross-reactive neutralizing antibodies – but rather on a T-cell-based vaccine which would be intended to provide a durable reduction in viral load, and thereby retard disease progression and reduce the likelihood of transmission to others [9].

5. **Immune evasion:** The HIV accessory protein Nef interacts indirectly with the cytoplasmic tail of HLA A and B alleles, leading to endocytosis and a down-regulation of class I expression on infected cells [10]. This impairs the ability of cytotoxic T lymphocytes to recognize infected cells, and has been shown to have functional significance on the ability to contain HIV replication [11]. Neutralizing antibodies are unable to recognize the variants that arise *in vivo* [7, 12, 13], so that the humoral immune response is always playing "catch-up." In addition, mutations arising within targeted CD8+ T-cell epitopes also lead to either a loss of recognition by the T-cell receptor (TCR) of established responses, or to a loss of binding of the epitope to HLA class I, allowing immune escape.

1.3
What Immune Responses will be Required for an Effective AIDS Vaccine?

A fully preventive HIV vaccine would almost certainly require the induction of broadly cross-reactive and highly potent neutralizing antibodies, which would have to prevent the infection of cells and the establishment of latent infection. There is widespread agreement that this is not likely to occur, for the reasons outlined below. Indeed, most – if not all – vaccines currently in use do not achieve this level of protection. This reality has directed the field toward vaccine strategies that would prevent disease progression rather than prevent infection – which, at least in theory, would cause the epidemic to contract – if the viral load could be kept low enough to limit both disease progression and transmission.

The challenges to this direction for vaccine development are compounded by the fact that we still lack an understanding of the correlates of immune protection, despite an intricate understanding of the molecular biology of the virus (Figure 1.1). Despite marked differences in disease outcome following infection, we lack a fundamental understanding of the mechanisms that account for these differences.

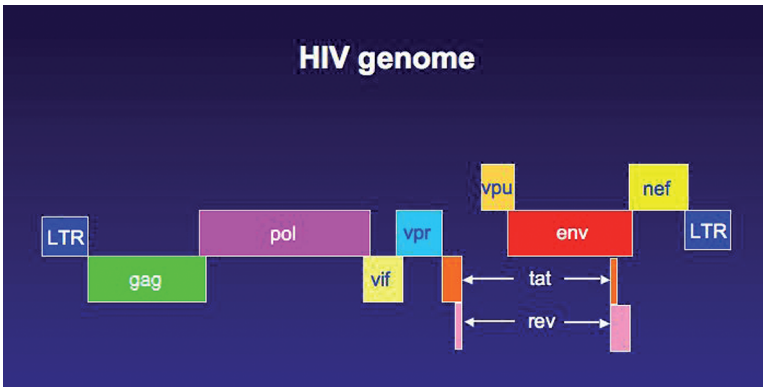

Figure 1.1 The HIV-1 genome. There are nine coding regions, in three different reading frames. *gag* is the main structural protein, *pol* encodes the replicative functions, and *env* encodes the heavily glycosylated outer envelope. The regulatory proteins include *vif*, *vpr*, *vpr*, *rev*, *tat*, and *nef*. LTR, long terminal repeat.

There is a growing body of data indicating that adaptive host immune responses play a role, but the key elements of protective immunity that would have to be induced by a vaccine are not known. What *is* known is that some persons are able to maintain successful control of HIV viremia for 30 years or more without therapy. This, in turn, provides some level of optimism that a vaccine might be able to result in a similar equilibrium with durable control of HIV, even if a totally preventive vaccine is not possible [14]. In contrast, others progress from acute infection to AIDS within six months [15]. Whilst the factors that account for these dramatic differences in outcome remain elusive, a growing body of data is beginning to shed light on the rational induction of specific arms of the immune response for HIV vaccine design (Figure 1.2).

1.3.1 Cytotoxic T Lymphocytes

Following acute HIV-1 infection, the resolution of acute-phase plasma viremia to a semi steady-state level, or set-point, coincides with the activation and expansion of HIV-1- specific cytotoxic T lymphocytes (CTL), suggesting that virus-specific CD8 + T-cells may be responsible for reducing the levels of virus at this stage of infection [16–18]. Direct evidence for the role of CD8 + T-cells in mediating the decline in viremia during acute HIV infection has come from studies of the simian immunodeficiency virus (SIV)-macaque model. Here, the administration of CD8-specific monoclonal antibodies s(MAbs) resulted in a transient depletion of CD8 + cells in both the peripheral blood and lymphoid tissues. When administered during primary chimeric simian/HIV infections, the CD8 MAb caused marked elevations of plasma and cell-associated virus levels in both the peripheral blood and lymphoid tissues, and led to a prolonged depletion of CD4 + cells. Eliminating CD8 + lymphocytes from monkeys during chronic SIV infection resulted in a rapid and marked increase in viremia that was again suppressed coincident with the reappearance of SIV-specific

