Vaccines to prevent HIV and AIDS

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Introduction

HIV is exerting a devastating toll on individuals, communities, whole countries and economies around the world. An estimated 36 million people are currently infected with HIV worldwide, with the vast majority expected to die within 10 years (Figure 1). New HIV infections are increasing at an alarming 5.3 million per year (Figure 2). The vast majority of the global burden of HIV is occurring in Africa, however, Asia has an increasing number of new cases.

That the HIV epidemic has been allowed to spread basically untouched through so many countries is a tragic indictment of the world's current approach to global problems. Although drug treatments for HIV have advanced considerably in the last few years in wealthy countries, HIV frequently becomes resistant to treatments and long-term toxicities are now being observed to these very expensive drugs. Prevention of HIV is as critical as ever.

A vaccine to prevent HIV is widely viewed as an essential component to halting its spread. At present, there is no vaccine licensed and only one vaccine (a recombinant form of the surface envelope protein) has entered efficacy trials to date. The slow pace of vaccine development, up until the last couple of years, has been partly due to lack of commercial interest (since the most important target population of HIV vaccines is poor countries), lack of funding through governmental and philanthropic organisations, and lack of advocacy for HIV vaccine studies. This has started to turn around and the pace and interest in HIV vaccines has advanced considerably. This article highlights the status of several HIV vaccine strategies and issues of interest.

Humoral or cellular immunity or both?

Most vaccines that have entered clinical trials to date have been disappointing in their immunogenicity [1]. This is partly because many have focussed on inducing neutralising antibodies to HIV in an attempt to not allow any infection to occur (i.e. sterilising immunity). Although passive transfer of very high doses of neutralising antibodies obtained from infected individuals can prevent transmission of HIV in animal models, it is now clear that neutralising antibodies to primary HIV isolates are very difficult to induce by vaccination [2-3]. True neutralising epitopes are probably only exposed during a fleeting conformational event as HIV enters a cell, which has yet to be exploited in a viable vaccine [4].

Fortunately, the very significant control of HIV that can be exerted by T-cells against HIV by both CD4+ T-helper lymphocytes and CD8+ cytotoxic T-lymphocytes, is now also clear. Removal of CD8+ T-cells from monkeys results in brisk increases of SIV (the simian equivalent of HIV) viral levels [5, 6]. Individuals exposed to HIV-1, such as sex workers, but who do not become HIV-1 infected, can develop HIV-specific cellular immunity which plausibly plays a role in their resistance to becoming infected [7]. Treatment of subjects during acute HIV infection appears to result in maintenance of HIV-specific T-cell responses which are able to subsequently control virus replication off drug therapy [8]. Vaccine regimens that induce HIV-specific T-cells, but not neutralising antibodies, can protect against ongoing HIV replication in animal models [9, 10]. Vaccines that primarily induce HIV-specific T-cell responses cannot protect against any infection of the host since infection of cells is required for stimulation of the response (i.e. non-sterilising immunity is induced) [11]. Interestingly, T-cell responses against HIV are boosted by the exposure to the virus challenge in animal models, which contributes to the control of the virus [10].

Live-attenuated vaccines

Probably the most efficient way of inducing durable high level immunity to HIV would be via live-attenuated HIV vaccines. Such vaccines efficiently protect against SIV in animal models and induce high levels of both humoral and cellular immune responses [12, 13]. Curiously, a considerable duration of infection (over 20 weeks) is required to efficiently protect from wild-type virus challenge, the mechanism of which is not clear [14].

The most commonly studied attenuated strains contain deletions in the *nef* gene, a regulatory gene of several functions which is required for efficient replication of HIV-1 or SIV in resting CD4+ T-cells. An amazing 'experiment of nature' has been detected in a group of blood transfusion recipients in Sydney, all of whom received blood from a single donor, and all of whom experienced a non-progressive HIV infection over the first 10-12 years [15]. All virus strains sequenced from the cohort had deletions in *nef*.

There are, however, very serious safety concerns with attenuated HIV-1 and SIV strains that will preclude clinical trials of such vaccines for the foreseeable future. Infection of neonatal and, occasionally, adult monkeys with attenuated SIV strains can result in AIDS due to the attenuating strain [16]. Continued evolution of attenuated strains *in vivo* results in fitter viruses which have caused AIDS in both monkeys and humans after extensive (15+ years) of follow-up [17]. Unfortunately, very highly

attenuated SIV strains that do not persist for lengthy periods are not efficient at inducing immunity [18].

