Forum

The rationale behind a vaccine based on multiple HIV antigens

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Abstract

The viral diversity of HIV-1 is likely to require a vaccine strategy that induces broad cellular and humoral anti-HIV-1 immunity. Our strategy is based on multiple HIV-1 DNA immunogens together with adjuvant recombinant granulocyte-macrophage stimulating factor. This article describes pre-clinical and clinical work preceding the initiation of clinical HIV-1 phase I/II trials.
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1. Introduction

1.1. The evolution of HIV vaccine development

The first pre-clinical vaccine experiments were performed in the mid 1980s using whole inactivated simian immunodeficiency virus (SIV) in macaques and resulted in induction of sterilizing immunity [1]. Later it turned out that the protective immunity induced by inactivated SIV was, at least partly due to anti-host reactivity directed towards cellular components acquired during the viral cell culture process [2]. Due to the risk of reversion and/or transmission to immunocom-

promised individuals, the use of live attenuated HIV-1 in humans for vaccination has not been regarded as a realistic approach. Instead, the focus turned to subunit vaccines, aiming at inducing virus-neutralizing antibodies. Recombinant or patient-isolated HIV-1 envelope proteins (rgp120) were used to induce antibodies that theoretically should mediate protection, however this did not turn out to be the case upon experimental challenge of chimpanzees [3]. The early recombinant gp120 molecules were structurally monomeric, a feature that later was described insufficient in order to create the correct antigen conformation needed for induction of neutralizing antibodies. Further, heavy glycosylation of the gp120 molecule creates a glycan shield, protecting the gp120 from incoming neutralizing antibodies, an unknown phenomenon at the time of the first envelope immunizations [4]. Recent large-scale human trials with similar subunit based vaccines did not give significant protection against primary infection [5]. This suggests that the envelope antigen conformation is crucial. Another challenge has been that most antibodies directed against the envelope are strain specific,

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whereas breadth against different clades, and different strains within a clade are needed.

During the mid-1990s the focus of HIV-1 vaccine development shifted from induction of neutralizing antibodies, to that of stimulating cytotoxic T-lymphocyte (CTL) immunity. The small regulatory HIV-1 proteins Rev, Nef and Tat have been shown to be potent targets for CTL activity and numerous vaccine strategies targeting these three antigens have been pursued both in animal models [6,7] and clinical trials [8]. One of the most potent of all HIV-1 antigens in eliciting CTL reactivity is the Gag (p24) antigen, and ongoing trials with the Gag antigen show promising induction of cellular immunity in primates and humans (reviewed in [9]). The HIV-1 reverse transcriptase (RT) has also been evaluated and experimental challenge experiments suggesting that it is a potent vaccine target [10]. The generation of CTL against epitopes that are conserved among different strains of HIV is the rationale for focusing upon CTL and these antigens. Although more knowledge is constantly gathered on HIV-1 specific immune responses, the exact correlates of protection in HIV-1 infection remain unclear [11]. Neither humoral nor cellular immunity alone is likely to result in protection; instead current opinion on correlates of protection emphasizes the collective effect of CD4+ and CD8+ T lymphocytes, broadly neutralizing antibodies and innate immunity.

1.2. Viral diversity requires induction of broad immunity

A typical untreated HIV-1 infected patient has an average of half a million viral particles per milliliter of blood, where multiple variants of the virus are present at any one time. Complete turnover of this viral quasi-species population occurs in a matter of days, generating a continuous intra-patient virus evolution. It becomes obvious that any anti-HIV-1 intervention is literally dealing with a range of dynamic enemies, not just one single static agent. The diversity displayed by HIV-1 is further complicated by extensive recombination. There is however evidence that cellular and antibody cross-reactivity are sufficiently large to cope with this variation, described in both clinical and experimental studies [12]. Still, cytotoxic T-cells are strictly epitope-restricted and a slight change in the antigen/virus may lead to CTL immune escape [13]. Infection with one subtype of the virus may permit subsequent infection with another or even the same subtype of virus [14]. Taken together, this strengthens the importance of using multiple targets as well as multiple subtypes in a vaccine strategy. We, and others, believe that the development of multi gene/multi antigen HIV-1 vaccines are necessary in order to achieve broad antiviral immunity [3,15,16]. Nevertheless this approach may require the determination of which antigens are the most useful for generating immunity against a certain breadth of strains. In other words, the immunotype of the viruses or of particular epitopes may not correspond to the genotype upon which the clade classification is based.

1.3. DNA immunogens—bringing new hope to HIV-1 vaccination

HIV-1 vaccine development has over the years explored vaccine strategies involving inactivated and attenuated viruses, recombinant viral and bacterial vectors, recombinant proteins, synthetic peptides and most recently the strategy of genetic (DNA or RNA) immunization [17]. In HIV-1 DNA immunization, one or multiple HIV-1 genes are cloned into a mammalian expression plasmid that is delivered directly to the host, which in turn expresses the DNA encoded antigen within its own cells. This leads to induction of antigen-specific immunity [17,18]. Genetic vaccines are capable of inducing both neutralizing antibodies and, since there are endogenously processed though the cellular machinery, also high quality CTL clones [19].

An unexpected enigma of this new technology is that DNA immunization alone results in relatively weak immune responses, particularly in humans [20]. Many different approaches have been evaluated to enhance the immunogenicity of genetic vaccines involving adjuvants or carriers such as liposomes, bacterial endotoxins, macroglobulins, chromosomal proteins, mineral adsorbents, CpG oligodeoxynucleotides, peptides and polymers like poly lactide-co-glycolide (reviewed in [21]). Extensive efforts have been made to explore the possibility to use cytokines/chemokines to improve DNA vaccination [22]. We have focused on the molecule granulocyte macrophage-colony stimulating factor (GM-CSF), a cytokine that is known to attract bone marrow derived progenitor cells as well as to have the ability to induce maturation and activation of dendritic cells [23]. Dendritic cells are powerful antigen presenting cells highly capable of activating and priming antigen specific T-lymphocytes. Plasmid encoded GM-CSF has been described to enhance immunity induced by HIV-1 gp160 DNA in mice [24] and non-human primates [25]. We have shown that recombinant GM-CSF (rGM-CSF) co-delivered with the HIV-1 gp160 encoding DNA plasmid appears to be an even better option. This led to enhancement of both cellular and antibody mediated immunity in mice (A. Bråve, unpublished and [26,27]).

2. The envelope (gp160) DNA immunogen(s)

In late 1998 our laboratory constructed an HIV-1 gp160 subtype B DNA immunogen [27]. The furin proteolytic cleavage site in the subtype B plasmid was destroyed by site directed mutagenesis thus preventing the maturation of gp160 to gp120 and gp41. The gp160 molecule has higher antigenicity for patient sera than the gp120 and gp41 molecules separately or combined (unpublished data). By using homologous recombination we created a set of three HIV-1 gp160 DNA immunogens originating from subtypes A–C viruses. All three constructs are based on a subtype B gp160 backbone, although in the gp160 A and C constructs