Published in final edited form as:
Curr Opin Immunol. 2009 June ; 21(3): 346–351. doi:10.1016/j.coi.2009.05.016.

Heterologous Prime-Boost Vaccination

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Summary

An effective vaccine usually requires more than one time immunization in the form of prime-boost. Traditionally the same vaccines are given multiple times as homologous boosts. New findings suggested that prime-boost can be done with different types of vaccines containing the same antigens. In many cases such heterologous prime-boost can be more immunogenic than homologous prime-boost. Heterologous prime-boost represents a new way of immunization and will stimulate better understanding on the immunological basis of vaccines.

Introduction

It is not unusual that multiple immunizations are required for many vaccines to be successful. For pediatric population, up to five immunizations may be needed, as is the case for Diphtheria, Tetanus and Pertussis (DTP) vaccine, which is given three times during the first six months after birth, followed by a fourth dose in the second year of life, and a final boost between four and six years of age. Still, some of the vaccines need additional boosts even in adults who have already received the complete immunization series, for example, the Tetanus-diphtheria (Td) vaccine, for which a boost is recommended every 10 years throughout a person's lifespan. While it is not entirely clear why some vaccines require more immunizations than others, it is well accepted that multiple immunizations (i.e. "prime-boost") are critical for even the most successful vaccines. This principle applies to live attenuate vaccines (e.g., oral polio vaccine), inactivated vaccines (e.g., hepatitis A vaccine), recombinant protein subunit vaccines (e.g., hepatitis B vaccine) and polysaccharide vaccines (e.g., *Haemophilus Influenzae* type b vaccine). For these vaccines, the prime-boost is "homologous" because the same vaccines given in the earlier priming immunizations are used for subsequent boost immunizations.

Over the past decade, studies have shown that prime-boost immunizations can be given with unmatched vaccine delivery methods while using the same antigen, in a "heterologous" prime-boost format. The most interesting and unexpected finding is that, in many cases, heterologous prime-boost is more effective than the "homologous" prime-boost approach. The rapid progress of novel vaccination approaches, such as DNA vaccines and viral vector-

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based vaccines, has certainly further expanded the scope of heterologous prime-boost vaccination [1–3].

Early history of heterologous prime-boost vaccination

A 1992 landmark Science report was among the first to employ the heterologous prime-boost immunization technique in a non-human primate model [4]. In that study, *Macaca fascicularis* were first immunized with recombinant vaccinia virus expressing SIVmne gp160 antigen and then boosted with gp160 protein produced in baculovirus-infected cells. Animals were protected from intravenous challenge of SIVmne viruses and this became one of the most promising protection results in the early HIV vaccine development effort.

Shiu-Lok Hu, the lead scientist of the above study, and his collaborators demonstrated previously, in rodents, that priming with a live recombinant virus and boosting with a subunit recombinant protein was more effective than immunization by either immunogen alone [5]. In a separate study, Girard et al. also reported a significant increase in antibody titers in a chimpanzee primed with recombinant vaccinia virus and boosted multiple times with a mixture of recombinant HIV-1 proteins or synthetic peptides [6]. Furthermore, around the same time, in what may be the first human testing of the heterologous prime-boost immunization, Daniel Zagury of the Pierre and Marie Curie University in Paris inoculated himself with a recombinant vaccinia virus containing the HIV-1 Env gene and later gave a boost using a recombinant Env protein [7]. Early work in other non-HIV areas include small animal studies conducted by Eckhart Wimmer's group who used synthetic peptides and inactivated polio virus for prime-boost immunizations [8].

Heterologous prime-boost HIV-1 vaccines

Initial efforts in the use of a heterologous prime-boost immunization approach for HIV-1 vaccine development was based on the following rationale:

Recombinant envelope (Env) glycoproteins, while being able to elicit isolate specific neutralizing antibody responses, were unable to elicit cytotoxic T cell responses, and on the other hand, immunization with recombinant vaccinia expressing HIV-1 antigens could elicit good T cell responses but not high levels of protective antibodies. Therefore combined immunization including both of these two types of vaccines may be more effective than either immunogen alone [5].