How to safely induce T-cell responses against HIV?

How, then, can T-cell responses be safely and efficiently induced against HIV-1? Protein vaccines (such as the recombinant envelope proteins now undergoing efficacy trials), even with newer adjuvants, fail to induce anti-HIV T-cell responses in clinical trials [1]. The answer may lie in a new generation technology of vaccines that produce antigens from within host cells. The greatest of such vaccines studied are DNA vaccines and live virus vector vaccines.

DNA vaccine technology is revolutionising many vaccine fields. It is amazing that an *in vivo* injection of simple plasmid DNA designed to express particular genes results in transfection of a few cells, expression of the encoded proteins and the induction of immune responses [19]. Since the proteins are expressed from within cells, these vaccines are efficient at priming T-cell responses, although in general have been only modest at induction of humoral responses. Early clinical studies did not show impressive immunity from DNA vaccine alone and animal model studies demonstrated that simple DNA vaccines provided very limited protection from AIDS [20]. A great advantage of DNA vaccine technology, however, is the ability to use molecular techniques to further enhance immunogenicity. Co-expression of a number of immune-modulatory genes can enhance immunogenicity. DNA vaccines also expressing the cytokine, interleukin 2, have recently induced considerable protection from AIDS in monkeys, almost certainly via the induction of T-cell responses [21].

Live viral vector vaccines also show considerable promise as HIV vaccines. Non-pathogenic viruses or replicons are engineered to express HIV genes and, like DNA vaccines, can be engineered to express immune-enhancing molecules. Amongst the best studied are modified vaccinia viruses and avian poxviruses such as canarypox or fowlpox viruses [22]. These poxviruses undergo only an abortive replication cycle so are quite safe. Canarypox-HIV vaccines have shown moderate T-cell immunogenicity in clinical trials and are poised to enter efficacy trials in the near future [23]. Alphaviruses such as Venezuelean Equine Encephalitis (VEE) virus have been engineered as abortive replicons and appear highly immunogenic and partially efficacious in animal models [24]. VEE-based vaccines are poised to enter early human trials in South Africa soon.

A number of HIV vaccines have been studied in combination 'prime and boost' scenarios. Here, DNA vaccines are first used to prime a T-cell response and another live vector, such as vaccinia or fowlpox virus, is used to boost the responses [9, 10, 25, 26]. This DNA prime and poxvirus boost technique results in extraordinarily high levels of vaccine-specific T-cell responses (Figure 3). Using recently developed MHC-tetramer technology to specifically identify CD8+ CTLs, up to 20% of all circulating CD8+ T-cells are specific for the vaccine antigens following DNA prime and poxvirus boost approaches, compared to around 1% or less by most DNA or poxvirus vaccines alone [25]. Considerable protection from SIV and HIV-1 has been observed in animal model studies [9, 10]. It is possible that co-expression of immune-enhancing molecules could further enhance this

Figure 1. The global burden of total HIV cases in the year 2000 broken down by continent as estimated by UNAIDS.

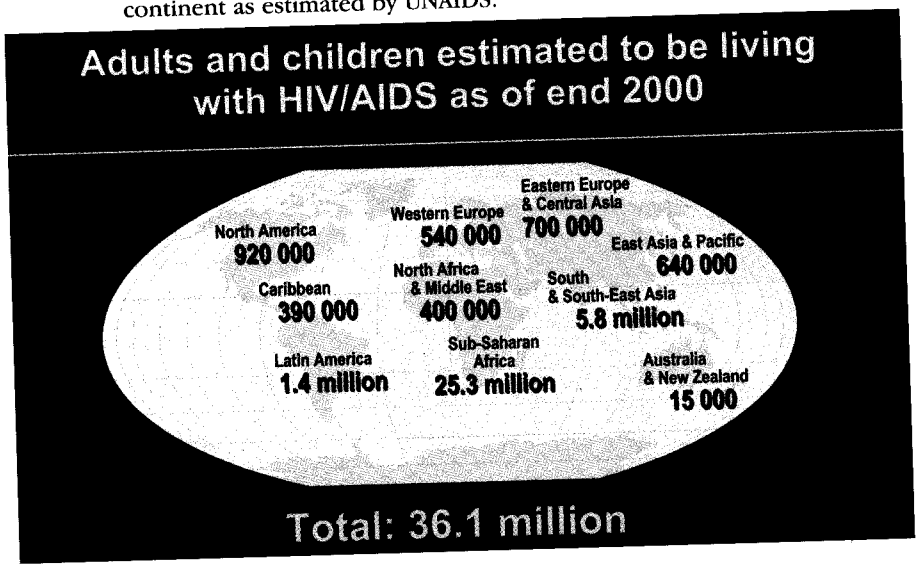


Figure 2. New infection with HIV during the year 2000 broken down by continent.

