A brief history of HIV vaccine research: stepping back to the drawing board?

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In September 2007, it was announced that the most promising HIV vaccine trial had to be stopped because it had failed to show the protection that was hoped for. Here, the history of HIV vaccine development from the discovery of HIV-1 in 1983 until 2008, the underlying ideas on protective immunity to HIV and potential avenues for vaccine research are discussed.

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The development of an efficacious vaccine against HIV is considered one of the greatest challenges to fight the AIDS epidemic. Ever since the discovery of HIV-1 in the spring of 1984, opinion leaders and administrators involved in HIV research have put down strong claims of the delivery of an AIDS vaccine in the next 5–10 years. In September 2007, it was announced that the STEP and Phambili trials conducted with the most advanced MRKAd5 Trivalent HIV vaccine, an adenovirus 5-based vector with HIV-1 subtype B Gag, Pol, or Nef, given three times in a 1:1:1 ratio were stopped. Despite promising results in the SIV macaque model [1], interim analysis of the human trial showed no protection from HIV infection and more importantly, not even a lowering of viral set point. In fact, those vaccinated who had high preexisting immunity to the Adeno-5 viral vector showed a trend towards increased risk for becoming HIV infected (http://www.iavireport.org/Issues/Issue11-5/Step.asp). Here, a brief critical review of the hypotheses that formed the main basis of the last decade of HIV vaccine research and development is presented.

Prologue

Neutralizing antibodies are the best, if not the only, correlate of protection for all successfully working vaccines known to date. Logically, neutralizing antibodies were the first choice for vaccine-induced immunity against HIV and much of the research in the first 10 years after 1984 focused on humoral anti-HIV immunity. This research program was questioned when in the early 1990s it appeared that antibodies, and also soluble CD4 molecules, that could efficiently block T-cell line adapted HIV-1 isolates failed to neutralize primary HIV-1 isolates. It became gradually clear that the classical vaccinology approach of a subunit envelope vaccine in aluminum hydroxide (alum) that by definition mainly induced an antibody response would not suffice. At that time, these novel insights and drawbacks significantly delayed planned trials with candidate vaccines and made scientists abandon the idea of neutralizing antibody-mediated protection elicited by a vaccine. In addition, an HIV vaccine delivering sterilizing immunity to infection was believed to be non-realistic and the hope was that a vaccine could induce immunity that would protect from progression to disease [2,3]. Evidence for T-cell protection came from early vaccine studies in rhesus monkeys by Hirsch *et al.* [4] followed some years later by seminal studies by Shiver and colleagues [1,5], in which although no sterile protection was shown, there was evidence of prolonged survival after homologous SIV challenge, and this appeared to be associated with a lower viral set point. Because cellular responses are believed to be crucial for

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inhibition of progression to disease the field shifted to become heavily focused on T-cell immunity.

To define 'protective' T-cell immunity, many laboratories set out to study patients at different stages of HIV infection. Important studies showed that antibody-mediated depletion of CD8 T cells resulted in increased viral load in SIV-infected rhesus monkeys [6], and a temporal relationship between control of viremia and cytotoxic T lymphocyte (CTL) responses in acute HIV infection was shown [7]. From 1994 onwards, a host of data was published on HIV-specific CD8 and CD4 T-cell immunity mapping responses to viral proteins and peptides, initially using the classical approaches of T-cell lines and CTL precursor (CTLp) limiting dilution assays [8]. The original thought that T-cell immunity to HIV-1 was in general rather poor came from CTLp and CD4 T-cell proliferation data. Long-term survivors, but not patients who had progressed to AIDS, had strong CTL responses [9,10]. These CTL were shown to select for viral escape mutations [11]. Interestingly, early in infection progressors appeared to have had strong CTL responses that were exhausted or deleted during progression the mechanism of which was related to enhanced T-cell turnover and perturbation of cellular immunity [12,13]. T-helper cells could be detected only in patients with a very low viral load when proliferation assays were used [14,15]. Interestingly, also in patients that were treated immediately during acute HIV infection with highly active antiretroviral therapy (HAART) CD4 T-helper responses apparently were preserved. This finding suggested that a proliferative T-helper response to HIV was associated with viral control and moreover that early treatment might be followed by periods of structured treatment interruption (STI) during which viral load would be contained by these now preserved HIV-specific CD4 T cells. Although the initial short-term effect looked promising, several subsequent studies showed no sustained control by the preserved T-cell immunity during STI as the viral load quickly rebounded in almost all patients [16–19].