This statement established a key principle for the use of heterologous prime-boost immunizations, i.e., to elicit both humoral and cell-mediated immune responses. Modern immunology has established that such a balanced immune response is important for protection not only against viral infections but also other types of pathogens. Traditional vaccines, particularly inactivated and subunit vaccines, are not very effective in eliciting T cell responses. This requirement is even more important for HIV vaccine development. An ideal HIV vaccine should be able to generate "sterilizing antibodies" to prevent the virus from establishing an infection that is more difficult to eliminate once HIV-1 is integrated into the genome of the host's peripheral blood mononuclear cells (PBMCs).

At the same time, T cell immune responses play a key role in controlling the scale of infection, which may affect the long-term mortality and morbidity of the host.

The discovery of DNA vaccines in early 1990s added a new vaccine modality to the traditional vaccine delivery approaches. After showing promising utility of DNA vaccines, mainly in small animal models, the immunogenicity of DNA vaccines in humans was soon realized too low to be used alone although DNA vaccination proved its worth as an excellent

priming modality [9]. As a result, DNA priming, along with various forms of boost, has been incorporated into almost every current major HIV-1 vaccine development effort.

Similar to the original vaccinia prime-recombinant protein boost HIV-1 vaccines which used antigens from laboratory adapted HIV-1 isolates, protein boost can also be given after the DNA prime [10, 11]. Actually, DNA prime and recombinant protein boost with primary HIV-1 Env antigens became the first approach to elicit positive neutralizing antibodies in rabbit sera against JR-FL, a fairly difficult to neutralize primary HIV-1 isolate [12, 13]. The same prime-boost approach can be used to deliver multiple Env antigens that can either be matched or not matched between the DNA prime and protein boost. Such polyvalent Env formulations were more likely to elicit cross-reactive neutralizing antibodies against a broad spectrum of primary HIV-1 viruses than the monovalent Env formulations [14]. The DNA prime-protein boost approach was also proven effective in non-human primates [15] and more significantly, in humans [16]. In these studies, four out of six rhesus macaques immunized with DNA prime-protein boost were able to achieve “sterilizing” protection [15]. In human volunteers, no antigen-specific antibody responses were detected after three DNA immunizations, but a quick rise of anti-Env IgG responses to titers of 1:104 to 1:105 were identified after only one protein boost in every volunteer that received this combination vaccine. These human immune sera were cross-reactive to Env antigens from every clade of HIV-1 isolates with positive neutralizing activities against primary HIV-1 from clades A to E [16]. This result is particularly striking as no other candidate HIV vaccine has been able to induce such a response in humans. More significantly, this DNA prime-protein boost formation also elicited high frequency of responders with HIV-1 antigen specific and polyfunctional T cell immune responses [16, 17].

Due to the failure of the VaxGen HIV vaccine trial, which only used recombinant gp120 proteins as immunogens, T cell-based vaccines have been the main focus in HIV vaccine development for the past decade. Due to safety concerns over the use of live attenuated HIV-1 vaccines, there are limited vaccine platforms to elicit high level T cell immune responses. Novel vaccine delivery approaches, such as DNA and viral vector-based vaccines, have become the main weapons to elicit T cell-mediated immune responses, particularly when these two approaches are delivered as a prime-boost. Results from a number of non-human primate studies have shown good immunogenicity and protection against chimeric SHIV challenge using the DNA prime-viral vector boost combination. Viral vector boosts have included MVA vector [18, 19], fowlpox vector [20], adenoviral vectors [21, 22] and Vesicular Stomatitis Virus (VSV) vector [23]. Some DNA prime-viral vector boost HIV-1 vaccines have also moved into human studies with clearly positive antigen-specific T cell immune responses. The Euro Vacc 02 phase I clinical trial provided the clear evidence in a comparative study that a DNA prime-NYVAC poxviral vector boost HIV-1 vaccine was more immunogenic than the NYVAC poxviral vector alone vaccine [24].

Using the similar strategy reported in early 1990s [4, 5], the ALVAC vector prime-protein boost HIV vaccine has been tested in an ongoing phase III clinical trial in Thailand. According to the phase 1/2 report, this combination HIV-1 vaccine was shown to be immunogenic as evidenced by positive antigen-specific antibody and positive CD8+ T cell responses [25]. While it is not clear whether this particular formulation will lead to a successful protective HIV-1 vaccine due to the selection of Env antigens that may have very limited cross-reactivity, the combination approach itself has proven successful in improving the immunogencity of otherwise two relatively weak vaccine components.