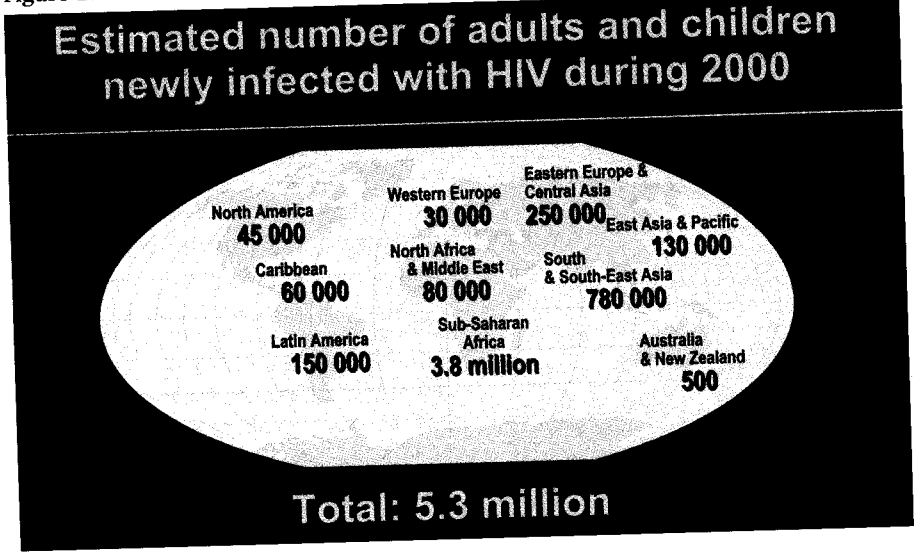
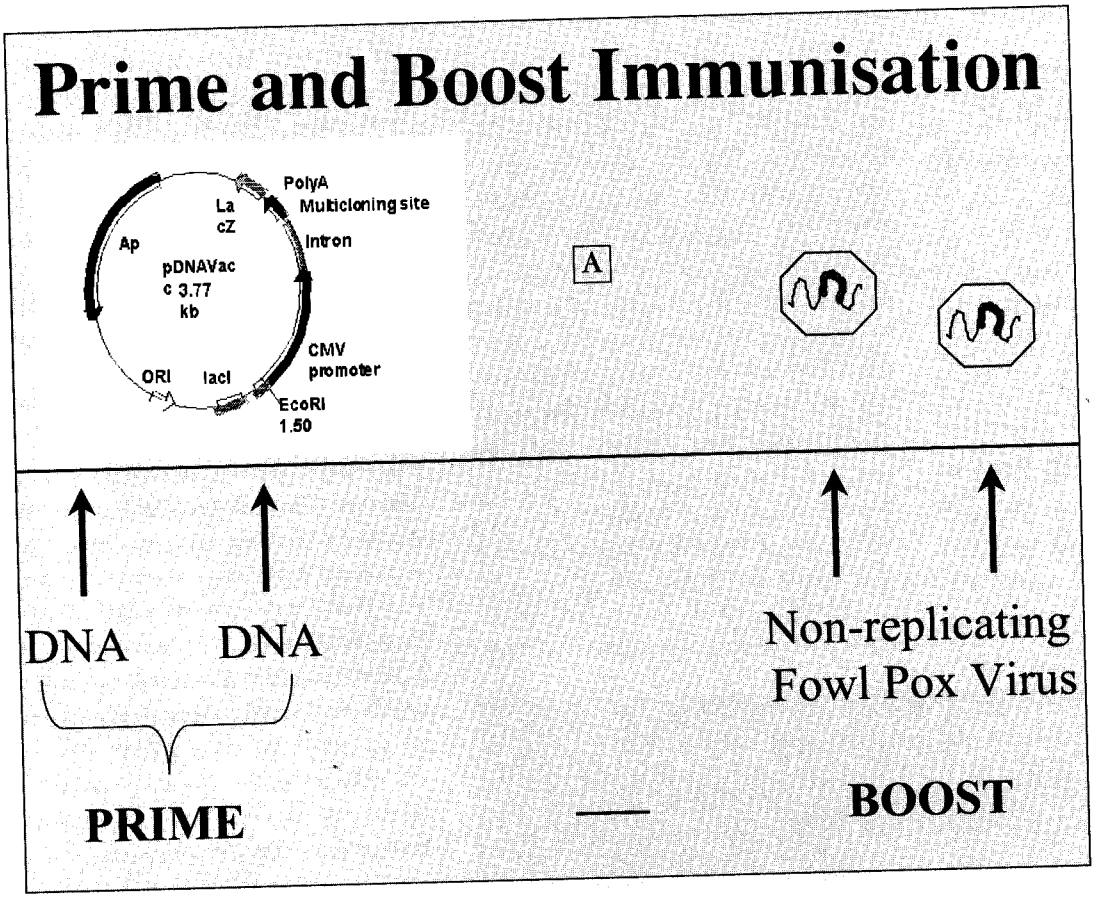