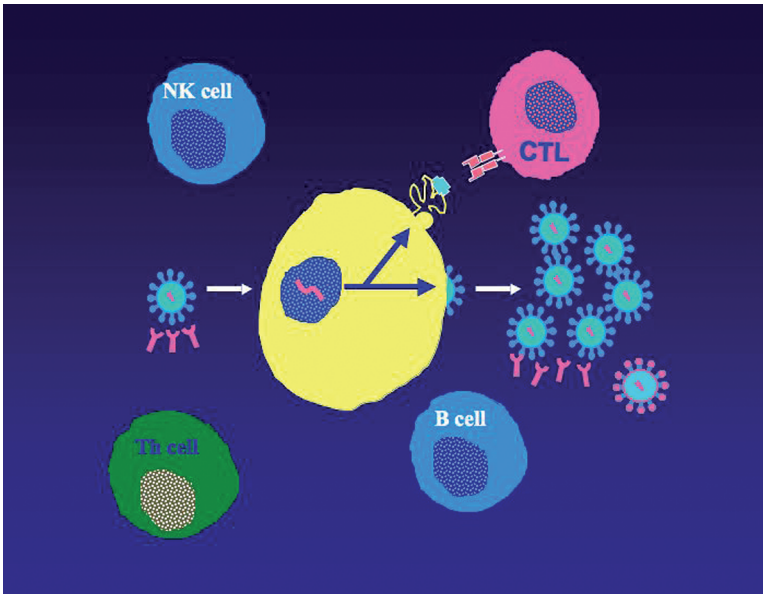

Figure 1.2 Immune responses to HIV. The B cells produce neutralizing antibodies, which are highly type-specific and poorly recognize diverse isolates, even those that arise within a single person due to reverse-transcriptase-induced errors in replication. The cytotoxic T cells (CTL) target virus-infected cells through recognition of viral proteins presented at the cell surface associated with HLA class I molecules, and deliver a lethal hit to the infected cell, ideally before progeny viruses are produced. Despite these responses, progression ensues in most persons. T helper (Th) cells, which express CD4 and CCR5, are the central orchestrator of effective cellular immunity, but are infected in large numbers in acute infection and never fully recover; they progressively decline over time until a CD4 count of 200 is reached, which defines AIDS. Natural killer (NK) cells target virus infected cells without requiring prior exposure; emerging data suggest that these may be important in HIV control.

CD8 + T-cells [19–21]. These results confirm the importance of cell-mediated immunity in controlling AIDS virus infection, and support the exploration of vaccination approaches for preventing infection that will elicit these immune responses.

An emerging body of data suggests that it is not just the magnitude but rather the specificity of the CTL response that may be critical for immune containment. Numerous population studies have determined that neither the total breadth nor the total magnitude of HIV-specific CD8 + T-cell responses correlate with the ability of an individual to control HIV-1 [22–24], which suggests that selected epitope-specific CD8 + T-cell responses play a relevant role. Large population studies conducted in South Africa have defined that a preferential targeting of Gag is associated with a lower viral load [25], while more recent data have indicated that the breath of the Gag-specific response is negatively correlated with the viral load in persons with chronic infection [26]. In contrast, broad Env-specific CD8 + T-cell responses are associated with a high viral load [26]. To some extent this may reflect differences in the quality of these responses, or in the relative efficacy of different responses to recognize and kill infected cells before progeny viruses are

produced [27]. The limited ability of these responses to provide durable containment may also be due to escape mutations emerging within targeted CD8+ T-cell epitopes, which arise during primary [28–31] and chronic [32, 33] HIV-1 and SIV infection, and demonstrates significant CD8+ T-cell pressure on these regions of the virus and impacts temporally on disease progression [33, 34]. In addition, functional impairment or exhaustion of these responses over time in the setting of chronic viral stimulation may play a role. The inhibitory receptor programmed death 1 (PD-1; also known as PDCD1), a negative regulator of activated T cells, is markedly upregulated on the surface of HIV-specific CD8+ T-cells, the expression correlating with impaired HIV-specific CD8+ T-cell function as well as with predictors of disease progression – positively with plasma viral load, and inversely with the CD4+ T-cell count [35]. In contrast, the inhibitory immunoregulatory receptor CTLA-4 is selectively upregulated in HIV-specific CD4+ T-cells, but not CD8+ T-cells, in all categories of HIV-infected subjects, except for a rare subset of individuals who are able to control viremia in the absence of antiretroviral therapy [36].

One of the strongest arguments in favor of a role for CTLs in the outcome of HIV infection is the association between certain HLA class I alleles and improved outcome [37]. Among these are the so-called protective alleles, the strongest of which include B*5701, B*5801, B51, and B*2705. These B alleles have in common that they are associated with strong immune responses to the Gag protein, and in some cases are associated with mutations that impair viral fitness [38]. Other HLA alleles, such as HLA B35, are associated with a worse outcome [39], although an understanding of the mechanism of this association remains obscure. One concern raised by these observations is that there may be genetic limitations to the efficacy of a particular vaccine candidate, in that it may be more immunogenic in certain HLA backgrounds, and may have limited immunogenicity in others. However, this concern remains unsubstantiated.