With the advent of new technologies, which are based on fundamentally different methods to detect antigen-specific T cells, it appeared that there was abundant T-cell reactivity. Strong T-cell responses were observed in asymptomatic and even still in late-stage patients when human leukocyte antigen (HLA) tetramers or interferon-gamma (IFN-γ) were used as a T-cell response marker [20,21]. The combination of these novel techniques allowed for detailed functional and phenotypic characterization of reactive T cells that appeared to correlate with disease progression markers such as CD4 T-cell numbers and viral load [22–24]. Pantaleo and coworkers [25], among others, observed a skewing in T-cell differentiation to less functional CD8 and CD4 T cells in progressors, which correlated with high viral load [26]. Several laboratories reported cross-sectional studies comparing HIV-specific T-cell properties in progressors and long-term nonprogressors (LTNP), showing a striking correlation between low viral load and preferentially polyfunctional [IFN-γ+ interleukin (IL)-2+] central memory (CD27+ CCR7+ IL-7R+) CD4 and CD8 T cells [24,27–30]. Although some reservations were sometimes made regarding causality, it was widely held that these memory T cells that were capable of producing multiple cytokines upon in-vitro restimulation were protective T-cell responses able to control HIV-1 during chronic infection. Most of these ideas were derived from or at least fueled by a host of data from murine infectious disease models in which IL-2 producing IL-7R central memory T cells were shown to protect against infection and disease by lymphocytic choriomeningitis virus (LCMV) [31]. On the basis of the correlative data from HIV natural history, in parallel, universal criteria for evaluation and monitoring of vaccine-induced T-cell immunity were agreed upon and developed among the various large vaccine-development consortia in the US and Europe.

Correlation but no causality?

The idea that a protective T-cell phenotype and function had been identified that could be used to monitor and define HIV vaccine-induced protective immunity was solely based on correlative cross-sectional data where the cause–effect relationship is unknown. In fact, several groups showed that high-level HIV viremia after STI might cause the subsequent loss of proliferative capacity and IL-2 production of HIV-specific CD4 T cells while leaving their capacity to produce IFN-γ intact [32,33]. Moreover, viral load increase after STI results in a 'non-protective' T-cell phenotype that, after restarting HAART and resumed control of viral replication, reverts back to the 'protective' T-cell type. These observations indicate that viral load during chronic infection might not be, or only partially be, controlled by T-cell immunity and moreover that antigen levels seem to determine T-cell function and phenotype, and not the other way around [34,35]. Increased expression of inhibitory receptors programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) on HIV-specific T cells from patients with high viral load is in good agreement with this [36,37].

The hypothesis that HIV-specific memory T cells that have the ability to produce IL-2 and high-level IFN-γ are truly protective and determine subsequent AIDS-free survival was tested for the first time in a prospective cohort study in 96 seroconvertors from the Amsterdam Cohort Studies. Applying Cox proportional hazard models and Kaplan–Meier survival analyses this hypothesis was refuted for both HIV-specific CD4 [38] and CD8 T cells (Schellens *et al.* Presented at 'Immunological correlates of protection from HIV infection and disease' meeting; Volendam, The Netherlands; August 2006

(submitted) [39]). In these studies, no correlation between T-cell immunity and viral set point early after infection was observed. These studies for the first time showed that abundant T-cell immunity, of the type that correlated with low viral load ('control') early in asymptomatic infection, neither prevented nor caused a delay in progression to AIDS. As it turned out progressors lose their HIV-specific T-cell immunity more rapidly during the course of infection than LTNP, this loss of immunity is most likely a consequence rather than a cause of disease progression. This confirmed data published more than a decade ago, using assays that detected memory CTL precursors, which showed that rapid progressors like LTNP had high CTL precursor frequencies early in infection which subsequently became lost during follow-up [7,8]. At that time, it was less clear how the early steady state with strong cellular immunity to HIV was disturbed and what could explain the deletion or exhaustion of these HIV-specific responses. Recently experimental evidence has reported that CD4 T-cell loss and disease progression are driven mainly by HIV-associated systemic immune activation that appeared to be independent from viral load [40–43]. Because of this, it appears that the pace and extent of exhaustion of the initial cellular immune response and loss of CD4 T-cells is determined by the strength of the systemic immune activation induced by HIV infection. Indeed, after the viral set point is established, the 'set point' immune activation is the best and independent predictor for progression, overriding viral load and early HIV-specific T-cell immunity as prognostic parameters [38]. This immune activation, next to HIV-induced activation of the adaptive immune system, has been shown to be induced by microbial products, including lipopolysaccharide (LPS), resulting from a breach of the gastrointestinal tract due to massive irreversible loss of memory CD4 T cells during acute infection [44]. It may be envisaged that polymorphisms in genes involved in innate immune pathways determine the level of immune activation and progression.