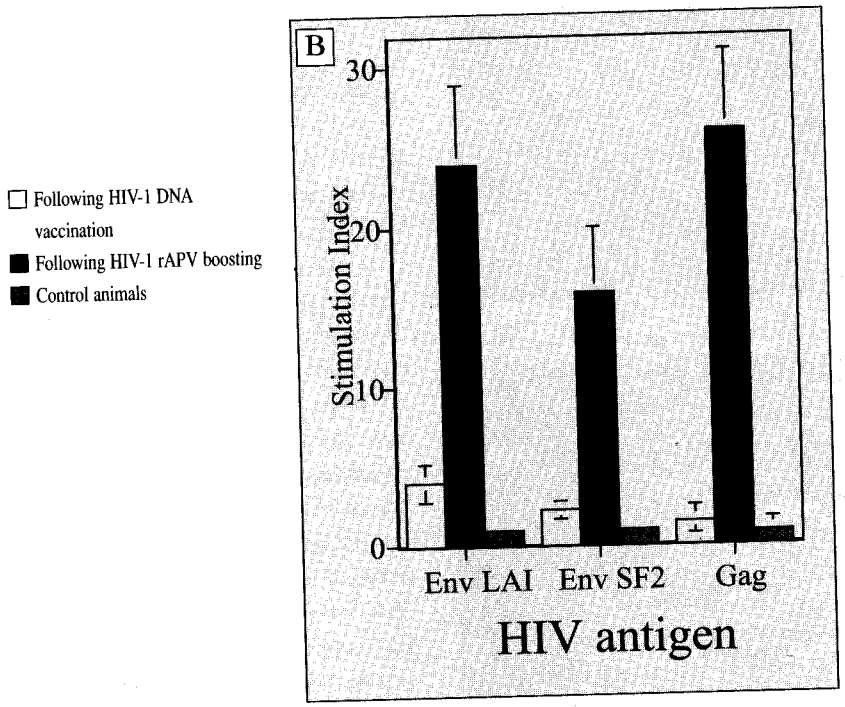