1.3.2 Neutralizing Antibodies

Following the identification of HIV as the causative agent of AIDS, it was predicted that a vaccine inducing neutralizing antibodies and thereby preventing infection would rapidly be available. Yet, a quarter of a century later an effective preventive HIV vaccine still eludes us. Neutralizing antibodies are induced by HIV, but fail to control viremia. Despite a pronounced antibody response to the viral envelope proteins, only a small fraction of these antibodies have neutralizing activity. This is partly due to the fact that the HIV-1 Env glycoprotein is a trimer on the virion surface with extensive N-linked glycosylation that effectively shields many conserved epitopes from antibody recognition [40]. Key conserved regions, such as the binding site of the chemokine coreceptor, are only formed after Env binds its cellular receptor CD4 and undergoes an extensive conformational change. The broadly reactive MAb b12 binds to the CD4-binding site, suggesting that this region of Env may represent a critical point of vulnerability that is potentially amenable to neutralization, although the CD4-binding site is recessed and only partially accessible to antibody binding. The membrane-

proximal external region (MPER) of gp41 is another conserved region, which represents the target of the broadly reactive MAbs 2F5 and 4E10 [41, 42]. However, MPER-specific neutralizing antibodies may be difficult to elicit by vaccination for multiple reasons, including tolerance control and immunoregulation, sequestration of the epitopes in the lipid membrane, exposure of the epitopes only transiently during viral entry, or possibly a combination of multiple factors.

HIV infection induces neutralizing antibodies directed against three major determinants: (i) the highly variable V3 loop; (ii) the CD4 binding domain; and (iii) the more conserved gp41 transmembrane protein. So far, most of the evidence [43, 44] suggests that these responses play only a minor role in immune containment in chronic infection as the antibody responses to autologous virus are typically weak. This applies also for persons who are able to control HIV infection without antiviral therapy [45, 46]. Furthermore, neutralization escape has been observed even in persons who persistently control viremia [47, 48]. The presumably minor role of antibodies in viral control is supported by a study in which B cells were depleted with anti-CD20 antibody in an acute infection primate model, and showed little impact on viral control. This intervention led to the delayed emergence of neutralizing antibodies and no change in early viral kinetics [49]. Despite the lack of protection, longitudinal studies of autologous neutralizing antibody responses indicate that the viral inhibitory capacity of these responses can be of sufficient magnitude to completely replace circulating neutralization-sensitive virus with successive populations of neutralization-resistant virus [12, 13]. It has even been shown that neutralizing antibody escape can exceed the rate of change observed with potent anti-HIV-1 drug selection pressure. Nevertheless, despite a gradual broadening of the neutralizing antibody response, it does not become sufficiently broad to neutralize the next population of virus to arise. Different means by which the virus evades antibody pressure have been proposed, including an evolving glycan shield and resultant steric hindrance [12]. Even so, these studies provide evidence that the neutralizing antibody responses are strong enough to drive immune escape, and also demonstrate how quickly immune escape from neutralizing antibodies can occur.

1.3.3 CD4 + T Helper Cells

One of the central immunological defects in most individuals with HIV-1 infection is a weak to absent HIV-1-specific CD4 + T-helper cell proliferative response [50], although when present, HIV-1-specific T- helper cell responses have been correlated with a decreased virus load [51]. Indeed, HIV appears to preferentially infect HIV-specific CD4 + T-cells [52]. It is likely that the mechanism behind this association between CD4 + help and disease outcome is due to the effect of these cells on CTL function. This has been well established in murine models of chronic viral infections, in which durable control by CTL is dependent upon the persistence of virus-specific T helper cells [53]. Several detailed studies have demonstrated that while the primary expansion of antiviral CD8 + T-cells can occur independently of CD4 + T-cell help, memory CD8 + T-cell numbers and secondary responses to bacterial

or viral challenge are decreased over time in CD4+ T-cell-deficient animal models [54, 55]. It has been shown that CD4+ help is particularly required for the long-term survival of memory CD8+ T-cells [56]. In the absence of CD4+ T-cells, memory CD8+ T-cells become functionally impaired and decrease in quantity over time.

1.3.4 Natural Killer Cells

Although natural killer (NK) cells have traditionally not been considered as a component of a vaccine approach, emerging data suggest that these cells may be critical. On the one hand, NK cells respond to Toll-like receptor (TLR) ligands and help to create the proper milieu for immune induction, whereas on the other hand, recent data suggest that at least some NK cell subsets can be endowed with memory properties, allowing for a more rapid expansion on subsequent encounters [57]. This recent discovery will no doubt influence future research directions in the HIV field.