Heterologous prime-boost vaccines against other pathogens

Over the past few years, the use of heterologous prime-boost approaches in vaccine research has gained significant momentum against a wide range of pathogens. Several features have become apparent for this trend.

First, it is common to use the heterologous prime-boost approach to address some of the most challenging vaccine development objectives including malaria and tuberculosis (for detailed information please see accompanying articles in this volume) due to the failure of other vaccination approaches. The idea is to focus on certain critical antigens and to elicit high quality immune responses involving different subsets of T cell immune responses. A DNA prime-MVA boost vaccine encoding thrombospondin-related adhesion protein partially protected healthy malaria-naïve adults against *Plasmodium falciparum* sporozoite challenge [26]. This study also highlights the importance of antigen selection for immune protection, made clear by the fact that the same combination vaccination using circumsporozoite protein, instead of the thrombospondin-related adhesion protein, did not elicit such protection.

For tuberculosis vaccine development, qualitatively and quantitatively different cellular immune responses have been elicited in rhesus macaques receiving a recombinant Bacille Calmette-Guérin (BCG) prime followed by an adenovirus 35 vector boost that expressed a fusion protein composed of Ag85A, Ag85B and TB104 [27]. Alternatively, BCG can be used as a boost following a DNA vaccine prime. In one study conducted in calves, DNA prime with Ag85B, MPT64 and MPT83 antigens followed by a BCG boost was able to elicit higher immune responses and better protection than BCG alone against *Mycobacterium bovis* challenge [28].

Second, a well-designed heterologus prime-boost approach can expand the scope of immune responses. When mice were primed with DNA vaccine expressing ESAT6 and later received the same antigen in the form of recombinant protein as boost, production of Th1-type cytokines was increased significantly, as was the IgG2 to IgG1 ratio [29]. In another murine study, prime with a DNA vaccine, expressing the gD antigen of herpes simplex virus type 2 (HSV-2), which preferentially induces Th1 type cellular immune responses, and boost with recombinant gD protein, which mainly induces Th2 biased responses, led to significantly enhanced antibody, T cell proliferation, and Th1 cytokine production [30].

Third, the prime-boost vaccine approach can also improve the effectiveness of existing vaccines. One example is the use of DNA prime, which increased antibody response levels, in animals later receiving boost with inactivated rabies vaccines [31]. Similarly, DNA prime can increase the titer and longevity of hyperimmune sera in animals to be immunized with the recombinant PA antigen against anthrax [32]. Adding a DNA prime, mice boosted with the licensed hepatitis B surface protein vaccine were able to produce stronger and more homogenous antibody responses in a study group when compare to groups only receiving recombinant protein alone. Higher IL-12 and IFN-γ secretion in splenocytes were also observed [33].

Finally, the prime-boost approach can have important practical applications in addressing vaccines with broad public health impact. In an animal model naïve to influenza infection, it has been shown that a heterologous one-time DNA prime and one-time inactivated influenza vaccine boost was more immunogenic than twice administered homologous prime-boost using either DNA or inactivated influenza vaccine alone [34]. This finding can be very useful for preparation against pandemic avian influenza. One of the key issues facing the development of influenza vaccines is the limited capacity and long cycle needed to produce traditional influenza vaccines. Usually two immunizations are needed for avian influenza

vaccines. It is feasible that targeted populations can first receive an avian influenza DNA vaccine prime long before any unexpected pandemic attack, which will greatly reduce the amount of vaccine needed at the time of outbreak of pandemic flu. This approach can also be useful for other forms of influenza, including human and swine influenza viruses. Adding a new strain of vaccine to the current trivalent influenza vaccines will require significant additional resources and time. A polyvalent DNA prime can cover a wide range of future potential viral strains at much lower cost.

Heterologous prime-boost vaccines as therapy to cancers

Similar to other novel vaccine forms, the heterologous prime-boost approaches have also been studied as potential treatments for cancer. Using a recently identified six-transmembrane epithelial antigen of the prostate (STEAP), a heterologous DNA prime and Venezuelan equine encephalitis virus-like replicon particles (VRP) boost was able to elicit better immune responses against STEAP, including INF-gamma, TNF-alpha, and IL-12, when compared to either vaccine modality alone. This vaccination regimen induced a modest but significant delay in growth of established, 31 day-old tumors in mice [35].