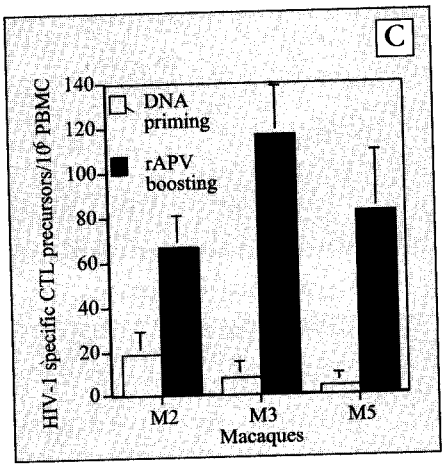
Figure 3. High levels of HIV-specific T-cell responses are induced by DNA prime and poxvirus boost HIV vaccine regimens in macaques [10].



A: The concept of first priming the immune system with a DNA vaccine (a simple plasmid designed to express HIV antigens) and then boosting with a recombinant fowlpox virus vaccine, engineered to also express HIV antigens.

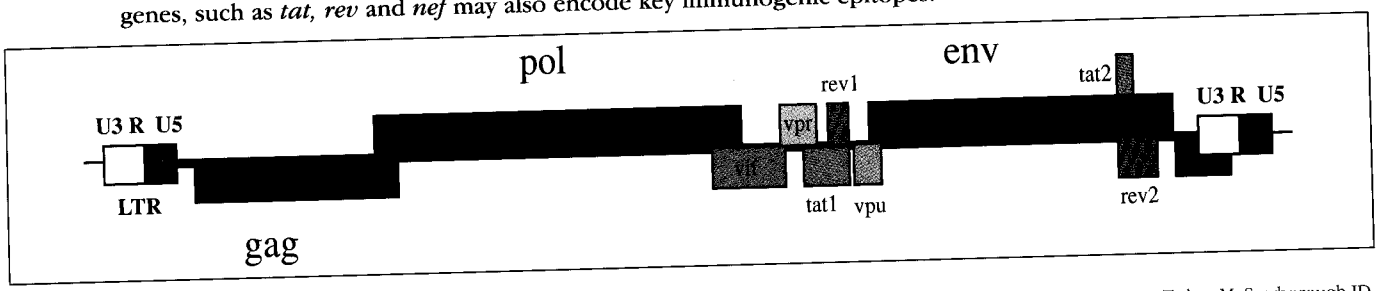


B: CD4+ T- helper responses to HIV antigens induced by DNA/prime and FPV boost regimens [10]. CD4+ T-helper cells are believed to be critical to the induction of sustained cellular immunity against HIV. In this experiment, PBMC are stimulated with HIV protein antigens (either Env or Gag proteins of HIV-1 strains) for 6 days and proliferation expressed as a stimulation index.



C: High levels of CD8+ CTLs are detected following FPV boosting of DNA primed animals [10]. CTLs are widely viewed as critical to the reduction in HIV viremia upon exposure. A limiting dilution assay measures the number of HIV-specific CTLs in PBMC of animals based on the ability of the PBMC to specifically kill target cells expressing HIV genes.

Figure 4. The genomic structure and open reading frames of HIV-1. Most vaccines have focussed on antigens from the large structural genes, *gag, pol* and *env* (in red). However, increasing evidence suggests some of the smaller regulatory genes, such as *tat, rev* and *nef* may also encode key immunogenic epitopes.



DNA prime/poxvirus boost strategy [22]. Such combination vaccine strategies are poised to enter early human clinical trials.

Which antigens of HIV should be targeted?

It is not clear which components of the nine genes encoded by the 10kb genome of HIV are required to induce protective immunity to HIV (Figure 4). Some evidence suggests that the more antigenic material encoded the better [27]. Envelope was an early focus of HIV vaccine efforts, however, not only is it difficult to induce neutralising antibodies with envelope vaccines, but envelope is one of the most variable of all the genes so escape from immune pressure is likely [28]. Recent focus has been on the large, more conserved *gag* and *pol* genes of HIV, as well as the smaller regulatory genes such as *tat, rev* and *nef,* as antigenic targets. Tat has been of interest lately since it is has been shown that rapid and early escape mutants evolve to escape CTLs directed towards Tat in animal model studies, implying that vaccine-induced immunity to Tat may prevent establishment of infection [29]. Furthermore, Tat-based vaccines are affording some protection in animal model studies [30]. Specific CTL epitopes strung together by a single vaccine also show considerable promise [31].

How will mucosal responses be induced?

In the vast majority of cases worldwide, HIV-1 enters via genital mucosal tissues and it would therefore be desirable to induce responses at mucosal sites. Sexual partners of HIV-infected individuals frequently have HIV-1 specific IgA present in genital mucosa [32]. It has long been observed that genital mucosal immune responses can be induced when vaccines are delivered at other mucosal surfaces such at the nose or orally, but not when delivered systemically such as intramuscularly. There is considerable interest in the mucosal delivery of HIV vaccines at present, including the use of engineered wart-virus like particles containing HIV antigens that are efficiently taken up at mucosal sites [33].

Who will receive access to HIV vaccines?

The intense worldwide effort now underway, coupled with promising animal model data, seems likely to yield at least a partially effective HIV vaccine sometime over the next 10-20 years. Attention is now being focussed on how these potentially expensive vaccines will be provided to poor countries in desperate need of them. Recent vaccine successes in rich countries, such as those for Hepatitis B and *Haemophilus influenzae* infection, licensed for up to 20 years, are still not widely available in developing countries. The World Bank, now facing increasing defaults from countries unable to pay back loans due to AIDS-associated economic instability in many countries, is considering setting up a purchase fund to supply HIV vaccines to developing countries. The will of the public worldwide to develop and provide HIV vaccines will hopefully promote fast access to HIV vaccines if and when they become available.

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