1.4 Models of Successful Vaccination?

Because of challenges to the development of a fully preventive vaccine, which would require the induction of potent and broadly directed neutralizing antibodies, the field has in part focused on development of T-cell-based vaccines. These would be intended not to prevent infection, but rather to prevent disease progression when a person becomes infected, by limiting the production of progeny virions from infected cells (Figure 1.3). Enthusiasm for such an approach comes from the observation that a small fraction of persons who become HIV infected are able spontaneously to control HIV replication and maintain normal CD4+ cell counts without medications – some now for more than 30 years after the initial infection (for a review, see Ref. [14]). This group of persons has been termed "HIV controllers,"

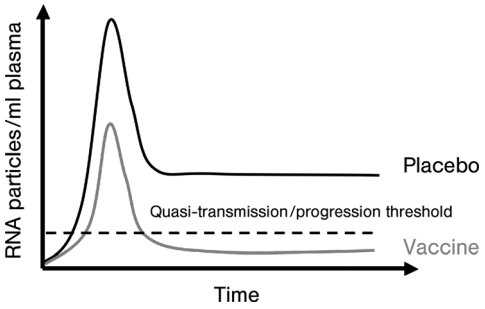

Figure 1.3 The theory behind T-cell vaccination. T-cell vaccines would be expected not to prevent infection, but rather to modulate the viral load after infection, reducing it to a level at which the likelihood of disease progression and transmission would be markedly reduced. This level is thought to be around 1000–2000 RNA copies per ml plasma.

and consists of both "elite controllers," who maintain plasma viremia less than 50 RNA copies ml^{-1}, as well as "viremic controllers" who maintain viral loads between 50 and 2000 copies, a level at which the likelihood of progression and of transmission are markedly reduced [14, 58]. Most studies suggest that durable HIV control occurs in less than 1% of infected individuals [59–61], and may be as low as one in 300. So far, no epidemiologic factors have been associated with complete or near-complete HIV control *in vivo*. Gender does not seem to determine the ability to contain the infection, as both male and female HIV controllers are defined [62]. Controllers have been identified within multiple ethnicities, infected with different virus subtypes, and via different routes of HIV acquisition [63]. This leads to the assumption that race, geographic location, and/or viral subtype independently are not impacting immunologic and virologic outcomes [63, 64].

Although the mechanisms by which elite controllers are able to contain viral replication are still being defined, there are emerging data which indicate that it is immunologically mediated. There is an overrepresentation of certain HLA class I alleles in these individuals, particularly HLA B57 and B27, and recent studies have shown that circulating CD8+ T-cells from these individuals are able to potently suppress viral replication in an *in vitro* assay [27, 65]. Most information is available for the subset of elite controllers who express HLA B*5701, in whom it has been shown that CTL responses select for mutations unique to those with elite control, which markedly impair viral fitness, while at the same time eliciting *de novo* CTL responses to the variant virus [66]. The results of recent studies have also suggested that the specificity of responses may be critical for the durable control of HIV infection, with multiple studies showing that preferential targeting of Gag is associated with a better outcome [26, 67]. This observation may be at least partially explained by immune-induced mutations, which would be expected to have a greater impact on viral fitness when arising in key structural or functional proteins, as opposed to the envelope protein, which is able to accommodate extensive sequence variation.

Durable control of AIDS virus infection has also been achieved with live attenuated vaccines, which by far have had the most impressive effect of any vaccine tested. So far, a live attenuated SIV represents the most successful nonhuman primate vaccine approach, and has consistently protected rhesus macaques against challenge with a homologous, pathogenic SIV [68]. Whilst this is a critical model to understand in terms of the correlates of immune protection, thus far it remains unclear how such protection is achieved. Moreover, even this approach potentially falls short of what would be required, given the need for protection against heterologous strains of virus. The protective effect of this vaccine against a heterologous SIV challenge has been addressed in only a few smaller studies, with mixed results [69, 70].

1.5
Human Trials of AIDS Vaccines

To date, only two trials of AIDS vaccines have been conducted that have reached endpoints, and both have been failures.

1.5.1 Antibody-Based Vaccination

1.5.1.1 VaxGen Trial of AIDSVax

The first of these investigations was the VaxGen trial of AIDSVax, a recombinant HIV-1 gp120 vaccine consisting of two rgp120 envelope subunits derived from the subtype B isolates MN and GNE8. The hypothesis was that antibodies directed against the envelope would bind, neutralize, and clear HIV particles before infection became established. The vaccines generated antibody responses in almost 100% of recipients in Phase I and II trials [71], and protected chimpanzees from intravenous and mucosal challenge with homologous and heterologous HIV-1 variants [72, 73]. However, after completion of the Phase III trial in 2003, analyzing 3598 vaccine recipients and 1805 placebo recipients, no effectiveness in the reduction of HIV infection or levels of plasma viremia could be observed. Neither could any differences in the time to ART initiation or to virologic failure or pre-ART viral load and CD4 + lymphocyte count be found between the vaccine and placebo arms. Furthermore, antibody response levels were comparable among low-risk or high-risk vaccine recipients [74]. Although a larger trial is currently under way combining AIDSVax with a canarypox vector designed to induce T-cell responses, it is largely felt that the prospects for preventing infection or attenuating disease are modest at best with this vector, and unlikely to be achieved with any candidate neutralizing antibody vaccines in development today.