Given all this, the lack of effect of vaccine-induced T-cell immunity on the viral load set point observed in the STEP trial, although disappointing, may not be totally unexpected. One could argue that T-cell immunity induced by viral vectors other than Ad5, or HIV genes different from HIV gene fragments than the ones included in the MRKAd5 Trivalent vaccine, might provide protection from disease progression. It may seem paradoxal that despite the fact that in acute and chronic infection T-cell immunity does exert effects on the virus, which is apparent from the strong selection for escape mutations in HLA class I restricted T-cell epitopes [11,45], cellular immunity cannot truly prevent disease progression. Indeed, in rhesus macaques, AIDS vaccine failure has been shown to be associated with viral escape from CTL [5,7]. In addition, clear correlations between B57 and B27 HLA alleles, low viral load set point and long-term AIDS-free survival have been documented, in particular in so-called LTNP. Although until now progression has ultimately been seen in many LTNP that have been studied before, it may still be envisaged that some as yet undiscovered aspect of the innate or adaptive immune response in very rare 'elite controllers' provides long-term protection and may be successfully exploited for vaccine development. It may be by targeting of particularly conserved Gag epitopes [46] or through early attenuating effects of B57 and B27 restricted CTL on the incoming virus. If that type of immunity would, however, be restricted to a rare subpopulation of carriers of HLA B57 or B27, as has by analogy been suggested in a recent study [47] in Mamu-A*01 typed macaques, this would impose a severe and probably inhibitory limitation on the efficacy and use of a potential vaccine in most populations. Apart from this, once infection becomes established, not only the lag time required for CD4 and CD8 T-cell memory reactivation and expansion may preclude protection against infection, but HIV will also rapidly escape from CTL, diminishing protective effects of vaccine-induced immunity. Taking all this into account, it seems that we may need to return to the ambitious goal of a protective HIV vaccine that provides long-lasting sterilizing immunity mainly by way of the induction of broadly neutralizing antibodies. In the past decade, major breakthroughs have been achieved in that field and immunological and biochemical problems to be solved have been clearly identified, although at this time a vaccine candidate is not near to phase II or III trial.

Reemphasise investigator-driven science

The failure of the antibody-inducing HIV vaccines in 1994 called for a reevaluation of the global vaccine effort. At that time, a plea was made for structures and incentives to get public and private partners together in a global initiative to develop various vaccines and to prepare vaccine test sites in Africa, South America and Asia. In 1996, the International AIDS Vaccine Initiative (IAVI) was created and additional levels of global coordination were added by the Global AIDS Vaccine Initiative. European Union vaccine consortia and later the Bill and Melissa Gates Foundation Grand Challenge Program and Center for HIV/AIDS Vaccine Immunology (CHAVI). These public–private partnerships and mega consortia attracted, compared to other medical research programs, very large sums of funding and hence set the agenda for the international AIDS vaccine field. Despite 25 years of excellent and highly devoted HIV research, no vaccine is available and none will likely become available for the decade to come. After the recent failure of the STEP trial, it seems we have to go back to the drawing board and reconsider every remaining possible option for a vaccine. This approach may require that, for the near future, funding now spent via large consortia with product-oriented research programs and much effort on vaccine site

logistics is redirected to small-scale investigator-driven and ideally multidisciplinary projects. New creativity and theoretical and practical breakthroughs will have to come from the various laboratories that are committed to perform basic and translational HIV research working from many different hypotheses and angels.

Main issues, among many other promising research topics, to be addressed will be the design of immunogens able to induce broadly neutralizing antibodies and the remaining options of live-attenuated HIV vaccines and replication competent viral vectors. The negative view of HIV-1-specific humoral immunity changed with the discovery of broadly neutralizing antibodies, and at present there are options that may be quite challenging but are still viable. The observation that antibodies such as b12, 2G12, 4E10, and 2F5 could potently neutralize HIV-1 variants from different subtypes implies that certain epitopes on HIV-1 envelope are conserved between subtypes and capable of eliciting potentially protective humoral immunity [48]. Administration of the b12 antibody to monkeys, either intravenous or intravaginally, protected from infection via the intravenous or vaginal route, supporting that preexisting neutralizing antibodies can indeed provide sterilizing immunity [49–51]. Unfortunately, although progress has been made in stabilizing trimeric envelope structures the classical approaches to elicit broadly neutralizing antibodies all failed. More broadly neutralizing antibodies should be isolated from HIV-infected individuals and their epitope specificities defined, to enlarge the panel of potentially interesting epitopes that may be included in a vaccine. One obvious question to ask is why broadly neutralizing antibodies are so rare especially when most primary HIV-1 variants are sensitive to their neutralizing effect, which implies exposure of the epitopes sometime during the viral life cycle. Recent studies have shown that one of the main epitope specificities in sera from individuals with broadly neutralizing activity is the CD4 binding site. A large panel of b12-like antibodies with slightly varying epitopes may provide an interesting antibody repertoire from which HIV is unable to escape without loosing in parallel its ability to bind CD4 and infect its target cell. In the field of cellular immunity, the contribution of cellular immunity in the very rare elite non-progressors should be further studied and finally, despite discouraging results with live-attenuated SIV virus vaccines in monkeys [52] and of superinfection with HIV of HIV-infected humans [53] studies on the role of immunity in superinfection deserves attention.

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