Mechanisms of heterologous prime-boost vaccines

A fundamental but still mysterious question is why the heterlogous prime-boost is more effective than homologous prime-boost even when the same vaccine components are used for each. One way to study this question is to determine the importance of order of administration of heterologous prime-boost vaccines. Using a *Mycobacterium bovis* model, it was demonstrated that the order of prime-boost vaccination of neonatal calves with BCG and DNA vaccine, encoding Hsp65, Hsp70 and Apa, was not critical for enhancing protection against bovine tuberculosis [36]. In a different model, with DNA prime-protein boost using murine HSV-2 gD antigen, it was clear that DNA priming is critical because a reversed protein prime-DNA boost regimen produced antibody levels similar to those following homologous protein-protein vaccination, and failed to further enhance Th cell proliferative responses or cytokine production [30]. In an even more detailed analysis using hepatitis C E2 as a model antigen, it was found that DNA prime-adenoviral vector boost elicited the highest level of Th1 CD4+ T cell responses when compared to the reversed adenoviral prime-DNA boost or homologous prime-boost with the same vaccines. More interestingly, the DNA prime-adenoviral vector boost regimen, but none of the other three possible prime-boost combinations, elicited CTL responses against three E2-specific epitopes and one of them was immunodominant [37].

In an extensive non-human primate study, presented at the 2008 AIDS Vaccine conference in Cape Town, South Africa by Dr. Shiu-lok Hu from University of Washington, Seattle, vaccinia viral vector or DNA prime, followed by protein boost, generated better antibody responses than boosting with DNA or various viral vector vaccines. These two heterologous prime-boost regimens, including a protein boost component, but not any of the other combinations, were able to elicit better neutralizing antibodies and sterilizing immunity against a high-dose intrarectal challenge by $SHIV_{sf162.p4}$ in ~40% of immunized animals, and protected animals against peripheral CD4+ T-cell depletion.

Some studies have shown that DNA prime was able to improve the avidity of antibody responses elicited by protein-based vaccines [11, 13]. Because DNA vaccines produce antigens *in vivo*, priming with a DNA vaccine may elicit memory B cells that are specific to sensitive conformation domains of an antigen. In a rabbit study, the delivery of primary HIV-1 gp120 antigens using the DNA prime-protein boost approach, but not the recombinant gp120 protein alone vaccine, was able to elicit conformation dependent CD4 binding site antibodies which are potentially important for neutralizing HIV-1 [38].

The immunogenicity of heterologous prime-boost can be further improved by including other factors that may further facilitate or enhance the effect of vaccines. For example, including plasmid cytokines and colony-stimulating factors could enhance the immunogenicity of DNA prime-viral vector boosting HIV-1 vaccines [22]. The potency of DNA vaccine prime can be enhanced by using a micorparticle based formulation followed with a protein boost [39]. However, it is not clear whether using different adjuvants for a protein vaccine as boost will make any difference.

Conclusions

Heterologous prime-boost vaccination, using both traditional and novel immunization approaches, provides exciting opportunities to elicit unique immune responses to allow for improved immunogenicity and/or protection. Research has shown that the heterologous prime-boost can take various forms and that the order of prime-boost administration may be important although this may be antigen-dependent and may be influenced by the host species and the type(s) of immune responses to be achieved. Future studies will need to focus more on the mechanisms behind the heterlogous prime-boost vaccination approach and solve practical issues related to a two-component vaccine, including costs of vaccines and any currently unidentified issues of safety.

Acknowledgments

This work is supported in part by NIH/NIAID grant R01 AI06250. Author would like to thank Michael Vaine, Shiu-lok Hu and Stanley Plotkin for discussion and support in providing related references.

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Table 1

Common Heterologous Prime-Boost Vaccinations

Prime immunization	Boost immunization	Representative references
DNA	Recombinant protein	[14,17]
	Inactivated Vaccine	[31,34]
	Viral vectors	[19–21,23,24]
	BCG	[36]
Viral vector	Recombinant Protein	[4]
BCG	Viral vector	[27]