1.5.2 T Cell-Based Vaccination

1.5.2.1 The STEP Study

The second vaccine concept that has completed clinical efficacy studies involved immunization with three replication-incompetent recombinant adenovirus serotype 5 (Ad5) vectors expressing HIV-1 Gag, Pol, and Nef. The Ad5 vector-based vaccines have shown to be among the most immunogenic of available cell-mediated immunity vaccines in early-phase clinical trials [75, 76], surpassing immune responses generated by DNA plasmids [77] and many poxvirus vectors [78]. The antigens, HIV-1 Gag, Pol, and Nef, were selected because they are fairly conserved across different HIV-1 clades and commonly recognized during natural infection. In a promising Phase I trial, the study vaccine elicited immune responses in immunocompetent participants, independently of their Ad5 serostatus [75]. The aim of this collaborative study between Merck and the HIV Vaccine Trials Network (STEP study) was to elicit HIV-1-specific cellular immune responses, with the goal of preventing disease progression, but with little expectation that the vaccine would reduce the acquisition of infection.

The vaccine candidate was being studied in a Phase IIb clinical trial known as "STEP," and was being extended to a cohort in Africa through the Phambili trial. STEP (HVTN 502, Merck V520/Protocol 023) was a multicenter, randomized, double-blind, placebo-controlled Phase IIb test-of-concept clinical trial. STEP included 34 clinical trial sites in North and South America, the Caribbean and Australia. The first of 3000 participants enrolled in the study in December 2004, and enrollment was

completed in March 2007. The second Phase II trial of this study vaccine, Phambili (HVTN 503, Merck V520 Protocol 026), began in 2007 in South Africa, the goal being to determine whether the study vaccine used would prevent infection or reduce viral loads in an area where HIV subtype C is common.

The STEP trial was successfully enrolled, and even expanded to include persons who were adenovirus seropositive, but was prematurely halted for futility based on an early review of incoming data. The first planned interim analysis showed that this vaccine failed to protect against infection, or to reduce viral loads after infection in participants with baseline Ad5 antibody titers of 200 or less, despite generating interferon-γ ELISPOT responses in most participants receiving vaccine. Surprisingly, the risk for infection was highest in the subgroups of men given vaccine who were both uncircumcised and had pre-existing Ad5 neutralizing antibodies when compared to the placebo cohort; the risk was intermediate in men with either one of these two factors [3, 79]. With the early termination of the STEP Study, the Phambili protocol team also stopped vaccinations. Several theories have been proposed to explain the apparently increased acquisition in the STEP trial:

- A replication-defective Ad5 vector is insufficient to stimulate cellular immune responses of sufficient breadth to control HIV-1 infection.
- Previous infection with Ad5 led to immunity against this virus, and a lack of induction of potent immune responses.
- Pre-existing Ad5-specific antibodies could reduce the number of infectious Ad5 particles, and therefore the amount of transgene-derived proteins produced by target cells.
- rAd5 vaccination of individuals with pre-existing Ad5-specific neutralizing antibodies may have resulted in activated memory Ad5-specific CD4 + T-lymphocytes that were increased targets for HIV-1 infection.
- In individuals previously exposed to an adenovirus, Ad5-specific memory CD8 + T-cells could potentially eliminate infected target cells and thereby reduce the potency and breadth of vaccine-induced HIV-1-specific CD8 + T-cell responses.
- The selection of HIV-1 antigens Gag-Pol-Nef may have been insufficient for inducing protective immune responses.
- Ad5 immune complexes facilitate dendritic cell (DC) maturation and also induce significantly higher stimulation of Ad5-specific cytolytic CD8 + T-cells. A recent study showed that Ad5 immune complexes caused significantly enhanced HIV infection in DC-T-cell cocultures than Ad5 vectors alone [80].

1.6 Recent Advances in Animal Models: Reasons for Optimism

Despite the failure of the first two large-scale vaccine trials for candidate AIDS vaccines, and particularly the concern regarding the apparent increased acquisition

in the STEP trial, there are reasons for optimism. These stem not only from the observation that some persons control HIV for more than 30 years without developing disease, but also from some recent results with animal models of T-cell-based vaccination.

1.6.1 Success against Heterologous Challenge

One of the greatest challenges for the development of an effective AIDS vaccine is the generation of broadly cross-reactive immune responses that will protect against an heterologous virus challenge. Recent data, albeit in a vaccination model that involves a live attenuated virus vaccine that is not likely to be a viable approach in humans, have suggested that this type of cross-protection may be achievable. The immunization of rhesus macaques with live-attenuated SIV has consistently induced protective immunity against a homologous pathogenic SIV challenge. However, an effective HIV vaccine should be able to protect against a wide variety of HIV isolates circulating worldwide. Only heterologous challenge studies can therefore try to predict the degree of protection that an HIV vaccine can achieve. A recent report addressed this issue in macaques when, following the administration of a live-attenuated SIV vaccine, the animals were re-challenged with a heterologous SIV isolate [68]. This strategy led to a 2-log reduction in viral replication in the vaccinated animals for up to 32 weeks post-challenge. Macaques expressing protective MHC class I alleles were even able to achieve complete suppression of the challenge virus in the acute phase. After depletion of peripheral CD8+ T-cells in four vaccinated animals during the chronic phase, an increase in virus replication was observed which supported the crucial role that CD8+ T-cells play in viral control. One drawback in these findings was the fact that the authors identified evidence of recombinant viruses emerging in some of the vaccinated animals. Taking this into consideration, the conclusion can be drawn that attenuated virus vaccines will not play a key role in future vaccine developments.

1.6.2 Heterologous rAd26 Prime/rAd5 Boost Vaccine Regimen

The disappointing results of the STEP trial were largely predicted by animal model experiments, which had shown that rAd5 vectors expressing SIV Gag failed to reduce peak or setpoint viral loads after SIV challenge of rhesus monkeys lacking the protective MHC class I allele Mamu-A*01 [81]. However, a recent study in monkeys [82] using two serologically distinct adenovirus vectors, demonstrated a substantially improved protective efficacy in this challenge model, and has raised hopes for a T-cell-based vaccine approach. The heterologous rAd26 prime/rAd5 boost vaccine regimen expressing SIV Gag elicited cellular immune responses with augmented magnitude, breadth, and polyfunctionality as compared to the homologous rAd5 regimen. Vaccinated monkeys, when re-challenged with SIV (MAC251), showed a 1.4-log reduction of peak and a 2.4-log reduction of setpoint viral load, as

well as decreased AIDS-related mortality, when compared to control animals. Of note, the breadth and magnitude of vaccine-elicited, Gag-specific cellular immune responses before challenge correlated with the control of set-point viral loads after challenge, which suggested a critical importance of Gag-specific T-lymphocyte responses in controlling viral replication. Furthermore, the vaccine used in this study expressed only a single SIV Gag antigen and did not include a homologous Env immunogen. It could therefore be argued that the observed protective effect was mediated by Gag-specific cellular immune responses rather than Gag-specific antibodies. However, the protective efficacy of this vaccine regimen against highly *heterologous* SIV challenges has to be examined in future studies.

1.6.3
Induction of Effector Memory T-Cell Responses at Viral Entry Sites

One major limitation of current vaccine candidates is the fact that induced memory T-cell responses transform increasingly into lymphoid tissue-based central memory cells. This might be due to the decreasing amount of antigen provided by the vaccine vectors, as most of these vectors are nonpersistent. Central-memory T-cells, however, are primarily lacking effector functions and require differentiation and expansion in the presence of antigen to respond effectively to virus. However, this might be too slow to prevent the systemic dissemination of HIV. A recent study therefore utilized a SIV protein encoding vector based on rhesus cytomegalovirus (RhCMV); this was chosen because these vectors induce lifelong effector memory T-cell responses. Rhesus macaques vaccinated with the RhCMV vector expressing SIV Gag, Rev/Nef/Tat, and Env maintained robust SIV-specific, CD4+ and CD8+ effector-memory T-cell responses, despite pre-existing RhCMV immunity. In addition, vaccinated Rhesus macaques were less susceptible to progressive SIV (mac239) infection upon intra-rectal challenge. Four animals were even able to control rectal mucosal infection and prevent the progressive systemic dissemination of the challenge virus [83].

1.7
The Current Vaccine Pipeline

The most immunogenic vaccine used to date has been the adenovirus; however, concerns regarding the possible enhancement of infection in persons with pre-existing adenovirus-specific immunity that were uncovered in the STEP trial, have made the future of this vector uncertain. In the following section we provide a brief review of the candidate delivery systems currently under investigation.

1.7.1
DNA

Although plasmid DNA vectors have elicited effective cellular immune and antibody responses in mice, HIV DNA vaccines could not replicate the same level of

immunogenicity in nonhuman primates, and have been even less immunogenic in humans. In order to improve the immunogenicity of these plasmid DNA vaccines, several approaches for improved *in vivo* expression and more robust protection against degradation have been investigated [84]. The coadministration of plasmid DNA immunogens with plasmids encoding cytokines has proven to increase vaccine-elicited cellular immune responses [85]. Furthermore, alternative strategies to deliver the DNA plasmids have been explored, including delivery of the plasmid by intramuscular injection followed by electroporation *in vivo* [86]. A recent study used DNA plasmids containing gp160, Rev, p17/24 Gag and RT from multiple HIV-1 subtypes and boosted with a heterologous vaccinia virus Ankara (MVA) containing Env, Gag, and Pol. Subsequently, 30% of the vaccinees developed CD8 + and CD4 + T-cell responses after the DNA prime. After the boost with MVA, T-cell responses could be measured in 92% of the vaccine recipients [87]. Further studies are required to investigate how to enhance DNA–induced immune responses.

1.7.2 Adenovirus

Adenovirus-vectored vaccines have the ability to induce strong cell-mediated immunity, and have therefore been considered primary candidates for further vaccine development. The results of early-phase trials were very encouraging, in that the rAd5 vector-based vaccines elicited cellular immune responses in monkey models and later in most human subjects, although these responses were partially suppressed in individuals with pre-existing Ad5-specific neutralizing antibodies [75]. However, even with the DNA prime–Ad5 boost, which was suggested to be more effective at controlling SIV replication than the Ad5 prime–Ad5 boost strategy, only macaques expressing a macaque MHC class I protein that had previously been associated with diminished viral replication (Mamu-A*01) showed some control of SIV (mac239) replication [81, 88]. In order to investigate whether cellular immune responses are able to control viral replication, eight Mamu-A*01-positive rhesus macaques were vaccinated with SIV Gag, Tat, Rev and Nef using a DNA prime–adenovirus boost strategy, excluding Env intentionally. A strong protective effect of the vaccine on peak viremia and viral set point was observed, which supported the idea that a vaccine that induces only cell-mediated immunity might be able to control viral replication [89]. These data, together with the recently demonstrated protective effect of an adenovirus prime boost regimen in macaques challenged with SIV, offers hope for this approach if concerns regarding possible enhancing effects can be overcome.

1.7.3 Peptides

The so-called OPAL study (Overlapping Peptide-pulsed Autologous CeLls) did not seek to prevent new infection with SIV, but instead followed new pathways in eliciting potent cellular immune responses in already infected animals. Instead of delivering HIV antigens with vectors to induce CTL and Nab responses, the OPAL approach has

shown promising results in SIV (mac251)-infected macaques. After exposure to overlapping SIV peptides, autologous blood cells were reinfused into the animals. Following immunization, robust SIV-specific CD4 + and CD8 + T-cell responses were observed, and SIV levels were up to 10-fold lower for one year in immunized animals compared to controls. Furthermore, the AIDS-related mortality in immunized animals was significant delayed. Interestingly, immunization with all SIV proteins did not show any better effect on viral outcome compared to animals which had been immunized against Gag alone, thus supporting the fact that Gag is an effective stimulator for T-cells [90]. A more advanced approach evaluated inactivated SIV pulsed fresh peripheral blood mononuclear cells in the same animal model. Strong SIV-specific CD4 + T-cell responses, but unfortunately lower SIV-specific CD8 + T-cell responses, could be measured. Interestingly, most of the functional responses were directed against Gag. In contrast to the previous study, no reduction in viral load was observed [91]. Human trials will undoubtedly follow.

1.7.4 Bacillus Calmette-Guérin

The attenuated, nonpathogenic *Mycobacterium bovis* (Bacillus Calmette-Guérin; BCG) is widely used as a vaccine for TB and leprosy [92], although its efficacy is rather limited. Nevertheless, mycobacteria have distinct characteristics that make them attractive as potential HIV-1 vaccine vectors; for example, their ability to stably express transgenes or to elicit longlasting cellular and mucosal immune responses [93, 94]. So far, recombinant BCG (rBCG) vaccine constructs have been used in multiple murine models to evaluate immunogenicity and protection against various infectious agents, including *Borrelia burgdorferi*, *Streptococcus pneumoniae*, *Bordetella pertussis*, rodent malaria, leishmania, and measles virus. Furthermore, rBCG was able to induce antibody as well as cellular responses against antigens derived from HIV and SIV in murine and monkey studies [95, 96]. A *Mycobacterium smegmatis* vector expressing full-length HIV-1 envelope protein was able to induce functional MHC-class I-restricted HIV-1 epitope-specific CD8 + T-cell responses in mice [97]. Furthermore, repeated immunization led to the expansion of central-memory virus-specific cells. Human studies with recombinant BCG-HIV vectors can be anticipated in the near future.

1.7.5 Listeria and Other Bacterial Vectors

1.7.5.1 *Listeria monocytogenes*

Listeria monocytogenes (Lm) is an intracellular bacterium with promising properties, as it infects and induces the maturation of DCs; it therefore has the capacity to stimulate innate as well as adaptive immune responses. Additionally, vaccine antigens encoded by *Listeria* are efficiently presented by both MHC class I and MHC class II molecules, as *Listeria* vectors deliver antigens directly to the DC cytosol, resulting in antigen-specific CD8 + and CD4 + T-cell activation [98, 99]. Another

advantage, although secondary, is the benefit that *Listeria*-derived vaccine vectors may be given orally as the natural route of *Listeria* infection involves oral exposure [100]. A *Listeria* vector containing two genes of feline immunodeficiency virus (FIV) showed that pre-existing immunity against Lm does not preclude the generation of immunity to foreign antigens expressed by the *Listeria* vector [101]. A recent study compared oral priming/oral boosting versus oral priming/intramuscular boosting of a live attenuated Lm expressing HIV Gag in rhesus macaques [102]. The latter was able to induce Gag-specific cellular immune responses as well as mucosal anti-Gag antibodies, whereas the former could only elicit cellular immunity. Another study showed that an attenuated, recombinant Lm-gag for priming, followed by a boost of a replication-defective rAd5-gag, induced a strong cellular immunity. By modulating the route of priming or boosting (oral, intrarectal, intravaginal, or systemic) differences in CTL activities in the different target tissues could be observed [103].

1.7.5.2 *Salmonella enterica*

Recombinant *Salmonella enterica* serovar Typhi can function as a live vector to deliver HIV antigens and induce both mucosal and systemic immune responses. Recombinant *Salmonella* Typhi vaccines are easy to produce and have been used as oral typhoid vaccines, which can induce mucosal, humoral, and cellular immune responses after immunizing via mucosal surfaces. A recent study used a recombinant *Salmonella* Typhi strain expressing HIV-1 Gag integrated into the bacterial chromosome and *gp120* gene carried by a plasmid, induced high titers of gp120 antibodies as well as Gag and gp120-specific CTL responses in mice [104].

1.7.5.3 **Shigella**

Invasive *Shigella* strains are able to gain access to the cytoplasm of infected cells, and are therefore attractive for DNA vaccine delivery. *Shigella* vectors with DNA encoding HIV gp120 were immunogenic after administration into mice [105]. Mice, which were immunized intranasally with live recombinant bacterial cells carrying a plasmid encoding HIV Gag, showed local and systemic immune responses [106]. *Shigella* vectors might become useful in prime-boost vaccination regimens with DNA vaccines, although additional more detailed studies, preferably in humans, are required.

1.7.6 Canarypox

In the past, poxvirus vectors have shown very variable efficiency in eliciting T-cell responses. In a study using recombinant DNA in combination with a modified vaccinia virus Ankara expressing Gag protein and some immunodominant CD8+ T-cell epitopes [107], only limited immunogenicity was observed. Although, using the vaccines in a higher dose resulted in an enhanced immunogenicity, the response rate based on *ex vivo* interferon-γ ELISPOTs remained limited and was due exclusively to CD4+ T-cells [108]. A very recent study, the EuroVacc 02 Phase I trial,

delivered a prime-boost regimen to evaluate the safety and immunogenicity of recombinant DNA and the poxvirus vector NYVAC, both of which were expressing Env, Gag, Pol, and Nef polypeptides from HIV clade C isolates. A functional analysis of the vaccine-induced T-cell responses indicated that the DNA/NYVAC vaccine combination elicited *ex vivo* T-cell responses in 90% of immunized volunteers, and that these responses were polyfunctional, broad, and durable. Env induced by far the strongest and most frequent T-cell responses (91% of vaccines), although in 48% of the vaccinees responses against Gag-Pol-Nef could also be observed. This led to the conclusion that regimens using DNA/NYVAC vaccine combinations are promising, and support the need for further clinical development [109].

1.7.7
Adeno-Associated Virus

Adeno-associated viruses (AAVs) are single-stranded DNA parvoviruses that infect both dividing and nondividing cells [110], but do not cause disease. The characteristic feature of the adeno-associated virus is a deficiency in replication, and thus its inability to multiply in unaffected cells. However, new AAV particles are successfully generated in the presence of selected proteins derived from the adenovirus genome [111], or other viruses such as HSV [112]. Although recombinant AAV (rAAV) vectors have been initially developed for gene replacement therapy, several characteristics have made these potential candidates for vaccine delivery [113]: (i) rAAV vectors are well tolerated; (ii) they fail to induce strong vector-directed inflammatory responses, although the induction of neutralizing antibodies has been described [114]; and (iii) rAAV vectors do not introduce any of their viral genes into the host cells. The latter point is due to the fact that the inverted terminal repeat (ITR) elements which flank the 4700 nucleotides of the AAV's single-strand DNA genome are minimally required in cis to generate rAAV vectors in which all other viral sequences are supplied in trans [115]. A Phase II clinical trial using a rAAV vector-based vaccine containing clade C HIV antigens was started in 2006 in South Africa (IAVI Report, http://www.iavireport.org/Issues/Issue9-5/VaccineBriefs.asp). However, a recent report suggested that T-cells generated by AAV vectors might have a limited proliferation potential [116].

1.8
Conclusions and Future Directions

The pathway to the development of a licensed HIV vaccine will no doubt be long. Even if a viable candidate vaccine were available today, the manufacture, testing and distribution of such a vaccine could easily take 10 years before it could be made available to healthcare providers for administration in endemic areas. The challenge of a fully preventive vaccine is not likely to be overcome with any of the products that are currently available, and thus a return to basic science to address fundamental concepts is needed. These were highlighted in a recent vaccine summit sponsored by

the US National Institute of Health, and prompted a re-allotment of funds to address some of the basic questions before any major advances are made in the HIV vaccine field. On the other hand, the recent success of a T-cell-based vaccine for an AIDS virus in a monkey model [68, 82] offers hope that the viral load set point might be altered by a vaccine that could function to delay or prevent disease progression. In the meantime, ongoing studies to determine the correlates of immune protection are of paramount importance, and the vigorous investigation of those persons who are able to control viremia in the absence of therapy may represent the most promise for providing the required insights. Meantime, major efforts should be expended not only to prevent new infections but also to implement procedures to slow the intersection of the TB and HIV epidemics.

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